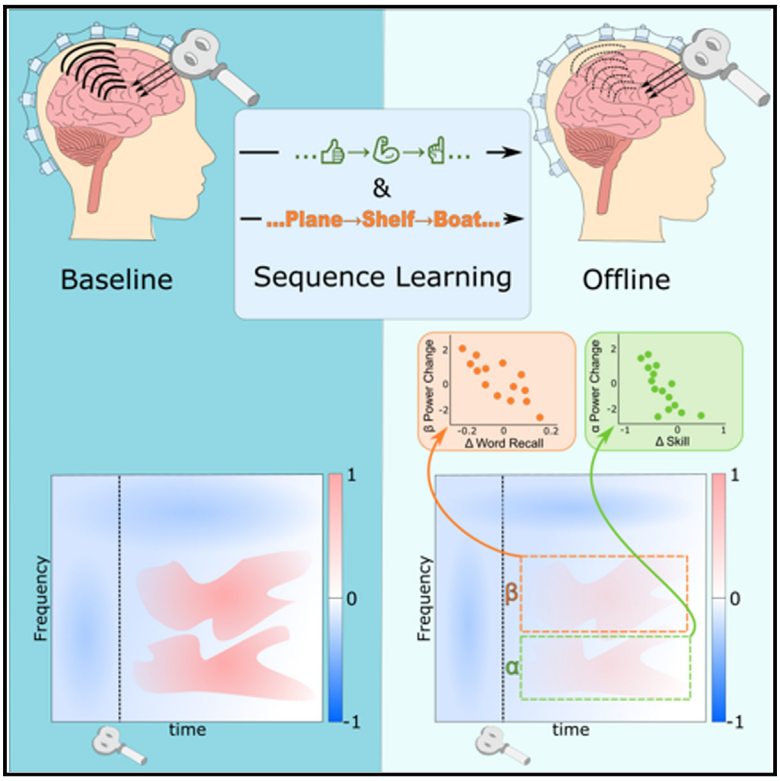
Report

Current Biology

Distinct frequencies balance segregation with interaction between different memory types within a prefrontal circuit

Graphical abstract



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In brief

Bracco et al. show how segregation between memory systems breaks down. Different memory types (action versus. word sequences) within a prefrontal network are linked to different oscillations (alpha versus beta frequencies), and the strength of these shape the shift from a segregated to an interactive memory organization.

Highlights

- How do interactions emerge between segregated memory systems (actions versus words)?
- Prefrontal oscillatory activity changed when different memories interacted
- Different oscillations were linked to the fate of different memory types
- Oscillation strength balanced the segregation interaction between memory types

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Report

Distinct frequencies balance segregation with interaction between different memory types within a prefrontal circuit

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SUMMARY

Once formed, the fate of memory is uncertain. Subsequent offline interactions between even different memory types (actions versus words) modify retention.[1–6] These interactions may occur due to different oscillations functionally linking together different memory types within a circuit.[7–13] With memory processing driving the circuit, it may become less susceptible to external influences.[14] We tested this prediction by perturbing the human brain with single pulses of transcranial magnetic stimulation (TMS) and simultaneously measuring the brain activity changes with electroencephalography (EEG[15–17]). Stimulation was applied over brain areas that contribute to memory processing (dorsolateral prefrontal cortex, DLPFC; primary motor cortex, M1) at baseline and offline, after memory formation, when memory interactions are known to occur.[1,4,6,10,18] The EEG response decreased offline (compared with baseline) within the alpha/beta frequency bands when stimulation was applied to the DLPFC, but not to M1. This decrease exclusively followed memory tasks that interact, revealing that it was due specifically to the interaction, not task performance. It remained even when the order of the memory tasks was changed and so was present, regardless of how the memory interaction was produced. Finally, the decrease within alpha power (but not beta) was correlated with impairment in motor memory, whereas the decrease in beta power (but not alpha) was correlated with impairment in word-list memory. Thus, different memory types are linked to different frequency bands within a DLPFC circuit, and the power of these bands shapes the balance between interaction and segregation between these memories.

RESULTS AND DISCUSSION

Different memory types are predominately processed within segregated systems (procedural versus declarative[19,20]). However, they interact "offline" following their formation, which modifies their fate (retained versus impaired[1–6,21]). A functional link between these otherwise segregated memory systems may support this interaction.[7–13] Such networks may become resistant to external perturbation because memory processing is driving their activity.[14] This is analogous to a child's swing driven by its own momentum resisting the external influence of a parental hand in its continued back-and-forth. We tested this prediction by perturbing brain activity using single pulses of transcranial magnetic stimulation (TMS) and simultaneously measuring its effects with electroencephalography (EEG[15–17]). Stimulation was applied over brain areas (right dorsolateral prefrontal cortex, DLPFC; left primary motor cortex, M1) that have been implicated in offline memory processing.[4,10,18,22] Specifically, the right DLPFC was selected because disrupting its function prevents interactions between different memory types without disrupting the individual memories.[3,4] We applied stimulation to these brain areas before (baseline) and after (offline) different behavioral tasks. The critical events that we are seeking to identify occur offline.[23–25]

DLPFC circuits linked to the interaction between different memory types

Initially, a motor task was followed by a word-list learning task. Participants learned a movement sequence (motor skill group) or else performed the same number of movements without any serial structure (control group) and then immediately learned the same word list (Figure 1; Comparison 1). We measured skill as the response time advantage of the sequential over random trials, which is a widely used sensitive and specific measure of sequence learning, while performance in the task without a serial structure was measured as a response time, and the word recall

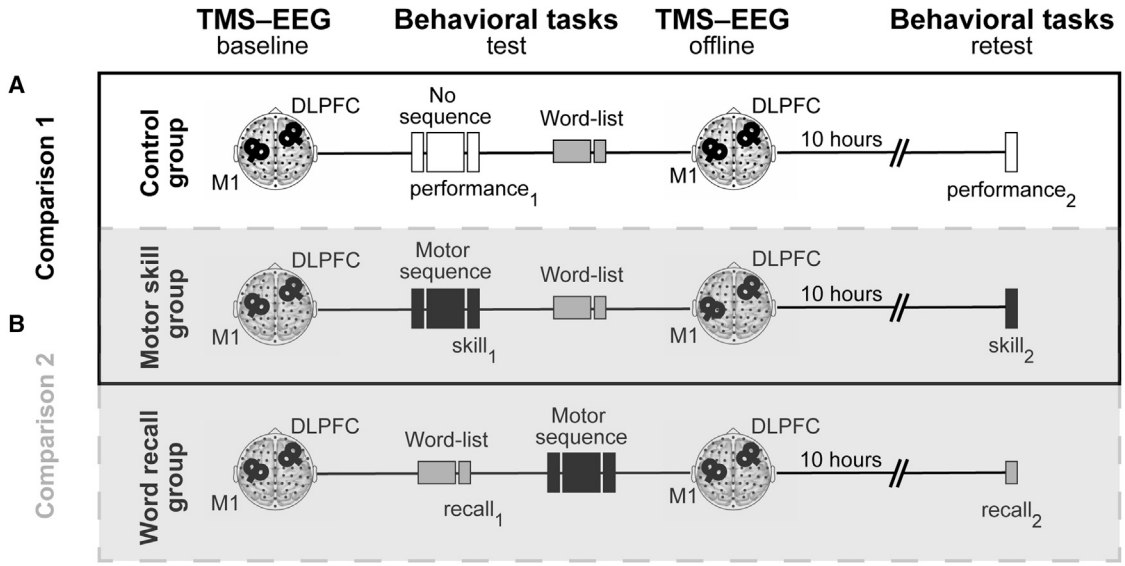

Figure 1. Experimental design

We applied single TMS pulses to the dorsolateral prefrontal cortex (DLPFC) or the primary motor cortex (M1) and detected the response using an EEG montage (black dots overlying a brain). This was done in all three groups before (baseline) and after (offline) participants performed two tasks in quick succession (n = 15 per group).

(A) A motor task without any serial structure was performed ($performance_1$), then a word list learned, and subsequently motor performance was retested ($performance_2$; control group). By contrast, in another group a motor sequence ($skill_1$) was learned, then a word list, which interacts with the motor sequence, impairing its retention ($skill_2$[1,4,6]; motor skill group). By comparing between these groups, we were able to identify how circuits were changed by tasks that do or do not interact (Comparison 1; control versus motor skill groups).

(B) We then compared learning the motor skill and word list in a different order (Comparison 2; motor skill versus word recall groups). A word list ($recall_1$) was learned, then a motor sequence, which interacts with the word list, impairing its retention ($recall_2$[1,4,6]). By comparing between these groups, we tested whether circuit changes were linked broadly to interactions or linked to a specific interaction affecting the fate of a particular memory type.

See also Figure S2.

was measured as the total number of correctly recalled words (from the 16-item list[1,26]). As expected, there was an interaction between the motor skill and subsequent word list (mean ± SEM; $recall_1$, 13.6 ± 0.4 words) with a significant decrease in motor skill between testing and subsequent retesting (motor skill group; $skill_1$ versus $skill_2$, 95 ± 15 versus 73 ± 14 ms, paired t test, $t(14) = 2.7$, $p = 0.017$). By contrast, enhanced skill develops over this offline interval when the motor skill is learned in isolation.[1,4,6,27–32] In the control group, there was no interaction between the control task and the word list (13.0 ± 0.5 words) because without a serial structure to the movements, a motor sequence memory was not formed, and hence, the performance (visual response time) was not impaired, but improved (control group; $performance_1$ versus $performance_2$, 408 ± 15 versus 367 ± 12 ms, paired t test, $t(14) = 4.0$, $p = 0.001$[1,4,6,33]). Otherwise, the motor skill and control groups were identical with the same number of movements being performed (in the motor tasks), which was followed by the same list of words being learned (in the word-list task). Thus, comparing between the groups revealed how an interaction between different memory types modified the response of brain networks (detected with EEG) to an external perturbation (from a single TMS pulse).

We found that the DLPFC circuit became resistant to perturbation due to an interaction between different types of memory. We found that the spectral response to single TMS pulses before (baseline) compared with after (offline) the behavioral tasks differed significantly between the groups (cluster analysis; time (offline versus baseline)*group (motor skill versus control), $p = 0.006$; Figure 2A). This change in the response to single-pulse stimulations was not correlated with motor skill ($skill_1$; all $p > 0.16$), which implies that the changed response cannot simply be attributed to motor skill. It originated from a fronto-temporal network (Figure 2A). The response to the single TMS pulses decreased significantly relative to baseline in the alpha/beta (6–23 Hz) band following an interaction between the memory tasks (offline versus baseline; motor skill group, $p < 0.001$; Figure 2B). Changes within this alpha/beta frequency band have been shown to occur during offline memory processing.[10,34] By contrast, when a motor performance task replaced the motor sequence task, which prevents the interaction between memory tasks, there was no significant change in the post-TMS spectrum within any frequency range (offline versus baseline; control group; all $p > 0.45$; Figure 2B). At baseline, before the tasks, the response to stimulation did not differ significantly across the groups (Figure S1). Thus, the reduced response of a DLPFC circuit to TMS was due to the interaction between different memory types, which implies that these offline interactions are processed within a DLPFC circuit.

Unlike the DLPFC circuit, the M1 circuit did not become resistant to external perturbation due to memory interactions. We found that the spectral response to TMS pulses before (baseline) compared with after (offline) the behavioral tasks did not differ significantly between the groups (cluster analysis; time (offline versus baseline)*group (motor skill versus control), all $p > 0.28$;

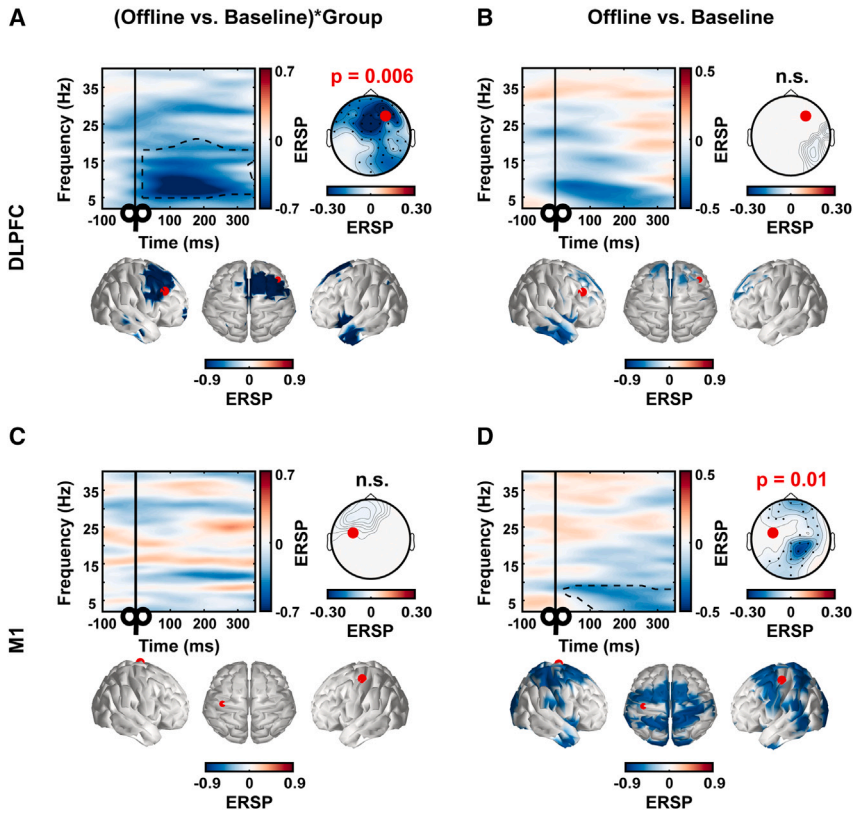

Figure 2. The DLPFC but not the M1 circuit changes due to an interaction between different memory types

We tested for changes in the response of different networks (DLPFC versus M1) to single pulses of stimulation before (baseline) and after (offline) different tasks. The tasks were either a motor skill followed by a word list (motor skill group) or a motor performance task followed by a word list (control group; Comparison 1; Figure 1).

(A) We found that the change in DLPFC response differed significantly between the groups ((offline versus baseline)*group(motor skill versus control), $p = 0.006$). The identified cluster (dashed box; time-frequency plot) corresponded to frequencies within the alpha/beta range (6–23 Hz) and localized to a bilateral fronto-temporal network (spatial plots).

(B) There was no significant change in the DLPFC response to stimulation (both groups combined; offline versus baseline; $p > 0.19$). The DLPFC response only changed significantly when there was an interaction between memory tasks (motor skill group; $p < 0.001$), whereas there was no significant change when there was no interaction between the tasks (control group; $p > 0.45$). Thus, the DLPFC response changes were specifically related to the interaction between different memory types.

(C) We found that the change in M1 response did not differ significantly between the groups (time (offline versus baseline)*group(motor skill versus control); $p > 0.284$).

(D) However, there was a significant change in the M1 response (offline versus baseline, $p = 0.01$). The identified cluster (dashed box; time-frequency plot) was within the theta/alpha frequency range (2–10 Hz) and localized bilaterally to premotor and parietal regions (spatial plot). In the time-frequency plots, the black vertical line and black coil indicate TMS-pulse onset (i.e., 0 ms). The black dashed boxes highlight the significant clusters (frequency range and duration). In the topographical plots, the red dots identify the position of the TMS coil (in sensor space), while the small black dots show those channels included in the significant clusters. Both the time-frequency and topographical plots show the same clusters visualized in different ways. The three-dimensional clusters (time, frequency, and channel) were collapsed across channel—to give the time-frequency plot—or across both time and frequency—to give the topographical plot. Finally, in the spatial plots (3D magnetic resonance imaging [MRI] average brain), a red dot indicates the position of the TMS coil (in anatomical space).

See also Figures S1 and S2.

Figure 2C). This suggests that the M1 circuit was not affected by the interaction between different memory types. It also implies that comparing between the groups yielded a minimal amount of motor skill because this would be processed offline, leading to M1 circuit changes (for example, Buch et al.,[10] Muellbacher et al.,[22] and Robertson et al.[29]). However, we did not observe changes in M1 responses unique to a specific group (i.e., motor skill group). Nonetheless, the cluster analysis did reveal a significant change in the response to the TMS pulse before and after the behavioral tasks (time (offline versus baseline), 2–10 Hz; $p = 0.01$). This reduction in response to the TMS pulse affected both the premotor and parietal cortices bilaterally (source analysis; Figure 2D). Thus, the decreased response from the M1 circuit, unlike the decreased response from the DLPFC circuit, was not due to the interaction between different memory tasks. Instead, it was due to task performance.

DLPFC and how memory interactions are produced

The DLPFC may have a general role in the interaction between different memory types. Hence, the response of applying a TMS pulse to the DLPFC, although modified by the presence or absence of an interaction (Comparison 1), would not be modified by how an interaction is produced. An interaction can be produced in different ways.[1,4,6] Here, learning a motor skill and then immediately learning a word list impaired subsequent skill retention (Comparison 1). Conversely, an interaction is also generated when learning a word list and then immediately learning a motor skill, which impairs word-list retention.[5,21,35] In each of these scenarios, the memory task order differs, and the memory impaired by the interaction differs (motor skill versus word recall, respectively); however, common between them is an interaction between different memory types. As a consequence, changing the memory task order provides a means to test whether changes in the response of the DLPFC to a TMS pulse are linked to a feature of a specific interaction, or more broadly to interactions between different types of memory. We set out to distinguish between these possibilities.

In the earlier comparison (Comparison 1), participants learned a motor sequence, then learned a list of words, and showed impaired recall for the motor sequence (motor skill group; Figure 1). This group was compared with another group, in which the task order was reversed, with word-list learning being immediately followed by motor sequence learning ($skill_1$, 84 ± 11 ms; Figure 1; Comparison 2). We found a significant decrease in

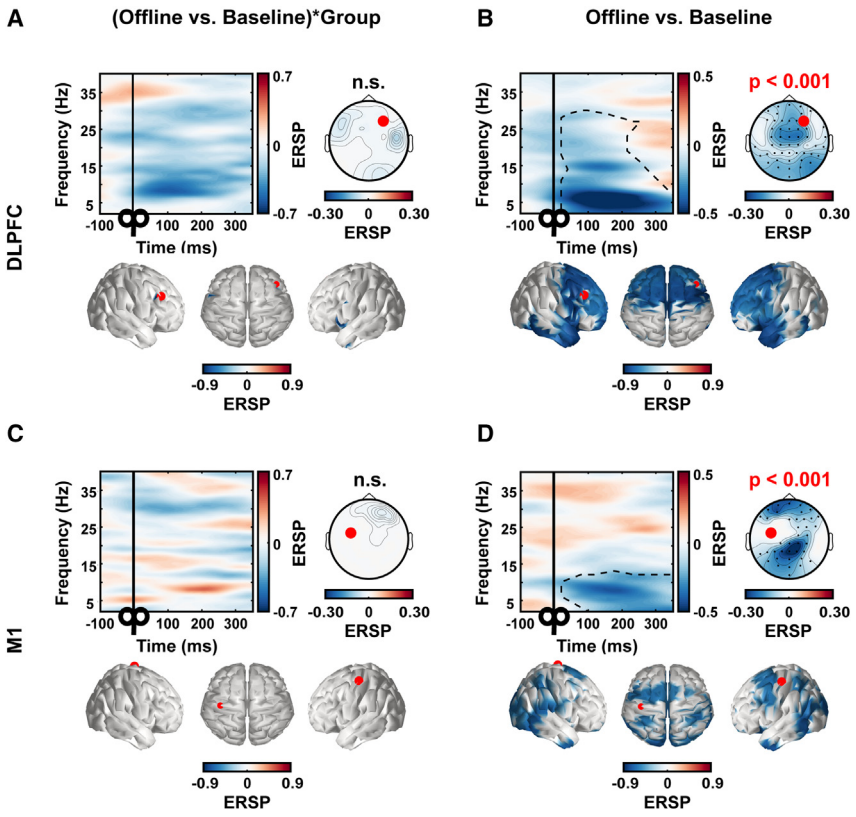

Figure 3. The DLPFC circuit response to TMS remains, regardless of how an interaction is produced between different memory types

We tested for changes in the response of different networks (DLPFC versus M1) to single pulses of stimulation before (baseline) and after (offline) different tasks. The tasks were either a motor skill followed by a word list (motor skill group) or a word list followed by a motor skill (word recall group; Comparison 2; Figure 1).

(A) We found that the change in response of the DLPFC did not differ significantly between the groups ((offline versus baseline)*group(motor skill versus word recall); all $p > 0.08$).

(B) Nonetheless, the change in the DLPFC response was significant (offline versus baseline; $p < 0.001$). The identified cluster corresponded to frequencies within the theta/alpha/beta range (2–31 Hz) and localized to a fronto-temporal network. Thus, the change in DLPFC response remained regardless of how the interaction between different memory types was produced.

(C) The change in the response of M1 did not differ significantly between the groups ((offline versus baseline)*group(motor skill versus word recall); all $p > 0.33$).

(D) However, the change in the M1 response was significant (offline versus baseline; $p < 0.001$). The identified cluster corresponded to frequencies within the theta/alpha frequency range (2–12 Hz) and localized to premotor and parietal regions (spatial plot). In each plot, we used the same conventions as applied in Figure 2.

See also Figures S1 and S2.

word recall between testing and subsequent retesting ($recall_1$ versus $recall_2$, 13.5 ± 0.5 versus 12.4 ± 0.6, paired t test, $t(14) = 3.23$, $p = 0.006$). Word recall is retained over this interval when the list is learned in isolation; however, by immediately acquiring the motor sequence after word-list learning, the different types of memory task interact, and subsequent word recall is impaired.[1,4,6,33] Common between these groups is the interaction between different memory types. However, how this interaction is produced is different (i.e., memory task order; Figure 1).[1,4–6,21,33] Thus, comparing between these groups tested whether the response of the DLPFC to a TMS pulse was specifically due to how the memory interaction was produced.

Comparing the two groups, we found that the resistance of the DLPFC circuit to a TMS pulse was not modified by how the memory interaction was produced. We found that the spectral response to TMS pulses before (baseline) compared with after (offline) the behavioral tasks did not differ significantly between the groups (cluster analysis; time (offline versus baseline)*group (motor skill versus word recall), all $p > 0.08$; Figure 3A). Instead, there was a significant decrease in the spectral response to single TMS pulses applied over the DLPFC, regardless of how the memory interaction was produced (i.e., unaffected by task order; time (offline versus baseline), $p < 0.001$; Figure 3B). This cluster largely overlapped with the circuit identified in the earlier comparison (Comparison 1) as being linked to the interaction between different memory types, both in terms of frequency range (including alpha to beta; 2–31 Hz) and spatial distribution (a fronto-temporal network revealed using source estimate analysis; Figure 2A). Thus, regardless of how an interaction is produced between different types of memory tasks, there is a decrease in a DLPFC circuit response.

The response of the M1 circuit to a TMS pulse was also not modified by how the memory interaction was produced. We found that the change in the post-TMS power spectra before (baseline) compared with after (offline) the memory tasks did not differ significantly between the groups (cluster analysis; time (offline versus baseline)*group (motor skill versus word recall); all $p > 0.33$; Figure 3C). However, there was a significant change in the post-TMS power spectrum before compared with after the tasks (time (offline versus baseline); $p < 0.001$; Figure 3D). There was a significantly decreased response within the theta/alpha frequency range (2–12 Hz) distributed over a premotor-parietal network (revealed using source-estimation analysis). This overlaps with the network identified earlier, also following TMS pulses applied to M1, as being insensitive to the interaction between different memory types (see Comparison 1). Thus, a similar M1 network was identified, unaffected by memory interactions, and instead modified by task performance.

Distinct DLPFC frequency bands and protection from interference

We found a decrease in the response of the DLPFC to single-pulse TMS within the alpha/beta band, which was linked to the interaction between different memories. We used cluster analysis to test for a correlation (positive or negative) between the change in the strength of the alpha or beta frequency bands

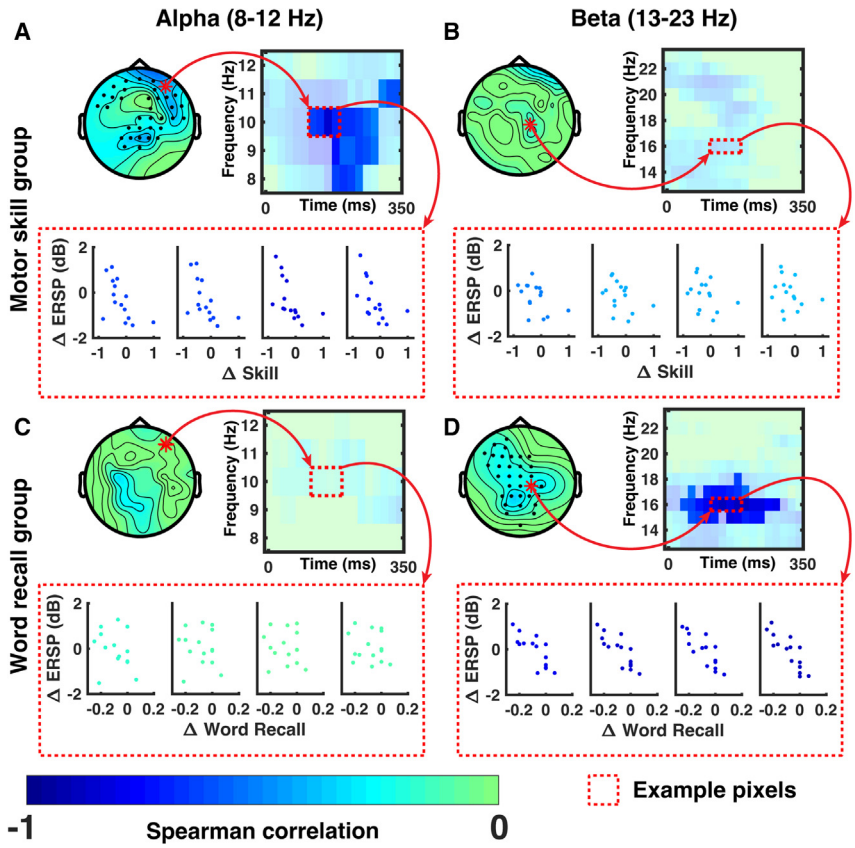

Figure 4. Different frequencies within the DLPFC circuit shapes the fate of different memory types

(A and B) We found a significant negative correlation between motor skill change and the change in the DLPFC response to a TMS pulse (A) within the alpha range (8–12 Hz; p = 0.04), but (B) not within the beta range (13–19 Hz; all p > 0.85).

(C) Conversely, we found no significant correlation between the word recall change and DLPFC response change within the alpha range (8–12 Hz; p = 0.82).

(D) However, there was a significant correlation in the beta range (13–19 Hz; p = 0.02). The topographies show the spatial distribution of the significant relationship between DLPFC response change and performance change (skill or words). These were identified using a cluster analysis (Spearman correlation) and each (three-dimensional) cluster collapsed across time and a specific frequency band (alpha or beta) to create the spatial plot. We selected a channel from within an identified cluster and used the same channel when there was no identified cluster (red asterisks). From these selected channels, we show the time-frequency plots, and four representative pixels (red dashed rectangle) have been used to illustrate the relationship between power change (ΔERSP) and the normalized change in memory task performance (Δ skill or Δ word recall). The color of the scatter dots and the corresponding time-frequency pixel indicate the Spearman correlation coefficient in the pixel.

and the behavioral expression of the memory interactions. To factor out differences in initial performance (i.e., $skill_1$ and $recall_1$), we used a normalized measure of impaired performance in the different memory tasks (for example, $(skill_2 - skill_1)/skill_1$). We found a negative correlation between the normalized decrease in motor skill with the power decrease in the alpha (p = 0.04), but not within the beta frequency band (all p > 0.85; Figures 4A and 4B). Conversely, we found a negative correlation between the normalized decrease in word recall with the power decrease in the beta (p = 0.02), but not within the alpha frequency band (all p > 0.82; Figures 4C and 4D). This showed that a memory (action versus words) was protected from being disrupted by another, different type of memory when the strength of a particular frequency band decreased (alpha versus beta, respectively). Overall, the interaction between different memory types was linked to the functional state of the DLPFC circuit.

We found that offline memory processing makes networks less susceptible to external influences. The creation of stable functional networks during offline processing attenuates the external influence of single TMS pulses upon brain activity. We found that an M1 circuit showed a decreased response due to task performance. By contrast, a DLPFC circuit showed a decreased response exclusively following memory tasks that interact, revealing that it was due specifically to the interaction, and not task performance (Figure 2). It remained regardless of how the memory interaction was produced, showing that it was due to interactions generally (Figure 3). Within the DLPFC circuit, the change in the strength of alpha (but not beta) was linked to minimizing the interaction of the motor skill with a word list and, consequently, maintaining motor skill. Conversely, it was the change in the strength of beta (not alpha) that was linked to minimizing the interaction of the word list with subsequent motor skill and so maintained word recall (Figure 4). Thus, the offline processing of different memories is linked to different frequency bands, and the strength of these within a DLPFC circuit shapes the interaction or segregation between the different types of memory.

We compared the effects of single TMS pulses upon EEG activity before (baseline) and after (offline) task performance. This design isolates those changes specifically due to performing the tasks (Figure 1). The same numbers of single TMS pulses were applied at the same stereotactically maintained positions (over right DLPFC and left M1) during both baseline and the subsequent offline recording. By comparing between these recording sessions, the general effects of TMS, such as sensory stimulation, were factored out to reveal specifically those changes in how brain activity responded to TMS pulses, which are due to task performance. What is identified using this approach is how the response to single-pulse stimulation changes due to task performance (Figures 2 and 3). Thus, the change in response to single pulses (baseline versus offline) reveals how the brain state is modified by task performance.

We found that single TMS pulses had less effect upon circuits during offline processing. The effect of a pulse upon brain activity was attenuated offline (compared with baseline). The

circumstances of this decrease depended upon the network. When TMS was applied to M1, the decrease simply followed task performance. By contrast, when TMS was applied to DLPFC, the attenuated power was due specifically to the interaction between the different memory tasks (Figures 2 and 3). This dissociation reveals the different contributions made by M1 and DLPFC circuits to memory processing and the importance of the DLPFC to interactions between memories. Although the attenuated response to stimulation occurs under different circumstances, nonetheless, it may be due to the same underlying mechanism.

Learning leads to the formation of neuronal ensembles.[36,37] These consist of cells distributed throughout the brain becoming flexibly linked together to create functional circuits.[12] Their formation due to learning may make the brain less sensitive to a TMS pulse because the ongoing oscillation resists the perturbing effects of the pulse.[38–40] This is analogous to a child's swing having sufficient momentum to resist an external influence, from a parental hand, when the swing is in motion.[14]

Being resistant to external perturbation explains how a neuronal ensemble has a consistent pattern of activity during memory processing.[41] However, these stable activity patterns will be disrupted by learning another, different type of memory, which when allocated to the same ensemble reduces its stability, making it less resistant to perturbation.[41] The interference between the different memories reduces the resistance of an ensemble to perturbation; consequently, the effect of stimulation is less attenuated and so returns to baseline. This leads to greater memory impairment (from interference) being linked to smaller differences in the response to single TMS pulses compared with baseline (i.e., a negative correlation; Figure 4). Thus, the emergence of ensembles during learning explains both how brain activity becomes resistant to the external perturbation from a TMS pulse and how this resistance is (negatively) correlated with impaired performance due to memory interactions.

The interaction between different memory types was shaped by the functional state of the DLPFC network. The motor skill was protected from interference from learning the word list when alpha (but not beta) strength decreased. Conversely, the word list was protected from interference from learning a motor skill when beta (but not alpha) strength decreased (Figure 4). Earlier studies have linked alpha power changes to motor skill learning and beta power changes to word recall.[42,43] Changes in these different frequency bands within a DLPFC circuit modified the interaction, resulting in interference between different memory types. The functional state of the DLPFC circuit can also be modified artificially to change the interaction between different memory types[4,5]; for example, directly modifying its state by applying repetitive TMS over the DLPFC or indirectly modifying its state by applying stimulation to functionally connected areas including M1.[44–46] Thus, changes in the DLPFC circuit's functional state provide a flexible organization in which the interaction or independence between different memory types can be changed for adaptive benefit.

Decreasing the interaction between memories could have an adaptive benefit. It would improve accurate memory recall by diminishing interference between memories. Although accurate recall is adaptive in some circumstances, for example, when a recall is rewarded,[47,48] an impaired recall also has adaptive benefits.[21,49] The interaction between, and the consequent interference of, a memory has been linked (both correlatively and causatively) to the sharing of abstract serial information between different memory types.[6,21,33,49] Accuracy is sacrificed to extract common features.[49] This allows information acquired in one situation (learning a sequence of words) to be applied in a new context to enhance the learning of a different type of memory (sequence of actions[6,21]). Thus, functional changes within a DLPFC circuit can flexibly modify the balance between segregation and interaction between different memory types for an adaptive benefit.

Overall, this work shows how the segregation between different memory systems breaks down. The DLPFC is central to this shift from memory independence to interactions. The response of the DLPFC circuit to a TMS pulse decreased exclusively when different memory types interacted and occurred, regardless of how this interaction was produced (Figures 2 and 3). The decrease is consistent with the formation of functional networks during offline processing, which because they are driven by ongoing processing are less affected by perturbation from single TMS pulses. This attenuation was specifically within the alpha/beta frequency range, which has been implicated previously in offline memory processing.[10,34] The attenuation in alpha (but not beta) strength was linked to the protection of the motor skill from interference from the word list; conversely, the attenuation in beta (but not alpha) strength was linked to the protection of the word list from interference from the motor skill (Figure 4). The protection from interference shows that the interaction between different memory types has reduced, and instead, they are being processed independently. Thus, the physiological state of the DLPFC circuit balances the independence or interaction between different memory types.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.05.027

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AUTHOR CONTRIBUTIONS

Conceptualization, E.M.R.; methodology, M.B., T.P.M., D.V., G.T., and E.M.R.; formal analysis, M.B., T.P.M., and E.M.R.; investigation, T.P.M. and M.B.; writing – original draft, E.M.R.; writing – review & editing, M.B., T.P.M., D.V., G.T., and E.M.R.; visualization, M.B., T.P.M., G.T., and E.M.R.; funding acquisition, E.M.R.

DECLARATION OF INTERESTS

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INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as a member of the LGBTQ+ community. One or more of the authors of this paper self-identifies as living with a disability.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Behavioral and TMS–EEG Data	This paper; Zenodo	https://doi.org/10.5281/zenodo.7891964
Software and algorithms		
Psychophysics Toolbox	https://github.com/Psychtoolbox-3/Psychtoolbox-3	RRID: SCR_002881
Brainsight	Rogue Research	RRID: SCR_009539
BrainVision Recorder	Brain Products GmbH	RRID: SCR_016331
MATLAB	MathWorks	RRID: SCR_001622
EEGLAB	https://sccn.ucsd.edu/eeglab/index.php	RRID: SCR_007292
FieldTrip	https://www.fieldtriptoolbox.org/	RRID: SCR_004849
TMS-EEG signal analyser (TESA) code repository	Rogasch et al.[50]	https://github.com/nigelrogasch/TESA.git
FastICA algorithm	Hyvärinen et al.[51]	https://research.ics.aalto.fi/ica/fastica/
Source-based artifact-rejection techniques	Mutanen et al.[52]	https://doi.org/10.1016/j.brs.2020.06.079
Nonparametric cluster-based statistical testing of EEG data	Maris and Oostenveld[53]	https://doi.org/10.1016/j.jneumeth.2007.03.024
Other		
The New York Head (ICBM-NY)	https://www.parralab.org/nyhead/	https://doi.org/10.1016/j.neuroimage.2015.12.019

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Edwin Robertson (edwin.robertson@glasgow.ac.uk).

Materials availability

This study did not generate any novel unique reagents.

Data and code availability

The data generated during this study have been deposited at Zenodo and are publicly available at the time of publication. DOIs are listed in the key resource table. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experimental design

We tested how the response of brain activity to an external perturbation changed during offline memory processing. All experiments had a similar design (Figure 1). Each used single pulses of Transcranial Magnetic Stimulation (TMS) to perturb networks, and this was measured concurrently with EEG. We compared the effect of a TMS pulse applied over the right dorsolateral prefrontal cortex (DLPFC) and left primary motor cortex (M1) upon EEG activity before (baseline) and after (offline) behavioral tasks. This comparison factored out the general effects of TMS, such as sensory and cutaneous stimulation to reveal specifically those changes in how brain activity responded to TMS pulses, which are due to performing the behavioral tasks. We were able to specifically isolate those changes due to an interaction between different memory types by contrasting the brain activity changes in response to an external perturbation across different behavioral tasks. All the experimental work was approved and overseen by the local (National Health Service; West Coast of Scotland) research ethics committee.

Different types of memory tasks were used. A motor skill and then a word-list were learned, which interact and lead to impaired motor skill retention (motor skill group[1,4,6]). Participants learned the motor sequence (~10am), the skill acquired was then measured on a test block ($skill_1$), participants then learned a word-list, had their recall tested ($recall_1$), and subsequently 10-hrs later had their skill retested ($skill_2$; motor skill group; Figure 1). This was compared against another group, in which the motor skill task was replaced

by a motor performance task (control group; Comparison 1; Figure 1). These tasks were identical in every respect, including being visually cued and having the same number of movements, however, the movements had no serial structure in the motor performance task. It is only when there is a consistent serial regularity that a motor sequence memory is formed, which interacts and is impaired by learning a subsequent word-list task.[1,4,6,33] Thus, comparing between these groups showed how networks response to an external perturbation (TMS pulse) changed depending upon whether or not there was an interaction between the tasks.

We compared learning a motor sequence (test; $skill_1$) and then a word-list (motor skill group) against another group (Comparison 2; Figure 1). The same memory tasks were performed, but in the reverse order. Participants learned the word-list (~10am), had a free recall test administered ($recall_1$), then learned the motor sequence, had their skill tested ($skill_1$), and subsequently 10-hrs later had their free recall retested ($recall_2$; word recall group; Figure 1). In both groups, the initial task (motor sequence, word-list, respectively) interacts offline with a different type of memory task impairing its retention (10 hours later, ($skill_2$ or $recall_2$)[1,4,6]). The interference between these different types of memory task was measured as a change in performance between testing and retesting ($skill_2 - skill_1$ or $recall_2 - recall_1$;respectively[1,4,6,21]). The memory task order differs; but, what is common between them is an interaction between different memory types.[1,4,6] Thus, comparing between these groups provides a test of how a network's response to an external perturbation (from a TMS pulse) changed depending upon how the interaction between the different memory types was produced.

Finally, a structural magnetic resonance imaging (MRI) of each participant's brain was used to identify target areas (in the case of the right DLPFC) and maintain coil position throughout the experiment with a frameless stereotactic system (please see Transcranial Magnetic Stimulation (TMS)).

Participants

We recruited 51 right-handed (as defined by the Edinburgh Questionnaire[54]), healthy participants, with no medical, neurological, or psychiatric history, normal or corrected to normal vision, who met the additional safety criteria for the use of MRI and TMS.[55,56] All participants provided written informed consent for the study, approved by the local institutional review board. Some of the participants (n = 6) were excluded from further analysis because they were able to recall four or more items of the motor sequence (please see Motor sequence learning task). This amount of recall can prevent a motor task being disrupted by word-list learning, and rather than impairing its subsequent recall, the motor skill is enhanced.[57] Using this exclusion criterion ensured that there was a consistent performance measure for the interaction between memory tasks (i.e., skill impairment). The remaining 45 participants (30 females, 23.9±3.6 years; mean ± std) were randomly and equally divided across the three groups (n = 15 per group). Similar number of participants (per group) have been used to successfully detect EEG changes in response to a TMS pulse, and interactions between different memory types.[1,4,58]

METHOD DETAILS

Motor sequence learning task

We used a modified version of the serial reaction time task (SRTT[26,59]). A solid circular visual cue (diameter 20mm, viewed from approximately 800mm) could appear at any one of four possible positions, designated 1 to 4, and arranged horizontally on a computer screen. Each of the four possible positions was indicated on screen by a circle with a narrow border and corresponded to one of the four buttons on a key-pad, upon which the participant's fingers rested. When a target appeared, participants were instructed to respond as quickly and accurately as possible by pressing the appropriate button. If the participant made an incorrect response, the stimulus remained until the correct button was selected. Once the correct response was made, the cue on the screen disappeared and was replaced by the next cue after a delay of 400 ms. Response time was defined as the interval between presentation of a stimulus and selection of the correct response.

Participants were introduced to the task as a test of reaction time. Yet, the position of the visual cue followed a repeating 12-item sequence (2-3-1-4-3-2-4-1-3-4-2-1). They were not told about the 12-item sequence, and there were no cues marking the introduction of the sequence. Learning started on an initial, short training block that contained 15 repetitions of the motor sequence (180 trials), and then on a longer training block that contained 25 repetitions of the sequence (300 trials). The skill acquired was accessed immediately after learning on a test block (180 trials; $skill_1$) and again subsequently (10-hrs later) on a retest block (180 trials; $skill_2$; Figure 1). Each of the blocks had a similar structure with an initial 50 random trials preceding the sequential trials, and these were then followed by another additional set of 50 random trials. The random trials contained no item repeats (for example, -1-1- was illegal), and each item had approximately the same frequency of appearance. Each set of random trials in each block was unique, which minimized the chance that participants might become familiar with the random trials. The short-training, training, and test block together took approximately 15-20 minutes to complete.

A free recall test was administered when participants had completed the motor task. Participants were asked if they had noticed a pattern to the visual cues of the task, and if so, to report verbally as many items of the sequence as possible.[1,4] It was scored as the longest, continuous and accurate verbally recalled segment of the sequence that was at least three items long (i.e., a triplet or more). Those recalling 4 or more items from the 12-item sequence in the correct order were excluded from subsequent analysis (n = 6; <12% of the sample). A greater recall (i.e., > 4 items) can prevent a motor task being disrupted by word-list learning, and rather than impairing its subsequent recall, the motor skill is enhanced.[57] By excluding those participants, we ensured that the interaction between the memory tasks had a consistent affect upon performance (i.e., skill impairment[1,4,6]).

Motor performance task

We also used a modified version of the sequence learning task (control task). It was identical in every respect to the motor sequence learning task, except that all the sequential trials were replaced by an equal number of random trials. The same random trials were used for all participants performing the task. Removing the serial structure of the sequence learning task prevents the interaction with the word-list task, whilst ensuring the performance of the same number of visually guided movements over the same duration.[1] Thus, using this task isolates the interaction between memory tasks from task performance (Comparison 1; Figure 1).

Word-list task

A single word, from a list of 16 words (drawn from the California Verbal Learning Task), was presented on a computer screen for 2s. The word was then removed and replaced by another word also drawn from the list of 16 words. This process continued until all 16 words had been presented. The list of words was: truck, spinach, giraffe, bookcase, onion, motorcycle, cabinet, zebra, subway, lamp, celery, cow, desk, boat, squirrel, and cabbage. These same 16 words were presented individually and in the same order for five iterations for each participant. At the end of each of these presentations, participants were asked to verbally recall as many of the words as possible. Participants were not prompted for particular words, nor were they told those words, if any, which they had failed to recall. Following the fifth recall, there was a ten-minute interval. A free recall test was then administered with participants asked to recall as many of the words as possible ($recall_1$), and this same test was administered 10-hrs later ($recall_2$; Figure 1). It took approximately 15 minutes to complete the learning (five iterations) and conduct the initial free recall test of the word-list.

Transcranial Magnetic Stimulation (TMS)

We tested how offline processing modified the effect of a perturbation applied as a single TMS pulse (over the left M1 or the right DLPFC). Stimulation was applied focally to these brain areas using standard figure-of-eight coils connected to two biphasic stimulators (Magstim Rapid2, Magstim Company) at an intensity of 80% of the individual resting motor threshold (rMT). This intensity was chosen because: (1) it is sufficient to produce measurable EEG responses[60]; while being (2) unlikely to evoke MEPs when TMS is applied to M1, avoiding peripheral somatosensory potentials in EEG; and (3) elicits only small scalp muscle contractions, minimizing the muscle-artifact contamination of the EEG.[61]

We identified the left M1 as the optimal location for inducing motor evoked potentials (MEP; recorded using surface electromyography) in the first dorsal interosseous (FDI) muscle of the relaxed right hand. The rMT was defined as the lowest intensity that was capable of inducing at least ten measurable MEPs (>50 μV) in the contralateral FDI muscle following 20 single TMS pulses.[62] This method has been widely used and shown to consistently and accurately target the hand area of M1.[63] Participants held both of their hands relaxed during the rMT-estimation.

Right DLPFC was defined based on earlier functional imaging studies identifying DLPFC activations.[64] This same location was targeted successfully with repetitive TMS to modulate the interaction between different types of memories (Talairach co-ordinates; x = 40, y = 32, z = 30[4,23]).

To ensure accurate and consistent targeting, we first acquired individual anatomical T1-weighted MRI scans (MP-RAGE imaging sequence) using a 3T MR scanner (Magnetom Trio Siemens, Erlangen, Germany). The scans were then used to both guide the TMS targeting, and ensure that the location was maintained via a frameless stereotactic system (Brainsight TMS, Rogue Resolutions Ltd).

Combined simultaneous single pulse TMS and EEG recording

EEG was continuously recorded with a TMS-compatible EEG system (BrainAmp, Brain Products) from 62 Ag/AgCl-sintered electrodes (EasyCap GmbH, Herrsching, Germany) mounted on an elastic cap according to the International 10–10 system. AFz and TP9 served as reference and ground, respectively. To monitor eye movements, an additional electrode was placed on the outer canthus of the left eye and referenced offline to Fp1. We kept all electrode–skin impedances below 5 kΩ to ensure high-quality recordings, and a high sampling rate (5 kHz) to minimize the duration of the TMS-pulse artifact in EEG.

To protect participants' hearing and suppress potential TMS-related auditory responses, the participants wore foam earplugs through which masking noise was played[65] (3M E-A-RTONE Insert Earphone 3A 410-3002). We tailored the noise level for each participant by gradually increasing the volume until they could not hear the coil click anymore or until any further volume increase would be uncomfortable. During the recordings, participants sat on a comfortable chair in a dimly illuminated and electrically shielded room. To minimize muscular artifacts in the EEG signal, participants rested their heads on a chin support and were asked to remain relaxed. Participants were also instructed to stay focused on a provided fixation cross throughout the entire session to minimize their eye-movement artifacts and the drowsiness-related prominent posterior alpha activity.

Both baseline and subsequent offline TMS–EEG sessions consisted of 126 TMS pulses applied over the right DLPFC and the same number applied over the left M1 (252 single pulses in total, randomly distributed between sites). They were delivered with an interstimulus interval (ITI) of 4-6 s (4.9±0.6 s, mean ± std). The 252 pulses were distributed across three blocks of 7 minutes each with a 1-minute break between blocks (~23 min in total).

QUANTIFICATION AND STATISTICAL ANALYSIS

All the performance data was graphically explored using MATLAB (2017a, The MathWorks, Natick, Massachusetts, United States of America (USA)). Specifically, we examined the distribution of the data using histograms, normal probability plots, and verified that the data followed a normal distribution using the Shapiro-Wilk test.

In the sequence learning task (i.e., the SRTT), response times were defined as the time to make a correct response. Any response time in the top one percentile (i.e., $\alpha = 0.01$) of a participant's data was identified using a Grubbs' Test and removed. We quantified the amount of sequence learning by subtracting the average response time (RT) of the final 50 sequential trials from the average response time of the 50 random trials that immediately followed.[59,66] The difference between random and sequential RT is a widely used learning measure, which is both sensitive and specific to learning of the motor sequence (for example, Nissen and Bullemer,[59] Willingham et al.,[66] and Boyd and Winstein[67]; for review, Robertson[26]). It is a measure of the RT advantage of the sequential over the random trials, and so it increases as performance of the sequence improves. Accuracy in this task even with limited experience is very high (>95%[27,31,66]). The free recall of the motor sequence was scored as the longest, continuous and accurate verbally recalled segment of the sequence that was at least three items long (i.e., a triplet or more). In the other motor task (the performance task) there was no serial structure, and so no distinction between sequential and random trials. We used the average RT of the final 100 trials in each block to quantify performance. These are the same final number of trials within the test and retest blocks as used in the sequence learning task to quantify skill (i.e., 50 sequential plus the subsequent 50 random trials). For the word-list learning task, we analyzed the total number of words correctly recalled (i.e., total recall[1,4,6]).

The change in performance between testing and subsequent retesting (i.e., $skill_1$ vs. $skill_2$ plus $recall_1$ vs. $recall_2$) was used as a measure of interference with the other type of memory task (i.e., word-list and motor skill learning task, respectively). We used paired t-tests to determine the significance of these performance changes within groups. All the statistical tests used in the analysis were two-tailed unless otherwise stated.

EEG Analysis

Initially, TMS-related artifacts were removed (preprocessing) from the EEG signal, which was then transformed into time-frequency domain. Specifically, we transformed the EEG signal into event-related spectral perturbation (ERSP), which is a measure quantifying how an external event, i.e., a TMS pulse, perturbs a circuit's spontaneous oscillatory activity.[68] By comparing the ERSP at baseline and the subsequent offline sessions, we could assess how memory interactions modulate the response to external perturbation in the probed networks (Figure 1). Analyses were performed using custom MATLAB (Version 2017a, The Mathworks, Natick, Massachusetts, USA) scripts, combined with the EEGLAB toolbox (UC San Diego, La Jolla California, USA[69]), TMS–EEG signal analyzer plugin (TESA[50]) and the fieldtrip toolbox[70] (http://fieldtriptoolbox.org).

Preprocessing

We removed the typical TMS-related noise and artifact signals from the EEG data. The data were segmented into 2000-ms long epochs, each consisting of 1000ms of data around the TMS pulse. The artifact removal process was threefold. First, for each trial, the TMS-pulse (from -2 to 6 ms with respect to the TMS pulse) was cut and replaced with a 'mirrored' version of the baseline data right before the TMS pulse (-8 to -2 ms[50]). In addition, the recharging delay, which depends on the stimulation intensity, was visually identified for each dataset, removed and interpolated using a spline function.[71] Second, noisy channels were detected and corrected accordingly by means of the source-utilized noise-discarding algorithm (SOUND[52,72]). Finally, eye-movement-, muscle-, and exponential-decay artifacts were removed with the combination of the independent component analysis (ICA[51]) and the signal-space-projection–source-informed-reconstruction method (SSP-SIR[52,73]), tailored for muscle-artifact rejection.[74,75] The data analysis scripts were based on the open-source EEG and TMS-EEG data analysis toolboxes, EEGLAB and TESA, respectively.[50,69]

Analysis of the oscillatory response to single pulse stimulation

To analyze the oscillatory response to external perturbation, we computed the ERSP, a measure that quantifies the ability of a specific event, e.g., the TMS pulse, to perturb the spontaneous spectral activity.

Each trial was first transformed into time-frequency domain by applying fast Fourier transform to 500ms sliding time-windows (Hanning taper) moving in 20ms steps. The analysis was restricted to 2-40 Hz and for 0-350ms following the pulse because cortical waves are typically identified below 40 Hz following a TMS pulse and last for about 300ms.[76,77] The power values at each frequency were transformed into decibels with respect to the corresponding frequency-specific mean power during 500ms to 100ms before the TMS pulse. For each participant, session, and condition, the obtained time-frequency transformations were finally averaged across the trials to obtain the corresponding four ERSP-conditions (baseline and offline-session for the right DLPFC stimulation plus baseline and offline-session for left M1 stimulation). The offline conditions (right DLPFC and left M1) were normalized with respect to the corresponding baseline conditions (right DLPFC and left M1, respectively). Normalization was conducted on each participant's ERSP data by dividing the conditions (both baseline and offline) by the scalar normalizing factor. This was calculated from baseline condition as the Frobenius norm of the three-dimensional ERSP matrix (channels x time x frequency[78]). Frobenius norm robustly quantifies the overall power in signals that are centered around zero, such as ERSP. This factored out variability across participants in the ERSP at baseline.[79–81]

We statistically compared changes in the TMS-triggered ERSP due to memories interaction by using 2-by-2 non-parametric cluster-based statistics analysis. Time (offline vs. baseline) as a within-group factor and Group as a between-group factor (Comparison 1; motor skill vs. control groups or Comparison 2; motor skill vs. word recall groups) including all channels and frequencies (2-40 Hz) and individual time points between 0-350ms, separately.[53] The possible interaction between these factors (Time*Group) specifically identifies ERSP changes that are due to the interference between different types of memory.

To test whether the memory interaction shapes the TMS-triggered ERSP in a particular way, we ran the following cluster statistics to test the Time*Group interaction (Comparison 1; motor skill vs. control groups; Comparison 2; motor skill vs. word recall groups). This was achieved by computing the ERSP change from baseline to offline session for each participant. Next, we ran two-tailed unpaired t-tests (significance level $p<0.05$) at each sample to compare the ERSP changes between groups (across all 62 channels; 0-350ms; 2-40 Hz). Any neighboring significant samples were then assembled into clusters. The resulting clusters were tested for significance by comparing them to the permuted cluster distribution, obtained by shuffling the participants across the groups to produce two surrogate groups for every permutation (10000 permutations; two-tailed tests; significance level $p<0.05$). Differences between the shuffled surrogate groups were simply due to random stochastic variations. If the actual clusters, describing the group difference in how ERSP changed over time, belonged to the most positive or most negative 2.5 % in the permuted maximum cluster distribution, the Time*Group interaction was considered significant.

We also tested the effect of Time (offline vs. baseline) on the TMS-triggered ERSP. This test will identify changes attributable to performing tasks in succession. To test the effect of time, we combined both groups (i.e., for Comparison 1 motor skill and control groups, and for Comparison 2 motor skill and word recall groups). Next, we ran two-tailed paired t-tests (significance level $p<0.05$) at each sample to compare the ERSP changes between the baseline and offline sessions. Any neighboring significant samples were then assembled into clusters. The resulting clusters were tested for significance by comparing them to the permuted cluster distribution, obtained by randomly shuffling the baseline- and offline-session samples of each participant to produce two surrogate sessions for each group (10000 permutations; two-tailed tests; significance level $p<0.05$). If the actual clusters, describing the difference between the baseline and offline sessions belonged to the most positive or most negative 2.5% in the permuted maximum cluster distribution, the effect of time was considered significant.

To visualize the clusters resulting from both interaction (Time*Group) and time (offline vs. baseline) contrasts, we collapsed the three-dimensional signal (time, frequency, channel) either across channels – to give the time-frequency plot – or across both time and frequency – to give the topographical plot (Figures 2 and 3). Topographies were masked by the most significant cluster, i.e., in the case of significant contrasts, the significant cluster was selected, whereas for non-significant contrasts, the cluster closest to significance was selected. As a result, the spatio-spectral–temporal samples that did not belong to the selected cluster were set to 0 and, therefore, are white in the topographical plot.

When appropriate, post-hoc cluster tests were run for exploring the associated effects of time (offline vs. baseline) within each group. These cluster tests were run similarly as when testing the effect of Time.

Finally, to exclude the possibility that different baseline levels could explain the observed differences between the groups, cluster statistics, using an ANOVA, were run to compare the baseline-session ERSPs across the groups (please see Figure S1).

Source analysis

We performed source-space analysis to estimate the activity changes directly at the cortical level. As a source model, we used the 2000-dipole version of the New York Head with free dipole orientations.[82] To save computational time, the data were downsampled to 125-Hz sampling frequency prior to minimum-norm estimation. Then, to obtain the cortical ERSP estimates, each TMS–EEG trial of each participant was first transformed to current space using minimum-norm estimates with singular-value-truncated regularization (the 30 most significant dimensions were included[83]). Next, using the obtained cortical-current curves, we calculated ERSP, within the 6-30Hz and 0-350ms ranges after the TMS onset, for each of the 2000 sources. Finally, each of the source-specific ERSP map was normalized with respect to the appropriate baseline session.

We used a non-parametric permutation approach to test for statistically significant differences in the cortical ERSP maps.[84] When comparing two groups, the subject-specific ERSP maps were pooled together, from which random partitions were drawn 1000 times. Using the permuted partitions, the maximum statistics across the whole cortical space were formed for each time-frequency point. Finally, the real ERSP difference at each time-frequency-spatial point was compared to the corresponding maximum statistics. All ERSP differences larger than 95% of the maximum statistics were considered significant. When running within-group tests comparing the baseline and offline ERSP maps, the permutation approach was similar, except now for sampling the random partitions, each subject-specific ERSP-map pair (offline vs. baseline) was randomly shuffled at each round. The resulting cortical source maps showed the net ERSP-change across the significant time-frequency points (restricted to 6-30Hz and 0-350ms after the TMS onset).

Testing the relationship between neurophysiology and behavior

We tested the relationship between ERSP changes, and the change in memory performance. This was achieved using cluster statistics, with a two-tailed Spearman correlation as the test statistic. The identified ERSP and memory-performance changes were the dependent and independent variables, respectively. We used the Spearman rank correlation because it only assumes a monotonic relationship between the EEG measure and behavioral performance, as opposed to the more restricted requirements of a linear relationship.

We ran cluster-correlation tests. This was done for the normalized change in motor skill, and word recall due to interference from the other type of memory (word-list learning or motor skill learning; respectively[1,6]). Using a normalized measure of memory impairment factored out differences in initial performance. It was calculated as the difference in performance between retesting and earlier testing, and divided by the earlier performance at testing (i.e., ($skill_2$-$skill_1$)/$skill_1$ or ($recall_2$-$recall_1$)/$recall_1$).

At each time-frequency-channel point we ran two-tailed correlation tests (significance level $p<0.05$) to relate the ERSP change with the corresponding memory-performance change. Any neighboring significant samples were then assembled into clusters. The resulting clusters were tested for significance by comparing them to the permuted cluster distribution, obtained by shuffling the participant-specific memory-performance measures to random participants (10000 permutations; two-tailed tests; significance level $p<0.05$). With this permutation strategy, any correlation manifested in the permutation rounds could only reflect spurious ERSP-memory-performance-change relationships. If the actual clusters, describing how ERSP correlated with the behavioral performance change, belonged to the most positive or most negative 2.5 % in the permuted maximum cluster distribution, the correlation was considered significant (please see Figure 4, which shows the correlation analysis).

We mapped the clusters across all the channels (62) and all the time points between 0–350ms. However, we run the cluster statistics separately for the different frequency bands implicated in the interference between the different memory types (i.e., alpha and beta frequency bands; for more details please see results and discussion). Only by conducting separate cluster analysis for each frequency band was it possible to test for a frequency based dissociation in memory fate.[85]

To visualize the three-dimensional clusters (time, frequency, channel), we collapsed across time and a specific frequency band (alpha or beta) to create the topographical plots. From within the significant cluster we selected a representative channel and show the time-frequency plots (the other two dimensions of a cluster). Finally, from these we selected four representative pixels, which are shown as scatter plots of the power change (ΔERSP) against the normalized change in memory task performance (Δ skill or Δ word recall; see Figure 4).

Finally, we also tested for a relationship between motor skill and any cluster identified as being significantly different between the motor skill and control groups (i.e., a significant time*group interaction; Comparison 1). This was to determine whether any difference to single pulse stimulation between the groups was due to motor sequence skill (acquired in the motor skill group). We tested for a relationship using a Spearman correlation between initial skill and ERSP changes. The ERSP changes were within the channels, frequency bands and time domain identified as being significantly different between the groups in their response to single pulse stimulation (i.e., the time*group cluster; Comparison 1). At each time-frequency-channel point, we ran two-tailed correlation tests (significance level $p<0.05$) to test for a relationship between initial skill ($skill_1$) and the ERSP change. Any neighboring significant samples were then assembled into clusters. The resulting clusters were tested for significance by comparing them to the permuted cluster distribution, obtained by randomly assigning participant-specific initial skills across the participants (10000 permutations; two-tailed tests; significance level $p<0.05$). With this permutation strategy, any correlation between skill and ERSP found in the permutation rounds would be spurious. If the actual clusters, describing how ERSP correlated with the initial skill, belonged to the most positive or most negative 2.5 % in the permuted maximum cluster distribution, the correlation was considered significant.

Analyses of spontaneous oscillatory activity

We examined how spontaneous oscillatory activity was affected by task performance (see Figure S2). This was achieved by analyzing oscillatory activity not affected by the TMS pulses, before (baseline) and after (offline) task performance.

We restricted the analysis to 2-40 Hz and from -450 to -100 ms before the pulse (and 4-6 s after the previous pulse). This provided a window identical in duration as in the ERSP analysis (total duration of 350 ms), and as far as possible from the influence of the earlier TMS-pulse. For each participant and session, we merged the data between sites of stimulation (DLPFC and M1). The focus was on spontaneous activity changes, as opposed, to those in response to single pulses of stimulation. As in the main analysis, we averaged the power values of the time-frequency transformations across trials and normalized with respect to the corresponding baseline session, which factored out inter-individual power variability. However, the normalized power values were averaged across time (-450 to -100 ms). With spontaneous changes time becomes irrelevant, as there is no event. We used cluster-based statistics to identify spontaneous activity changes before (baseline) and after (offline) the different tasks across the different groups. We compared spontaneous changes between the motor skill and control groups (Comparison 1) and between the motor skill and word-recall groups (Comparison 2; Figure 1). At each channel and across each frequency (62 channels; 2-40 Hz with 1-Hz frequency bins), we used two-tailed unpaired t-tests (significance level $p<0.05$) to compare the changes in the spontaneous oscillatory activity between groups, and two-tailed paired t-tests (significance level $p<0.05$) to compare changes between the baseline and offline sessions. When appropriate, post hoc cluster tests were used to explore the effects of time (offline vs. baseline) for each group. Finally, to exclude the possibility that different baseline levels could explain the observed differences between the groups, cluster statistics, using two-tailed unpaired t-tests, were run to compare the baseline spontaneous oscillatory activity between the groups.