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Offset-related brain activity in the left ventrolateral prefrontal cortex promotes long-term memory formation of verbal events

Angela Medvedeva [a, b], Rebecca Saw [c], Carla Silvestri [c], Miroslav Sirota [d], Giorgio Fuggetta [c], Giulia Galli [a, *]

[a] Department of Psychology, Kingston University, Penrhyn Road, Kingston Upon Thames KT1 2EE, United Kingdom
[b] Vivian L. Smith Department of Neurosurgery, University of Texas Medical School at Houston, Houston, TX, 77030, United States
[c] Department of Psychology, University of Roehampton, Holybourne Avenue, London, SW15 4JD, United Kingdom
[d] Department of Psychology, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, United Kingdom

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ABSTRACT

Background: Recent evidence suggests that brain activity following the offset of a stimulus during encoding contributes to long-term memory formation, however the exact mechanisms underlying offset-related encoding are still unclear.
Objectives: Here, in three repetitive transcranial magnetic stimulation studies (rTMS) we investigated offset-related activity in the left ventrolateral prefrontal cortex (VLPFC). rTMS was administered at different points in time around stimulus offset while participants encoded visually-presented words or pairs of words. The analyses focused on the effects of the stimulation on subsequent memory performance.
Results: rTMS administered at the offset of the stimuli, but not during online encoding, disrupted subsequent memory performance. In Experiment 1 we found that rTMS specifically disrupted encoding mechanisms initiated by the offset of the stimuli rather than general, post-stimulus processes. Experiment 2 showed that this effect was not dependent upon rTMS-induced somatosensory effects. In a third rTMS experiment we further demonstrated a robust decline in associative memory performance when the stimulation was delivered at the offset of the word pairs, suggesting that offset-related encoding may contribute to the binding of information into an episodic memory trace.
Conclusions: The offset of the stimulus may represent an event boundary that promotes the reinstatement of the previously experienced event and episodic binding.
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Introduction

The type of brain activity engaged when individuals are exposed to new information is crucial to determine whether that information will be later remembered. Although research has traditionally focused on neural processes occurring online during the presentation of an encoding event, recent studies have demonstrated that peri-encoding activity –brain activity immediately preceding or following encoding– is also relevant to long-term memory formation [1]. On the one hand, EEG and fMRI research has demonstrated that brain mechanisms occurring during the anticipation of to-be-remembered information can predict subsequent retrieval [2,3–5,6,7]. On the other, a separate line of research revealed that immediate post-stimulus processes are also critical for memory encoding [8,9,10,11]. In fMRI studies, brain activity occurring within seconds after the termination of a visual stimulus in a set of regions including the hippocampus correlated with subsequent memory performance, possibly reflecting the binding of event features into cohesive episodic representations [8,9].

fMRI, however, is not the optimal technique to examine the temporal dynamics of memory formation given the sluggish hemodynamic response and the correlational nature of fMRI data. Instead, repetitive Transcranial Magnetic Stimulation (rTMS) allows causal inferences on the necessity of targeted brain regions at given time intervals by temporarily interfering with neural activity in those regions at specific points in time. rTMS studies demonstrated that the engagement of the lateral prefrontal cortex at different points in time during encoding, including post-stimulus time

* Corresponding author.
E-mail address: g.galli@kingston.ac.uk (G. Galli).

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windows, is necessary for memory formation [12,13]. Importantly, one recent study showed that rTMS administered over the left ventrolateral prefrontal cortex (VLPFC) within 100 ms of the offset of word stimuli disrupted the accuracy of retrieval in a subsequent memory test [10]. This effect was not evident when the stimulation was delivered during the presentation of the words or at later points in time (i.e., 200–400 ms) after their offset. That study was not designed to examine offset-related brain activity *per se*, but the findings suggested that the offset of a stimulus plays a key role in the formation of new verbal memories by triggering encoding-related activity in the left VLPFC, which is implicated with the encoding of verbal information (for reviews, [14,15,16]. This is in line with recent research showing that event boundaries –and the offset of a visual stimulus can be considered as such– promote episodic memory formation by reinstating and binding the contents of the previously experienced episode [17,18].

This study aims to characterize the response of left VLPFC to the offset of verbal stimuli and its role in long-term memory formation in three rTMS experiments. In our previous work [10] the time of rTMS administration varied while the duration of word stimuli was set to 1000 ms in all conditions. As a consequence, it was not possible to ascertain whether rTMS effects observed immediately after word offset were driven by the offset itself, or by offset-invariant post-stimulus processes occurring around 1000 ms after the onset of the words, therefore temporally coinciding with word offset. To adjudicate between these two competing explanations, in Experiment 1 we systematically varied word duration and time of rTMS administration. In Experiment 2 we delivered the stimulation to the left VLPFC and to the homologous site on the right hemisphere to demonstrate the neuroanatomical specificity of any observed effect and to control for rTMS-induced somatosensory effects. We also explored the idea that offset-related activity supports the binding of event features into an episodic representation [8,9]. To this end, in Experiment 3 we administered VLPFC rTMS during relational encoding and hypothesized that rTMS administered at the offset of word pairs would impact subsequent associative memory. The involvement of the left VLPFC in offset-related encoding relative to online encoding was further assessed with a meta-analysis conducted on the data from Experiment 1 and 3 of the current study and on the data reported in Ref. [10].

Materials and methods

Experiment 1

Participants: 24 participants (14 females; mean age ± SD: 21 ± 3 years; range: 18–29 years) took part in Experiment 1. We calculated that a minimum of 22 participants was required to detect an effect size of $d = 0.74$ (as in Ref. [10]; assuming $\alpha = 0.05$ and 1- $\beta = 0.95$, one-tailed paired-samples *t*-test), and adjusted the final sample size upwards to account for possible attrition rate. Two subjects were tested but excluded from the analyses due to low memory performance based on a-priori determined exclusion criteria (±2 SD from the average). Participants received course credits or monetary compensation for their participation. All participants gave written informed consent. The study was approved by the University of Roehampton Ethics Committee.

Materials: Stimuli were 288 seven-letter words (mean word frequency = 23.03, SD = 39.86 [19]; extracted from the MRC psycholinguistic database [20]. For each subject, 180 words were randomly selected from this pool to be presented as old items during the study phase and 108 words served as new items in the test phase.

Behavioral task: At study participants viewed a total of 180 words, presented one at the time, and were asked to indicate whether the word was pleasant or unpleasant by pressing one of two keys on the keyboard with their right or left index finger. This task ensured that participants attended to each word and encouraged deep encoding of the stimuli. Participants were also instructed to memorize the words in view of a subsequent memory test. Trials started with a fixation mark that stayed on the screen for 1000 ms followed by the presentation of the word. The duration of the word varied as a function of the experimental condition (see *rTMS protocol and experimental conditions* below and Fig. 1A), and the inter-trial interval was varied accordingly to achieve a trial duration of 5600 ms in all conditions. Words were presented in six study blocks of 30 words each, corresponding to the six stimulation conditions. In the test phase, the words from each study block were interspersed with 18 new words and presented again for the recognition memory task, resulting in six test blocks of 48 items each. The presentation of blocks in the test phase followed the same order of the study phase (e.g., words that were presented in the first block in the study phase, were presented in the first block of the test phase). For each word, participants had to decide whether or not they had seen the word during the study phase by pressing one of two keys with their right or left index fingers. The assignment of old responses to the left or right hand was counterbalanced across subjects. Each trial started with a 1000 ms fixation, followed by a word that stayed on the screen for 1000 ms. The time in between the offset of the word and the onset of the following trial was 1500 ms. In Experiment 1 and all experiments, fixation marks and words were presented in a white uppercase Helvetica on a gray background using the Cogent 2000 toolbox (http://www.vislab.ucl.ac.uk/cogent.php).

rTMS protocol and experimental conditions: Trains of 600 ms 20 Hz rTMS were delivered in the study phase through a MagStim Super Rapid stimulator with a biphasic current waveform (Magstim, UK). A figure-of-eight 70-mm coil was used for the stimulation. The coil was placed tangentially to the scalp, with the handle pointing backwards and laterally at a 45° angle of the middle sagittal axis of the participants' head. Prior to rTMS, single magnetic pulses were delivered to the hand area of the left motor cortex to establish the individual excitability threshold for the first dorsal interosseous muscle [21]. The resting motor threshold was determined on visual inspection of muscle twitches evoked by the stimulation. For each subject, the intensity of the stimulation during the experiment was set to 90% of the individual resting motor threshold. The stimulation site was identified on the scalp using a TMS-magnetic resonance imaging coregistration system (SofTaxic, Italy). Coordinates were automatically estimated by the Navigator System, on the basis of an MRI-constructed stereotaxic template. MNI coordinates for the left VLPFC (−52, 45, 10) corresponded to those used in previous rTMS studies of subsequent memory effects [22].

We used four VLPFC stimulation conditions, corresponding to four combinations of word duration/time of rTMS onset (Fig. 1A). In the *Offset* 1000 ms and *Offset* 1100 ms conditions, rTMS was delivered at the offset of 1000- and 1100-ms words respectively. In the two online conditions, rTMS was delivered at the same timing of the offset conditions but while the words were still on the screen. More specifically, in the *Online* 1000 ms condition rTMS was delivered 1000 ms after the onset of 1200-ms words. In the *Online* 1100 ms condition rTMS was delivered 1100 ms after the onset of 1300-ms words. Participants additionally performed one block of the task without rTMS and received vertex stimulation, which made six experimental conditions in total (Fig. 1A). The vertex stimulation site was defined as a point midway between the inion and the nasion and equidistant from the left and right intertragal notches. Since this region is not involved in learning and memory processes, it was considered a control site for possible unspecific

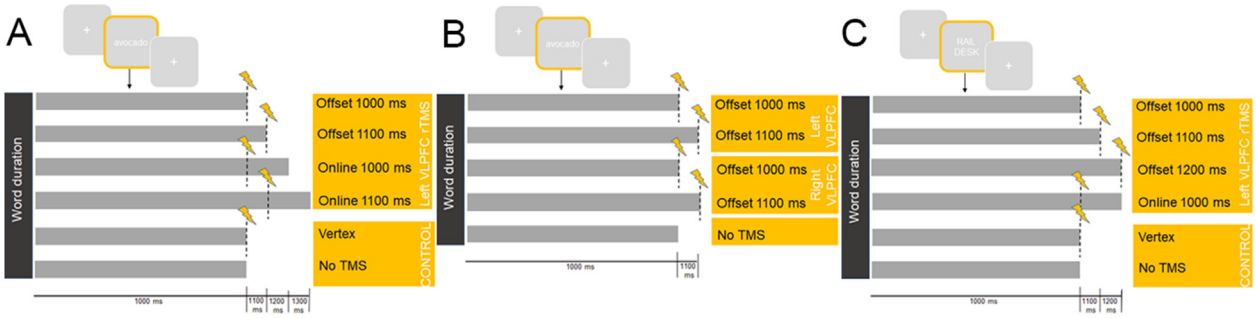

Fig. 1. Schematic illustration of the rTMS conditions in Experiment 1 (A), Experiment 2 (B) and Experiment 3 (C).

somatosensory, acoustic, or arousal effects of active TMS [11]. Throughout the study, participants received 1800 rTMS pulses and the interval between each stimulation train was 5000 ms in all experimental conditions. The four PFC conditions were administered in succession to avoid coil dispositioning and their order was randomized for each participant. The order of the PFC, Vertex and No-rTMS conditions was counterbalanced in a balanced Latin Square design. To assess the effect of any discomfort induced by rTMS in all experiments we administered a TMS sensation screening questionnaire (adapted from Ref. [11].

Experiment 2

Experiment 2 was conducted on 24 participants (19 females; mean age ± SD: 21 ± 3 years; range: 18–30 years). Two subjects were tested but excluded from the analyses due to low memory performance (±2 SD below the average). Methods were identical to Experiment 1 with the exception that *i)* stimuli were 290 seven-letter words (mean word frequency = 22.95, SD = 39.76, [19]) presented in five blocks of 36 words in the study phase and five blocks of 58 words in the test phase and *ii)* we tested the effects of rTMS on the left VLPFC site and the homologous right VLPFC (MNI: +52, 45, 10) site using the two offset conditions of Experiment 1 *(Offset* 1000 ms*, Offset* 1100 ms*)* and a no TMS control condition. Altogether then, Experiment 2 involved 5 experimental conditions (Fig. 1B). Throughout the study, participants received 1728 rTMS pulses and the interval between each stimulation train was 5000 ms in all experimental conditions.

Experiment 3

Participants: 24 participants (17 females; mean age ± SD: 20 ± 1 year; range: 18–23 years) took part in Experiment 3. One subject was tested but excluded from the analyses due to low memory performance (±2 SD below the average).The study was approved by the University of Roehampton Ethics Committee.

Materials: A list of 720 words of three or fewer syllables was extracted from the MRC psycholinguistic database and used to create 360 word pairs matched for frequency and imageability. For each subject, 240 word pairs were randomly selected to be presented as old pairs during the study phase and the remaining 120 word pairs were used as novel word pairs.

Behavioral task: In Experiment 3, at study participants viewed a total of 240 word pairs, presented one at the time in six consecutive blocks corresponding to six stimulation conditions (see *rTMS protocol and experimental conditions* below and Fig. 1C). The word pair was presented at the center of the computer screen, one word above the other. Participants were asked to create a mental image incorporating the concepts represented by both words and press one of two keys on the keyboard with their right or left index finger to indicate whether the quality of the mental image was good or bad. Participants were also instructed to memorize the word pairs and it was emphasized that the relationship between the two words of each pair was important for the following memory test. Each study trial started with the presentation of a fixation mark for 1000 ms, followed by the presentation of the study pair. As in Experiments 1 and 2, the time the word pair remained on the screen varied as a function of the experimental condition, and the inter-trial interval was varied accordingly to achieve a trial duration of 5600 ms in all conditions. In the test phase, the 40 pairs of studied items from each study block were presented again and intermixed with 20 novel word pairs, resulting in six test blocks of 60 word pairs each. Of the 40 pairs of studied items in each block, 20 remained in the same pairing as at study ('intact' pairs) and 20 were rearranged such that the studied items were the same but reassembled in different pairs ('rearranged' pairs). Participants were asked to decide whether the items had been paired together at study (intact judgment), presented at study but on separate trials (rearranged judgment), or not presented at study (new judgment). Each trial started with a 1000 ms fixation, followed by a word that stayed on the screen for 1000 ms. The time in between the offset of the word and the onset of the following trial was 1500 ms.

rTMS protocol and experimental conditions: rTMS protocol and experimental conditions of Experiment 3 were similar to those employed in Experiment 1. The main difference was that in Experiment 3 we removed the *Online* 1100 ms condition and added an additional offset condition (*Offset* 1200 ms) to investigate offset-related effects at later temporal windows. As in Experiment 1, this resulted in six experimental conditions (Fig. 1C). Throughout the study, participants received 2400 rTMS pulses and the interval between each stimulation train was 5000 ms in all experimental conditions.

Statistical analyses

The effects of rTMS at encoding on subsequent memory accuracy and RTs was investigated by comparing each stimulation condition with the control conditions, using Bonferroni-corrected pairwise comparisons (we report corrected Bonferroni-corrected p values throughout the manuscript). We used one-tailed t-tests for test performance (accuracy and RTs) because we expected a reduction of memory accuracy and increase in reaction times following rTMS administration, based on previous findings using a similar protocol [10,23,11]. We were agnostic about the direction of effects in the study phase and used two-tailed t-tests for encoding RTs. As in our previous study [10], to reduce the number of comparisons and achieve a unitary baseline we collapsed the *Vertex* and the *No TMS* control conditions in the three experiments, after checking that there were no significant differences between the two conditions in all analyses ($ps > 0.065$, see Table 1 for memory

performance separately for the two conditions). Our main interest was in recognition accuracy, established with the discrimination index *Pr* (the proportion of hits minus the proportions of false alarms; [24]). Analysis of memory accuracy in Experiment 3 focused on associative hits as index of associative memory performance ('intact' responses to intact word pairs). Furthermore, in all experiments we analyzed response times (RTs) at study and test. Finally, we meta-analyzed the data from Experiment 1 and 3 reported here and from Ref. [10] to estimate offset-related rTMS effects with higher precision. Data from Experiment 2 were not included as the study did not include an online condition. We compared the means aggregated across the collapsed online conditions (vs. control) and offset conditions (vs. control). We first calculated standardized mean change measures for individual studies between online/offset and control conditions and then meta-analyzed them using a random model (restricted maximum likelihood estimator) as implemented in 'metafor' R package [25]. We then used time (online/offset conditions) as a moderator variable.

Results

Encoding

rTMS did not affect the time taken to give a response at encoding (Experiment1, *ps* > 0.982; Experiment 2, *ps* > 0.989; Experiment 3 *ps* > 0.084, Table 1). There was no correlation between encoding RTs in each stimulation condition and the size of rTMS-induced memory effects in the corresponding condition at test (all *ps* > 0.075).

Retrieval

Memory accuracy. The administration of rTMS to the left VLPFC at the offset of 1100-ms words impaired subsequent item memory performance in Experiment 1 (t_{21} = 2.79, p = 0.020, d = 0.49; Fig. 2A) and Experiment 2 (t_{21} = 2.60, p = 0.032, d = 0.55; Fig. 2B). In Experiment 3 rTMS disrupted subsequent associative memory performance when administered at the offset of 1200-ms word pairs (t_{22} = 2.99, p = 0.014, d = 0.57; Fig. 3).

In Experiment 2, the stimulation of the right VLPFC did not impair recognition accuracy in either offset condition (*Offset* 1000 ms, p = 0.997; *Offset* 1100 ms, p = 0.896; Table 2), suggesting that offset-related rTMS effects are specific to the left VLPFC and are not affected by sensory phenomena induced by rTMS. When rTMS was delivered to the left VLPFC at the offset of 1000-ms words or during online encoding there was no significant difference with the control condition (*ps* > 0.064, Table 2). When analyzed separately, there was no significant effect involving hits or false alarms with the exception that in Experiment 1 rTMS administered at the offset of 1100-ms words significantly decreased the proportion of hits (t_{21} = 2.75, p = 0.020, d = 0.59; Table 2).

Reaction times. There was no significant effect of VLPFC rTMS on test phase RTs in Experiment 1 (*ps* > 0.115) and Experiment 2 (*ps* > 0.956). In Experiment 3 three additional subjects were excluded from the analyses due to technical difficulties. RTs for associative hits were slower in the *Offset* 1200 ms condition (t_{19} = 2.91, p = 0.040, d = 0.66). None of the other pairwise comparisons was statistically significant (*ps* > 0.108).

Table 1
Response times at encoding. Times are expressed in milliseconds, standard deviations are displayed in parentheses.

	Experiment 1	Experiment 2	Experiment 3
Left VLPFC			
Offset 1000 ms	958 (413)	902 (315)	1351 (648)
Offset 11000 ms	961 (362)	927 (340)	1410 (669)
Offset 1200 ms			1549 (745)
Online 1000 ms	943 (380)		1388 (718)
Online 1100 ms	961 (368)		
Right VLPFC			
Offset 1000 ms		909 (320)	
Offset 1100 ms		910 (348)	
Control conditions			
Vertex	965 (346)		1453 (633)
No TMS	949 (293)	956 (278)	1370 (535)

Meta-analysis

For the discrimination index, as expected, we found a small non-significant effect in the online conditions (z = 1.76, p = 0.079) and a large statistically significant effect in the offset conditions (z = 3.69, p < 0.001, see Table 3). For estimation purposes, we also run a moderation analysis; this should not be interpreted as confirmatory test due to insufficient power, the recommended minimal number of comparisons for a moderation analysis is typically at least ten (e.g., Ref. [26]). We found a non-significant medium-sized difference (b = 0.40, 95% CI [-0.11, 0.91], QM(1) = 2.36, p = 0.124) without substantial residual heterogeneity (QE(2) = 0.66, p = 0.720, I^2 = 0.0%, τ^2 = 0).

The pattern was similar for hit rates, with a statistically non-significant effect in the online conditions (z = 0.56, p = 0.577) and a medium-sized and statically significant effect in the offset conditions (z = 3.23, p = 0.001). Again, the moderation analysis showed a medium effect size difference between the two effects that did not reach statistically significance (b = 0.38, 95% CI [-0.002, 0.75], QM(1) = 3.79, p = 0.052) and without substantial residual heterogeneity (QE(4) = 1.06, p = 0.901, I^2 = 0.0%, τ^2 = 0).

TMS sensation questionnaire

Fig. 4 reports participants' responses to the TMS sensation questionnaire. We found no significant correlation between the intensity of the discomfort and its perceived effect on the task (in both cases coded on a scale from 1 to 4) with rTMS-induced changes in statistically significant conditions (all *ps* > 0.158).

Discussion

The left VLPFC has been frequently implicated with the encoding of verbal information (for reviews, [14,15,16]. Previous rTMS studies had demonstrated that brain activity in the left VLPFC occurring after the termination of a stimulus is critical for memory formation [10,11]. Here, in three experiments we demonstrated that rTMS did not disrupt subsequent memory performance when delivered during online encoding (i.e. during their presentation), rather, the involvement of the left VLPFC during the formation of new verbal memories was triggered by the offset of word stimuli. A meta-analysis combining the data from three experiments (Experiment 1, Experiment 3 and data reported in Ref. [10]) identified a medium-sized difference in the effect attributable to rTMS disruption of memory performance when delivered on the left VLPFC offline compared to online.

What could be the specific mechanisms underlying offset-related encoding activity in the left VLPFC? The results of Experiment 3 help provide an answer to this question by showing that offset-related mechanisms may be related to the binding of information into an episodic representation. In a task that required participants to memorize the association between word pairs, we observed a large decrease in associative memory accuracy when

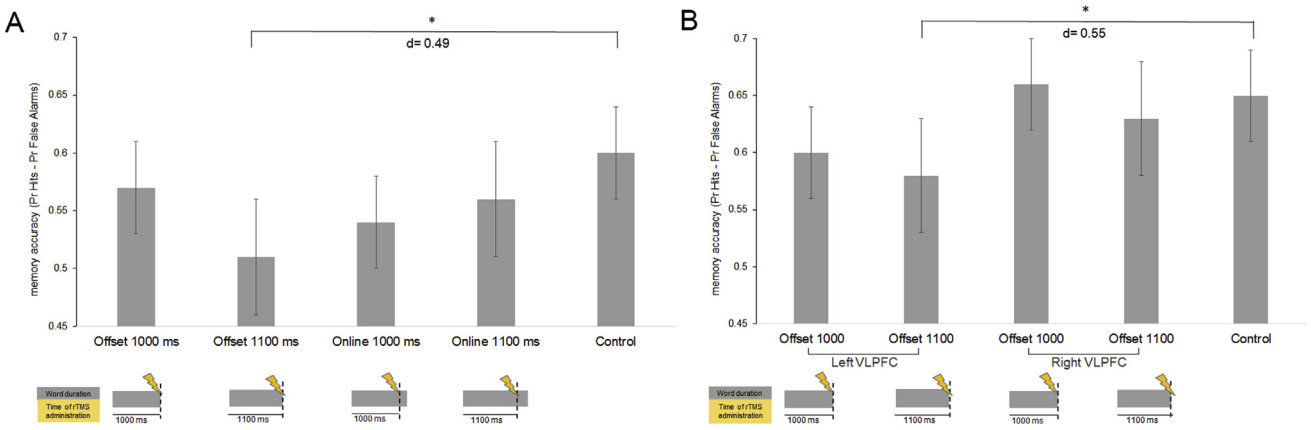

Fig. 2. Memory performance as a function of rTMS administration in Experiment 1 (A) and 2 (B). A decrease in memory accuracy is evident when the stimulation was administered at the offset of 1100-ms words in both experiments. For Experiment 1, the baseline (far right column) is based on the collapsed vertex and no-TMS conditions. * denotes $p < 0.05$ (Bonferroni-corrected). Effect sizes are shown as Cohen's d. Error bars depict standard errors.

rTMS was delivered at the offset of the pairs during encoding, along with an increase in the time taken to give memory judgments. If rTMS did not disrupt the binding of the word pairs into an integrated memory trace, we would not have observed this decrease. We speculate that the offset of a word stimulus triggers episodic binding and associative encoding processes in the left VLPFC, which in turn contribute to the formation of the memory trace. Although associative encoding is more prominent when there is a specific instruction to associate different features or items into a unique memory trace, such as in Experiment 3, it could also occur in single-item encoding in the absence of explicit associative task demands. It is notable that although associative encoding is typically associated with activity of the hippocampus [27], other studies have revealed an equally relevant role of the left VLPFC [28]. We could not examine the role of the hippocampus because the depth of TMS prevents a direct stimulation of medial temporal lobe structures. However, studies have shown that the left VLPFC has functional and anatomical connections with the hippocampus [29] and that PFC stimulation can modulate network dynamics and propagate to distant brain regions, including the hippocampus [30,31]. One hypothesis thus is that in the current study VLPFC stimulation at word offset interfered with memory formation through an indirect effect on medial temporal lobe structures.

Another explanation for the current findings, which is not necessarily mutually exclusive with the interpretations above, takes into account the role of event boundaries in memory formation. Studies on sequential learning have demonstrated that memory encoding is enhanced for information presented at event boundaries, for instance when shifts in stimulus category, perceptual context or object location occur, and that these memory enhancements are related to neural activity in the hippocampus and left VLPFC (e.g., Ref. [32]; Horner et al., 2017; [33,17,18]). It is reasonable to assume that event offsets are experienced as event boundaries and that VLPFC activation initiated by the offset

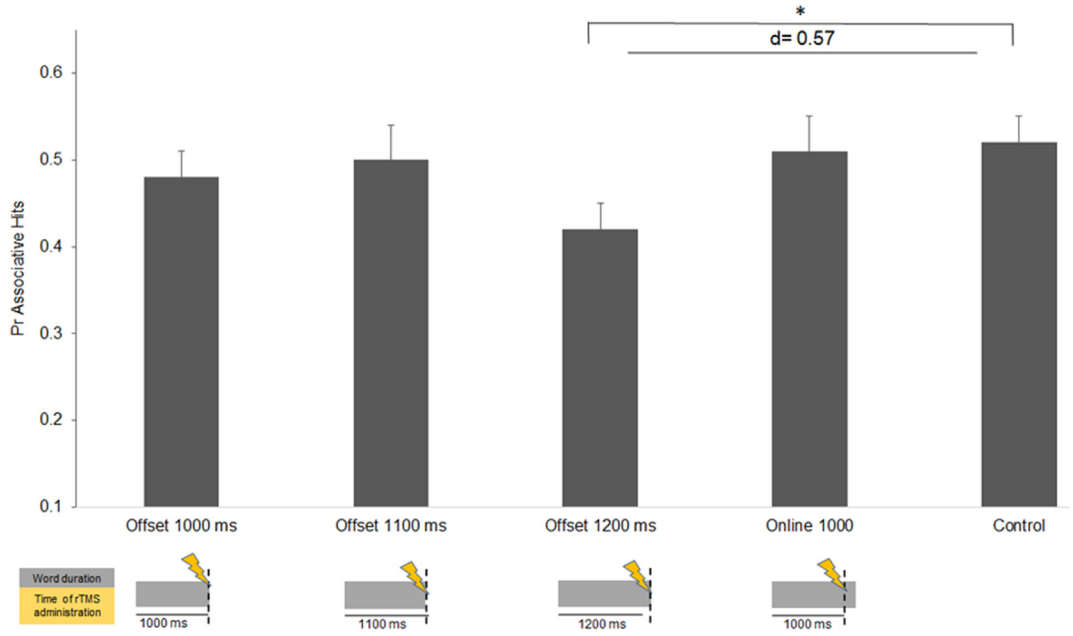

Fig. 3. Memory performance as a function of rTMS administration in Experiment 3. Subsequent associative memory performance decreased when rTMS was delivered at the offset of 1200-ms word pairs. The baseline (far right column) is based on the collapsed vertex and no-TMS conditions. * denotes $p < 0.05$ (Bonferroni-corrected). Effect sizes are shown as Cohen's d. Error bars depict standard errors.

Table 2
Effects of rTMS on memory accuracy. DI: Discrimination Index Pr (proportion of hits minus proportion of false alarms, [24]. FA: False Alarms. Associative Hits: 'intact' responses to intact pairs. Standard deviations are displayed in parentheses.

	Experiment 1			Experiment 2			Experiment 3
	Hits	FA	DI	Hits	FA	DI	Associative Hits
Left VLPFC							
Offset 1000 ms	0.81 (0.12)	0.24 (0.15)	0.57 (0.18)	0.82 (0.17)	0.22 (0.16)	0.60 (0.22)	0.48 (0.16)
Offset 11000 ms	0.77 (0.13)	0.26 (0.14)	0.51 (0.22)	0.84 (0.11)	0.26 (0.15)	0.58 (0.21)	0.50 (0.19)
Offset 1200 ms							0.42 (0.16)
Online 1000 ms	0.81 (0.12)	0.27 (0.18)	0.54 (0.19)				0.51 (0.21)
Online 1100 ms	0.79 (0.17)	0.23 (0.14)	0.56 (0.22)				
Right VLPFC							
Offset 1000 ms				0.86 (0.09)	0.21 (0.13)	0.66 (0.17)	
Offset 1100 ms				0.87 (0.09)	0.24 (0.15)	0.63 (0.18)	
Control conditions							
Vertex	0.83 (0.13)	0.20 (0.16)	0.63 (0.20)				0.48 (0.15)
No TMS	0.82 (0.14)	0.26 (0.16)	0.57 (0.23)	0.89 (0.09)	0.23 (0.19)	0.65 (0.21)	0.54 (0.16)

Table 3
Meta-analytical effect of online (vs. control) and offset (vs. control) conditions on discrimination index and hit rate.

	Online (vs. control) Conditions		*Offset (vs. control) condition*	
	Hedge's *g*	95% CI	Hedge's *g*	95% CI
Discrimination index				
[10]	0.19	−0.38, 0.77	0.89	0.22, 1.56
Experiment 1	0.37	−0.06, 0.81	0.62	0.17, 1.08
Overall effect	**0.31**	**−0.04, 0.65**	**0.71**	**0.33, 1.09**
Hit Rate				
[10]	−0.08	−0.64, 0.49	0.40	−0.18, 0.99
Experiment 1	0.23	−0.20, 0.65	0.53	0.08, 0.97
Experiment 3	0.01	−0.40, 0.42	0.40	−0.02, 0.83
Overall effect	**0.07**	**−0.19, 0.34**	**0.45**	**0.18, 0.72**

contributes to the recovery of the just-experienced events [34]. This idea is in line with the finding of similar activation patterns at the onset and immediately after the offset of visual stimuli that are successfully maintained in working memory [35].

One important consideration is that rTMS effects were evident only when the stimulation was delivered at the offset of 1100-ms word stimuli in Experiment 1–2 and 1200-ms word stimuli in Experiment 3. One hypothesis is that word offset triggers encoding-related brain activity in the left VLPFC only if earlier item- and task-specific encoding mechanisms have terminated. It could be that, at least in some participants, those encoding processes were yet to complete before associative encoding could initiate in the VLPFC. Interestingly, EEG studies showed that frontal subsequent memory effects reflecting episodic binding start to emerge 1000 ms post-stimulus and follow earlier modulations related to meaning-based and item-specific processing (e.g., Refs. [36,37]). We cannot test this hypothesis directly with the current dataset because response times provide only coarse information on encoding times. Across the three experiments however, effects were numerically larger in rTMS conditions with longer encoding RTs and in Experiment 3, where encoding reaction times were longer due to the nature of the encoding task, rTMS effects were evident at a later latency region. It will be interesting to explore the relationship between offset-related brain activity and individual differences in encoding times in future studies using finer-grained measures of encoding time, such as EEG or MEG. Future studies should also use a TMS protocol that allows a more precise chronometric approach, such as paired-pulse or triple-pulse TMS [12,38].

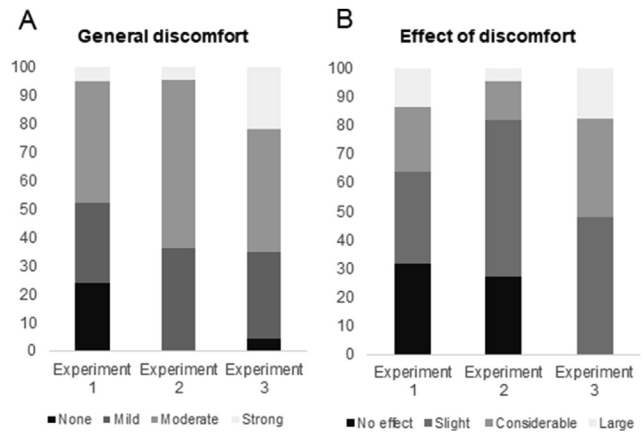

Fig. 4. Self-reports of TMS-induced sensations. Frequency of responses for the TMS sensation questionnaire regarding the level of perceived discomfort during rTMS administration (A) and the effect of the discomfort on the ability to perform the task (B).

Taken together, our findings offer insights into the temporal dynamics of memory formation and show that brain mechanisms in the left VLPFC induced by the offset of a verbal stimulus are responsible for the formation of verbal memories. Our results may be relevant for future clinical applications. Previous studies have demonstrated that the non-invasive stimulation of the PFC enhances long-term memory in young and healthy older adults [39,40,41], as well as in individuals with Mild Cognitive Impairment and Alzheimer's disease [42,43,44]. These studies have delivered the stimulation at different points in time during memory tasks. Future studies could test the idea that memory could be improved in clinical populations by administering facilitatory rTMS immediately after to-be-remembered items during encoding, either alone or in combination with memory training. By clarifying when and how memories are formed, our findings may thus help to refine neurorehabilitation programs in patients with memory disorders.

Declarations of competing interest

None.

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