Manipulating serial replay during memory retention rapidly alters human sequence memory

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Abstract

Temporarily storing sequences of events (sequence memory, SM), a fundamental ability in cognition, involves item-by-item serial reactivations during retention, yet the causal evidence is lacking. Here we develop a “behavioral temporal interference” approach to manipulate the item-specific reactivation and replay profile in the delay period when human subjects hold a list of items in working memory (WM). Eight experiments on more than two hundred subjects demonstrate that the manipulation successfully alters SM behavior. When items in the list are synchronized with each other and thus could not elicit time-dissociated responses, the serial position effect (SPE), a typical SM behavior, is disrupted. Reversing the temporal order of reactivations also alters SPE, supporting that the replay order is crucial as well. Our findings support the causal role of temporally ordered reactivation in mediating SM, and also provide a promising, efficient approach to manipulate the temporal structure of multiple items held in WM.

Keywords: working memory, sequence memory, serial position effect, recency effect, primacy effect, sequential replay

Introduction

The ability to hold temporal sequences of events in working memory (WM) (i.e., sequence memory, SM) is fundamental in many cognitive processes, such as language, movement control, episodic memory, and decision making[1,2,3,4]. A typical SM phenomenon is when presented with a sequence of items for subsequent recall, the memory performance would be dependent on the item's position in the list (serial position effect, SPE)[5]. For example, the last item tends to be recalled better ("recency effect"), and the item presented at the beginning of a long list also shows enhanced memory performance ("primacy effect"). SPE could be regarded as a robust behavioral index for SM.

How does the brain maintain and represent a list of temporally ordered items in WM? Neural evidence and theoretical models suggest that SM is mediated by item-by-item sequential reactivations in the delay period[6,7,8,9]. Recently, the serial reactivations have been further shown to be associated with recency effect (one type of SPE), but not with the overall memory accuracy[10], further supporting an essential connection between sequential replay and SM. In other words, remembering a list of items structured in sequence would require item-by-item reactivations that should be dissociated and ordered in time during memory maintenance. Accordingly, disruption or modification of the sequential replay would presumably deteriorate or alter the sequence information retained in WM, while not necessarily affecting the overall memory performance. However, the causal evidence, especially in human subjects, is completely lacking.

On the other hand, we lack an "optogenetics-like" tool in human studies, and it is technically difficult to modulate the item-specific reactivations and to precisely interrupt the replay profile in human subjects. The typical non-invasive interference approaches (e.g, TMS, tDCS, tACS, etc.) fail to meet the requirement, since they could not specifically manipulate the relationship between the neural representations of multiple items. Motivated by previous findings, here we designed a new "behavioral temporal interference" approach to address the issue. Specifically, previous studies demonstrate that task-irrelevant color feature would be automatically

bound to the to-be-memorized feature (e.g., orientation) in WM when they belong to the same object[11,12,13,14,15], and these task-irrelevant color probes could even tag memory reactivations[10,16]. Therefore, we could in principle modulate the ongoing temporal relationship between these color probes, which are presumably bound to each of the sequentially memorized items respectively, to interfere with or even modify the item-by-item serial reactivations. We then examined whether the temporal manipulation in retention would alter the subsequent SM behavior in retrieval, with a specific focus on SPE (e.g., recency effect, primacy effect), as typically observed in SM.

Two types of temporal manipulation – synchronization and order reversal –have been applied on the task-irrelevant but memory-associated color probes. First, when the luminance sequences of the color probes were synchronized with each other in time so that the associated memorized items would also be coordinated in time and could not be reactivated at different time, SPE was significantly disrupted (Experiment 1-4, 105 subjects; “synchronization manipulation”), thereby advocating the critical function of time-dissociated reactivations for SM. Second, when the luminance sequences of the probes were temporally shifted with each other in an order that was either the same or reversed as the stimulus sequence, they also showed distinct SPE (Experiment 5-7, 78 subjects; “order reversal manipulation”), indicating that the temporal order of the serial replay is vital as well. To our knowledge, our findings constitute the first causal evidence in humans supporting that retaining sequence information in WM is implemented by item-by-item reactivations that should be dissociated in time and follow certain temporal order.

Results

"Behavioral temporal interference" approach: synchronization during sequence memory retention

Subjects performed a SM task in all the experiments. As shown in Figure 1A, participants viewed two serially presented bar stimuli and were instructed to memorize the orientations of the two bars as well as their temporal order ("encoding period"). After 5 sec memory retention, participants rotated a horizontal bar to the memorized 1st or 2nd orientation based on the instruction ("recalling period"). Importantly, the two bar stimuli, in addition to having distinct to-be-memorized orientations, also had different colors. During the "maintaining period", subjects performed a central fixation task, while two discs that had color feature of either the 1st or the 2nd memorized bars (1st memory-related probe, SM1; 2nd memory-related probe, SM2) were displayed at left or right side of the fixation. The color (red, green, or blue) and the spatial locations (left or right) of the two probes (SM1 and SM2) were balanced across trials.

Crucially, throughout the 5 sec retention period, the luminance of the two color probes (SM1 and SM2) was continuously modulated according to two 5 sec temporal sequences respectively (i.e., Seq1 for SM1, Seq2 for SM2), the relationship of which varied in three ways – Baseline, Alpha-band synchronization (A-Sync), or Full-spectrum synchronization (F-Sync). Specifically, as shown in Figure 1B, the two luminance sequences (seq1 and seq2) were two independently generated random time series (Baseline; left), or the same random temporal sequence (F-Sync; right), or the same alpha-band time series (A-Sync; middle). The luminance sequences were generated anew in each trial. Using this manipulation, we aimed to examine whether the temporal synchronization (A-Sync and F-Sync relative to Baseline) of the two task-irrelevant but memory-related probes during retention would modify the subsequent SPE behavior in retrieval. We used Paul Bays' model[17] to quantify the memory performance for the 1st and the 2nd orientation and calculated the target probability (see the precision results in Supplemental Figure 1). We next converted

the target probability results using an arcsine square root transformation[18] to make it normally distributed.

Figure 1 about here

Temporal synchronization disrupts recency effect in 2-item sequence memory (Experiment 1 – 2)

Twenty-seven subjects participated in Experiment 1 (Figure 2A). First, the two-item SM showed typical recency effect ($2^{nd} > 1^{st}$ item) for the Baseline condition, when Seq1 and Seq2 were not related with each other, consistent with previous results[19]. In contrast, when Seq1 and Seq2 were temporally synchronized with each other (A-Sync and F-Sync), the recency effect was largely disrupted. Two-way repeated ANOVA (position * sync) revealed significant interaction effect ($F_{(2,52)} = 3.337$, $p = 0.043$, $\eta_p^2 = 0.114$), but nonsignificant main effect for either position ($F_{(1,26)} = 0.372$, $p = 0.547$, $\eta_p^2 = 0.014$) or sync condition ($F_{(2,52)} = 0.268$, $p = 0.766$, $\eta_p^2 = 0.010$). Post-hoc comparisons further confirmed the disruption of recency effect for both A-sync and F-sync compared to Baseline (2-way repeated ANOVA; A-Sync and Baseline: interaction effect, $F_{(1,26)} = 5.274$, $p = 0.030$, $\eta_p^2 = 0.169$; F-Sync and Baseline: interaction effect, $F_{(1,26)} = 3.503$, $p = 0.073$, $\eta_p^2 = 0.119$). Moreover, the two synchronization conditions did not show significant difference, indicating their similar effects (2-way repeated ANOVA; A-sync and F-syn: interaction effect, $F_{(1,26)} = 0.494$, $p = 0.488$, $\eta_p^2 = 0.019$).

To further confirm the disruption effects, we ran a separate group of subjects (N = 24) for only the A-Sync and Baseline conditions (Experiment 2). As shown in Figure 2B, the results were consistent with Experiment 1. The Baseline condition again showed recency effect (paired t-test, $t_{(23)} = -2.721$, $p = 0.012$, Cohen's d = -0.555), whereas the A-sync condition did not (paired t-test, $t_{(23)} = 1.300$, $p = 0.207$, Cohen's d = 0.265). Two-way repeated ANOVA (2 positions * 2 sync) revealed significant interaction effect ($F_{(1,23)} = 7.493$, $p = 0.012$, $\eta_p^2 = 0.246$) but nonsignificant main

effect for either position ($F_{(1,23)} = 0.659$, $p = 0.425$, $\eta_p^2 = 0.028$) or sync condition ($F_{(1,23)} = 0.283$, $p = 0.600$, $\eta_p^2 = 0.012$), further supporting the synchronization-induced disruption of recency effect.

Moreover, to examine whether the disruption effect was specifically introduced by the memory-related probes, we ran another experiment by using memory-irrelevant color probes (Experiment 2-IC, N = 25). As shown in Figure 2B (right), now we did not observe any difference between the Baseline and A-Sync conditions (two-way repeated ANOVA, interaction effect, $F_{(1,24)} = 0.006$, $p = 0.938$, $\eta_p^2 < 0.001$). Two-sample two-way ANOVA (across Experiment 2 and Experiment 2-IC) showed marginally significant interaction effect (Experiment*Position*Sync, $F_{(1,47)} = 3.391$, $p = 0.072$, $\eta_p^2 = 0.067$), supporting that SM behavior was modulated by the memory-related probes but not by the memory-irrelevant ones.

As summarized in Figure 2C, temporal synchronization of the memory-associated probes during maintenance essentially disturbed the SPE for the two-item SM (i.e., recency effect here) (see similar but nonsignificant trend when spatial locations were synchronized, Experiment 8, Supplementary Figure 2). It is noteworthy that the color probes were completely task-irrelevant in terms of both short-term memory (memorizing orientations) and attentional task (central fixation task). Furthermore, this disruption effect was not accompanied by any overall memory performance changes (i.e., no main effect), indicating that the manipulation does not interrupt memory retention at a general level but indeed specifically modulates the relative memory strength or the temporal association of multiple items held in WM.

Figure 2 about here

Temporal synchronization disrupts primacy and recency effect in 3-item SM (Experiment 3 – 4)

We next investigated whether the temporal synchronization would also disrupt SPE in three-item SM task (Experiment 3-4). As shown in Figure 3A, subjects were

instructed to memorize the orientations of three consecutively presented bars and their temporal order (3-item SM). During the 5 sec maintenance period, subjects performed a central fixation task, while three discs that had color feature of either the 1st, the 2nd, or the 3rd memorized bars (SM1, SM2, SM3) were presented, the luminance of which was modulated according to three random sequences respectively (Seq1, Seq2, Seq3). Similarly, the three temporal sequences were either nonrelated with each other (Baseline) or share the same time series (A-Sync in Experiment 3; F-Sync in Experiment 4). Notably, considering the large number of trials that would be required to include all the 3 synchronization conditions for a 3-item SM task in a repeated design, as well as the observed similar effects for F-Sync and A-Sync conditions, we employed a two-sample two-way repeated design. Specifically, Experiment 3 assessed Baseline and A-Sync conditions, and Experiment 4 tested Baseline and F-Sync conditions.

Two separate groups of subjects participated in Experiment 3 (N = 26) and Experiment 4 (N = 28) respectively. As shown in Figure 3B, when Seq1, Seq2, and Seq3 were three nonrelated time series (Baseline), memory performance showed typical recency effect (3rd > 2nd) and primacy effect (1st > 2nd) for 3-item SM[20]. In contrast, when the luminance of the three color probes was modulated by the same temporal sequences (A-Sync in Experiment 3; F-Sync in Experiment 4), both the recency and primacy effects largely decreased (Figure 3C). A two-sample two-way repeated ANOVA (Experiment*Position*Sync) revealed significant Position*Sync interaction effect ($F_{(2,102)} = 6.895$, $p = 0.002$, $\eta_p^2 = 0.119$), but nonsignificant Experiment*Position*Sync interaction effect ($F_{(2,102)} = 0.024$, $p = 0.977$, $\eta_p^2 < 0.001$), indicating the synchronization-induced SPE disruption in both experiments. Further tests across the two experiments revealed significant SPE for the Baseline condition (two-sample two-way repeated ANOVA, Experiment*Position, position effect: $F_{(2,102)} = 8.843$, $p < 0.001$, $\eta_p^2 = 0.148$; interaction effect: $F_{(2,102)} = 1.430$, $p = 0.244$, $\eta_p^2 = 0.027$), but not for the Synchronization condition (position effect: $F_{(2,102)} = 1.640$, $p = 0.199$, $\eta_p^2 = 0.031$; interaction effect: $F_{(2,102)} = 1.475$, $p = 0.234$, $\eta_p^2 = 0.028$).

Therefore, temporal synchronization of the three memory-related color probes during retention also disrupts the SPE in a 3-item SM task (i.e., primacy and recency effect).

Taken Experiment 1-4 together, temporal synchronization of memory-associated color probes in the delay period successfully alters SM behavior (i.e., SPE) without affecting the overall memory performance. Therefore, time-dissociated reactivations are necessary for representing and maintaining the ordinal structure in WM.

Figure 3 about here

Reversing replay order disrupts serial position effect (Experiment 5-7)

We next examined the function of the temporal order of the reactivations in SM. Specifically, if SM is mediated by serial replay that follows certain temporal order (i.e., forward or backward), forming temporal profiles that have the same (Seq1 leads Seq2) or reversed (Seq1 lags Seq2) temporal order as the physical sequence in the delay period would presumably result in distinct SM behavior.

Three experiments (Experiment 5-7) were performed to test the hypothesis. The same experimental paradigm as Experiment 1 was used (see Figure 1A), except that the luminance sequences of the two color discs were modulated in a different way. Specifically, Seq1 was a temporally shifted version of Seq2, either leading (Same, upper panel) or lagging Seq2 (Reversed, lower panel) by 200 ms (Figure 4A). The selection of 200 ms was motivated by previous neurophysiological studies revealing a central role of theta-band rhythm in organizing memory replay[7,8,10,21]. Two separate groups of subjects participated in Experiment 5 (N = 24; Same, Reversed, Baseline) and Experiment 6 (N=27; Same, Reversed, F-Sync) respectively. First, as shown in Figure 4B, consistent with previous results (Experiment 1-4), recency effect persisted for Baseline condition (paired t-test, $t_{(23)} = -1.835$, $p = 0.0790$, Cohen's d = -0.356; left panel) and vanished for Synchronization condition (paired t-test, $t_{(26)} = -0.366$, $p = 0.718$, Cohen's d = -0.0704; right panel). Most crucially, the recency effect significantly decreased for Reversed compared to Same condition (paired t-test, Expt. 5: $t_{(23)} = 2.184$, $p = 0.039$, Cohen's d = 0.446; Expt. 6: $t_{(26)} = 1.854$, $p = 0.075$,

Cohen's d = 0.357) in both experiments (Figure 4B). Two-sample two-way repeated ANOVA (2*2*2, Experiment*Position*Order) revealed significant Position*Order interaction effect ($F_{(1,49)} = 7.543$, $p = 0.008$, $\eta_p^2 = 0.133$), supporting that the order reversal of reactivations indeed altered the SM behavior. We further combined the Same and Reversed conditions across Experiment 5 and 6, and as shown in Figure 4C (left panel), the pooled results (N = 51) showed clear decrease and even reversal in recency effect for the Reversed condition compared to the Same condition (paired t-test, $t_{(50)} = 2.787$, $p = 0.007$, Cohen's d = 0.390). It is noteworthy that the only difference between the Same and Reversed conditions was the relative order of the two luminance sequences (i.e., Seq1 leads Seq2, Seq2 lead Seq1), yet they led to distinct SPE, thus advocating the vital role of reactivation order in mediating SM.

Finally, we assessed whether introducing a temporal lag outside of the theta-band rhythm would influence the SM as well. Instead of 200 ms, Experiment 7 (N = 27) employed a 500 ms temporal lag between Seq1 and Seq2. As shown in the right panel of Figure 4C, the Same and Reversed conditions now did not show SPE difference (paired t-test, $t_{(26)} = 0.331$, $p = 0.743$, $d = 0.064$). Thus, the replay order manipulation should be applied within certain neurophysiological-relevant temporal window (e.g., period of theta-band rhythm) to successfully influence SM.

Figure 4 about here

Meta-analysis

Finally, to increase the detecting sensitivity and examine the overall effects, we performed a meta-analysis (Comprehensive Meta-Analysis Software, CMA) across all the 8 experiments (Expt. 1-7 as well as the spatial experiment), focusing on the recency effect, given its presence in both 2-item and 3-item SM experiments. As shown in Figure 5, the Baseline condition (Experiment 1, 2, 3, 4, 5, 7, 8) showed significant recency effect (N = 182; $p = 3e^{-8}$), whereas the Synchronization condition (Experiment 1, 2, 3, 4, 6, 8) did not (N = 158; p = 0.601). Furthermore, Baseline and Synchronization conditions (Experiment 1, 2, 3, 4, 8) revealed significant difference

(N = 131; p = $2.11e^{-5}$). The meta-analysis on precision results across all 8 experiments showed the same trend (Supplementary Figure 3), although not as strong as the target probability estimation.

Figure 5 about here

Discussion

We developed a "behavioral temporal interference" approach to manipulate the item-specific reactivations when human subjects retained a sequence of items in WM, by modulating the ongoing luminance of memory-related color probes in the delay period. Our motivation is based on previous findings showing that the color feature would be automatically bound to each of the items in the list respectively and could tag item-specific reactivations in retention[10,11,12,13,15]. Eight experiments (N > 200) consistently demonstrate that this temporal manipulation approach – temporal synchronization (Experiment 1-4) and order reversal (Experiment 5-7) – successfully and rapidly altered subsequent SPE in SM tasks while leaving the overall memory performance intact. Our findings constitute new causal evidence supporting that SM is essentially mediated by an item-by-item serial replay that should be appropriately ordered within temporal scales associated with short-term synaptic plasticity. Our results also provide a promising and efficient non-invasive behavioral approach to precisely manipulate the neural representations of multiple items maintained in WM.

It has long been posited that SM is implemented by sequential replay activities during memory maintenance[6,22,23,24,25,26,27,28]. Theoretical model for WM proposes a theta-gamma coupling mechanism for multi-item WM such that each item is encoded at different phase within a theta-band cycle[7,8,9]. Animal neuronal recordings have provided evidence advocating the theoretical framework. For example, firing sequences of place cells in the rodent hippocampus are reactivated during spatial memory task as well as in sleep, in the same or reversed firing order as experienced[6,24,29,30]. Monkey recordings show that two serially presented objects are encoded at different phase within the beta-band activities[31]. Furthermore, neuroimaging studies on human subjects have revealed neural responses that are consistent with the cross-frequency coupling model, suggesting that multiple memorized items are encoded at different phases of low-frequency neuronal oscillation[8,32]. Recently, by tagging memory-related color probes in a SM task, we have also revealed a serial reactivation profile during maintenance and its association to recency effect[10]. However, most of these studies have remained at the descriptive

level to link neuronal activity to behavioral performance. Here we demonstrate that modifying the serial replay in the delay period would specifically influence the SM behavior (i.e., SPE) without affecting the overall memory performance, thus providing new causal evidence supporting that memorizing a sequence is indeed dependent on the item-by-item serial reactivation in a correct order.

Previous studies, by using behavioral or non-invasive stimulation approaches in the encoding or delay period, have successfully modulated the recalling performance[33,34,35]. For example, stimulating category-specific brain regions could enhance the object recognition performance specifically for that category[33]. Moreover, repetitive TMS at certain frequency bands has also been found to modulate short-term visual memory capacity[34] and enhance auditory memory performance in a manipulation task[35]. Recently, a very interesting study demonstrates that single pulse of TMS could successfully reactivate latent working memories that are potentially task-relevant at later stage and influence the memory performance[33], advocating a synaptic theory of WM[36,37,38,39]. Here, benefiting from the precise temporal modulation on item-specific reactivation responses and on the associations between items in the delay period, our study largely differs from previous interference findings in two aspects. First, previous studies mainly focus on the modulation of general memory performance while our study aims at particularly manipulating the sequence information without affecting the overall memory level. Second, by creating time-resolved luminance sequences, our approach could precisely modulate the temporal relationship between items in the SM list (e.g., synchronization in Experiment 1-4, 200 ms or 500 ms temporal shifting with different directions in Experiment 5-7). Therefore, the overall response magnitude and the fine temporal organization of the multi-item reactivations in the delay period might represent contents (e.g., memory performance) and structures (e.g., temporal order) of SM respectively.

The present findings are closely associated with a Hebbian short-term potentiation (STP) WM model[40,41]. This model, different from previous short-term non-associative plasticity model[36,37], incorporates fast-expressing form of Hebbian-like synaptic plasticity through which new items and their associations could be quickly encoded. Interestingly, the model simulation for a multi-item WM task shows item-by-item sequential reactivations and predicts a profile resembling the SPE (i.e., recency and primacy effect). Combined with our results, when the two sequentially memorized items are manipulated to be reactivated simultaneously during the delay period (Expt. 1-4), they tend to be bound together through the STP mechanism and thus could not be separated in subsequent recalling (i.e., disruption of SPE). Furthermore, the STP in WM, like the spike timing-dependent plasticity (STDP) in long-term memory[42,43], might be endowed with direction-dependent asymmetrical plasticity that could in principle enable the short-term storage of temporal order in synaptic strength. As a consequence, reversing the replay sequence would presumably cancel out and even shift the reactivations to an opposite direction and in turn modifies the SM behavior (Expt. 5-6). Finally, the fact that temporal manipulation is only effective within certain temporal window (Expt. 7) is also commensurate with the STP time constants in the WM model (e.g., 200-300 ms), beyond which STP could not be successfully formed[41].

In conclusion, to represent and store multiple items as well as their temporal relationship in WM, it is important for the brain to reorganize the external events that are separated widely in time within an appropriate temporal window that is vital for STP mechanism to operate on[44,45]. The “behavioral temporal interference” approach should be matched with the intrinsic STP temporal characteristic (e.g., time constant, order-dependent, etc.) of the short-term memory formation, whereby the reactivation pattern could be efficiently manipulated and the SM behavior would be successfully modulated.

Conflicts of interest

The authors declare no conflicts of interest.

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Methods

Participants

Two hundred and thirty-four participants (100 males, age ranging from 18 to 26 years) took part in nine experiments (Expt. 1–7 and Expt. 2-IC in main texts, Expt. 8 in supplementary materials). One subject in Expt. 1, two in Expt. 2, two in Expt. 2-IC, five in Expt. 3-4, three in Expt. 5, four in Expt. 6, three in Expt. 7, and five subjects in Expt. 8 were excluded due to their overall low behavioral performance, or self-reported strategies, or not finishing the whole experiment, resulting in 27, 24, 26, 28, 24, 27, 27, 25, 26 subjects for Expt. 1 to Expt. 7, Expt. 2-IC, and Expt. 8 respectively. All the participants were naïve to the purpose of the experiments and had normal or corrected-to-normal vision with no history of neurological disorders, and have provided written informed consent before the experiments. All experiments were carried out in accordance with the Declaration of Helsinki and have been approved by the Research Ethics Committee at Peking University.

Stimuli and tasks

Sequence memory (SM) paradigm

Participants sat in a dark room, 58 cm in front of a CRT monitor (Display++ monitor in Experiment 4) with 100 Hz refresh rate and a resolution of 1024 * 768. Subjects performed a sequence memory task, with their head stabilized on a chin rest. Each trial consisted of three periods: encoding, maintaining, and recalling (Fig. 1A). In the "encoding period", subjects were serially presented with two (Experiment 1, 2, 5, 6, 7, 8) or three (Experiment 3–4) bar stimuli (0.38° × 1.15° visual angle) displayed at the center. Each bar stimulus lasted for 1 s with a 0.5 s inter-stimulus interval and had different orientation and color features. Subjects were instructed to memorize the orientations of the bar stimuli as well as their temporal order. In the "maintaining period", participants performed a central fixation task by monitoring an abrupt luminance change (within 25% of total trials) and reported if they detected changes after the maintenance period. Finally, in the "recalling period", an instruction cue was presented for 1.5 s to indicate which orientation should be recalled (1st or 2nd or 3nd

orientation), and participants needed to rotate a horizontal white bar to the memorized orientation as precise as possible, without time limit.

Experiment 1–2 (two-item SM with synchronization manipulation)

In each trial, after a 0.5 s fixation period, participants performed a two-item sequence memory task. The 1st orientation was chosen randomly between 0° and 180°, and the 2nd orientation was generated based on the 1st orientation with the tiled angles of ±17°, ±24°, ±38°, ±52°, ±66°, ±80° [46]. The color of the 1st and 2nd bar was different (randomly selected from red, green, or blue in each trial). During the maintenance period, after a 0.6-1 s blank interval, two task-irrelevant discs (3° in radius) that had the color feature of either the 1st or 2nd memorized bar, referred as the 1st memory-related probe (SM1) and 2nd memory-related probe (SM2) respectively, were displayed at the left or right side of the fixation (7° visual angle) for 5 secs. The color (red, green, or blue) and the spatial locations (left or right) of the two probes (SM1 and SM2) were carefully balanced across trials. Importantly, throughout the 5 s maintenance period, the luminance of the two color discs (SM1 and SM2) was continuously modulated according to two 5 s temporal sequences, respectively (i.e., Seq1 for SM1, Seq2 for SM2), ranging from dark (0 cd/m^2) to bright (15 cd/m^2). The Seq1 and Seq2 were generated anew in each trial.

In Experiment 1, the relationship between Seq1 and Seq2 during the maintenance period varied in three ways: Baseline, alpha-band synchronization (A-Sync), or full-spectrum synchronization (F-Sync). For Baseline condition, Seq1 and Seq2 were two independently generated random time series (Seq1≠Seq2). For F-Sync condition, Seq1 and Seq2 were the same random temporal series (Seq1 = Seq2). For A-Sync condition, Seq1 and Seq2 were the same alpha-band (8-11 Hz) time series (Seq1 = Seq2). Experiment 1 had 216 trials in total (72 trials for each of the three synchronization conditions), divided into three blocks. The three synchronization conditions were randomly mixed within each experimental block.

Experiment 2 only examined the Baseline and A-Sync conditions. Experiment 2

had 144 trials in total (72 trials for each of the two synchronization conditions), divided into three blocks. The two synchronization conditions were randomly mixed within each experimental block.

Experiment 2-IC employed the same design as Experiment 2 (2-item SM task; Baseline condition and A-Sync condition), except that the color probes during the delay period had memory-irrelevant colors. Specifically, when the to-be-remembered two bars were blue and red in color, the color probes presented in the delay period would be set to yellow and green, and vice versa.

Experiment 3–4 (3-item SM with synchronization manipulation)

In Experiment 3 and 4, participants performed a three-item sequence memory task. The 1st orientation was chosen randomly between 0° and 180°. The angle difference between the 1st and the 2nd orientations and between the 2nd and the 3rd orientations were set to a value from a list (±12°, ±24°, ±36°, ±48°, ±60°, ±72°, ±84°). During the 5 sec maintenance period, subjects performed a central fixation task, while three discs (3° in radius) that had the color feature of either the 1st, 2nd , or 3rd memorized bar (SM1, SM2, SM3) were displayed at the vertexes (7° in eccentricity) of a regular triangle. Importantly, the luminance of the three color probes (SM1, SM2, SM3) was modulated according to three random temporal series, respectively (i.e., Seq1 for SM1, Seq 2 for SM2, Seq 3 for SM3). The Seq1, Seq2, and Seq3 were generated anew in each trial.

The relationship between Seq1, Seq2, and Seq3 varied in three ways: Baseline, alpha-band synchronization (A-Sync), or full-spectrum synchronization (F-Sync). For Baseline condition, Seq1-3 were three independently generated random time series (Seq1≠Seq2≠Seq3). For F-Sync condition, Seq1-3 were the same random temporal series (Seq1 = Seq2 = Seq3). For A-Sync condition, Seq1-3 were the same alpha-band (8-11 Hz) time series (Seq1 = Seq2= Seq3). Notably, considering the large number of trials that would be required to include all the three synchronization conditions for a three-item SM task in a repeated design, we employed a two-sample two-way

repeated design. Specifically, Experiment 3 assessed Baseline and A-Sync conditions, and Experiment 4 tested Baseline and F-Sync conditions, and each experiment used different subjects. Both Experiment 3 and Experiment 4 had 216 trials in total (108 trials for each of the two synchronization conditions), divided into four blocks. We then assessed the behavioral results using a two-sample (Experiment 3 and 4) two-way (Position*Sync) repeated ANOVA analysis to examine the overall manipulation effect.

Experiment 5–7 (2-item SM with temporal order manipulation)

Experiment 5–7 employed the same 2-item SM paradigm as Experiment 1–2, except that the temporal relationship between Seq1 and Seq2 during the maintenance period were manipulated in a different manner. Specifically, Seq1 was a randomly generated temporal sequence, and Seq2 was a temporal shifted version of Seq1. Specifically, Seq1 either leads or lags Seq2 by 200 ms, corresponding to the “same” and “reversed” conditions, respectively, compared to the stimulus sequence. Furthermore, in order to replicate findings of Experiment 1-2, Baseline and F-Sync conditions were added to Experiment 5 and 6, respectively. Experiment 5 included Same, Reversed, and Baseline conditions, and Experiment 6 included Same, Reversed, and F-Sync conditions. We then assessed the behavioral results using a two-sample (Experiment 5 and 6) two-way (Position*Sync) repeated ANOVA analysis to examine the overall manipulation effect. Each condition had 72 trials, resulting in 216 trials for Experiment 5 and 6, respectively.

Experiment 7 only examined Same and Reversed conditions. Moreover, rather than 200 ms lag in Experiment 5-6, the Seq1-Seq2 temporal lag was set to 500 ms in Experiment 7. Each condition had 72 trials, resulting in 144 trials for Experiment 7.

Data analysis

A mixture-modelling analysis[17] was used to quantify the SM behavioral performance for each of the item in the SM, by quantifying the Von Mises distribution variability

of the reported error. The three-parameter mixture model calculates the precision of the reports, as well as the respective proportions of target, distractor and guess responses. In the present study, we used the target probabilities (see the precision estimation results in Supplementary Materials) which showed a more consistent trend across all the experiments. Moreover, the target probability was further normalized by using an arcsine square root transformation[18]. The normalized target probabilities were further used to examine the effect of temporal manipulation on SM.

The Meta-analysis were performed by using Comprehensive Meta-Analysis Software (CMA).

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Figures

Figure 1

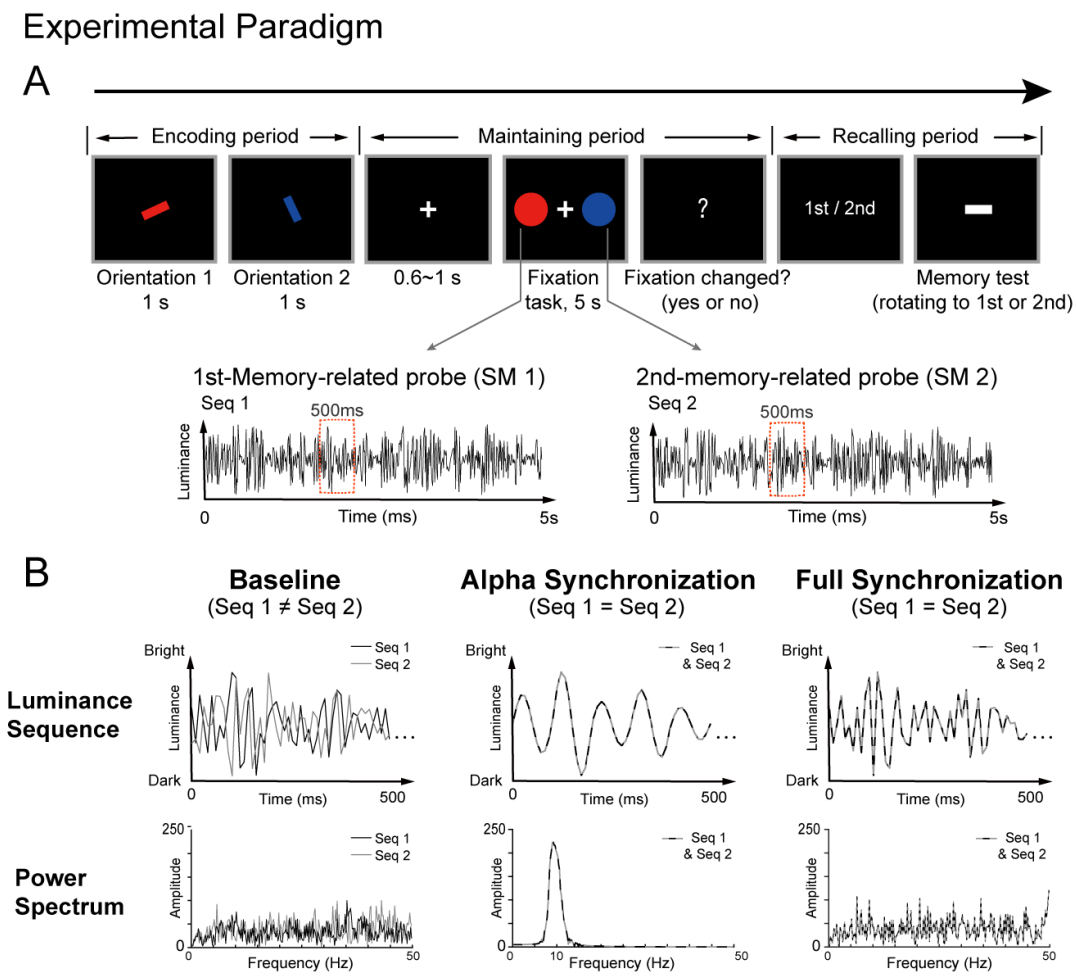


Figure 1

"Behavioral temporal interference" approach: synchronization during memory retention

(A) Two-item SM experimental paradigm. In each trial, in the "encoding period", two bar stimuli (item 1, item 2) were presented serially at the center. Subjects needed to memorize the orientations of the two bars and their temporal order (1st and 2nd orientation). In the "recalling period", participants rotated a horizontal bar to the memorized orientation based on the instruction (recalling the 1st or the 2nd orientation). During the 5 s "maintaining period", subjects performed a central fixation task, while two discs that had color feature of either the 1st or the 2nd memorized bars (1st

memory-related probe, SM1; 2nd memory-related probe, SM2) were displayed at left or right side of the fixation. The color (red, blue, or green) and the spatial locations (left or right) of the two probes (SM1 and SM2) were balanced across trials. Throughout the 5 s retention period, the luminance of the two color discs (SM1 and SM2) was continuously modulated according to two 5 sec temporal sequences respectively (Seq1 for SM1, Seq2 for SM2). (B) Temporal synchronization of SM1 and SM2 during the maintaining period. Upper: Illustration of 500 ms segment of the 5 s Seq1 (denoted in dark line) and Seq2 (denoted grey line). Lower: Spectrum of the 5 s Seq1 (dark) and Seq2 (grey). The temporal relationship between Seq1 (black) and Seq2 (grey) varied in one of the three ways (left: Baseline; Middle: A-Sync; Right: F-Sync) during memory retention. In Baseline condition (Left), Seq1 and Seq2 were two independently generated random time series. In A-Sync condition (Middle), Seq1 and Seq2 shared the same alpha-band (8-11 Hz) time series. In F-Sync condition (Right), Seq1 and Seq2 were the same random time series. Note that Seq1 and Seq2 were generated anew in each trial.

Figure 2

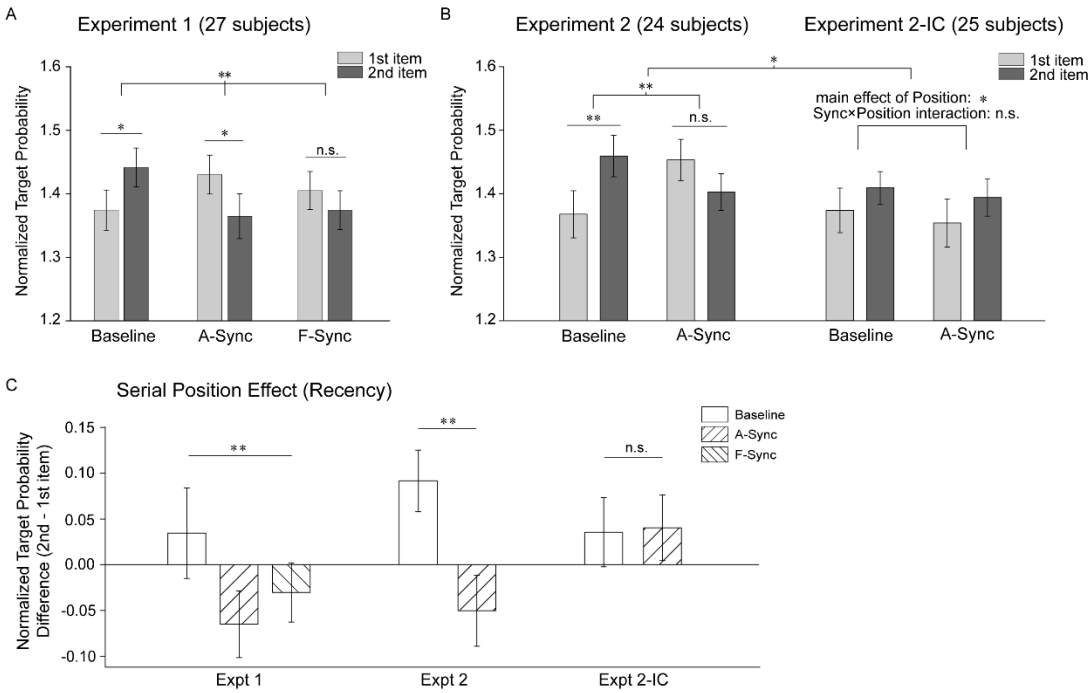


Figure 2

Temporal synchronization disrupts recency effect in 2-item SM

A probabilistic mixture model was used to quantify the memory performance for the 1st and the 2nd item. The target probability results were further converted using an arcsine square root transformation to make it normally distributed. (A) Experiment 1 results (N = 27). Grand averaged (mean±SEM) normalized target probability for the 1st (light grey) and 2nd (dark grey) orientation for Baseline, A-Sync, and F-Sync conditions. (B) Experiment 2 (N = 24) and Experiment 2-IC (irrelevant color probes) (N = 25) results. Grand averaged (mean±SEM) normalized target probability for the 1st (light grey) and 2nd (dark grey) orientation for Baseline and A-Sync conditions, in Experiment 2 (left) and Experiment 2-IC (right). (C) Grand averaged (mean±SEM) recency effect (2nd -1st) for Baseline (white box), A-Sync (rightward checked box), and F-Sync (leftward checked box) conditions in Experiment 1, Experiment 2, and Experiment 2-IC. Noting the significant synchronization-induced recency disruption in Experiment 1 and 2, but not in Experiment 2-IC. (**: p<=0.05, *: p<=0.1).

Figure 3

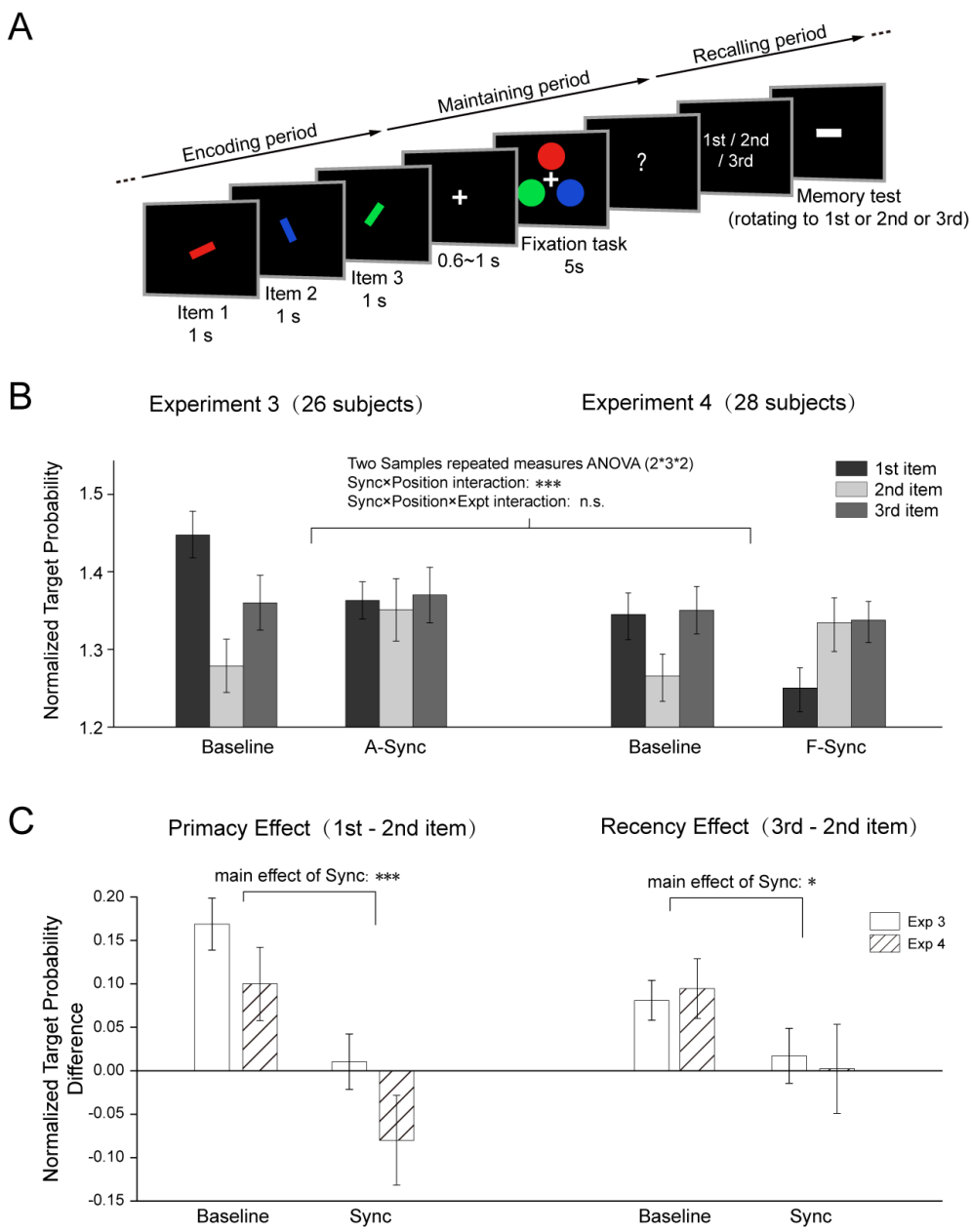


Figure 3

Temporal synchronization disrupts recency and primacy effect in 3-item SM

(A) Three-item SM experimental paradigm. In each trial, in the "encoding period", three bar stimuli (item 1, item 2, item 3) were presented serially at the center. Subjects needed to memorize the orientations of the three bars and their temporal order (1st, 2nd, or the 3nd orientation). In the "recalling period", participants rotated a horizontal bar

to the memorized orientation based on the instruction (recalling the 1st, the 2nd , or the 3nd orientation). During the 5 s “maintaining period”, subjects performed a central fixation task, while three discs (SM1, SM2, SM3) that had color feature of 1st, 2nd, or the 3nd memorized bars were presented. Again, throughout the 5 s retention period, the luminance of the three color discs was modulated according to three 5 sec temporal sequences respectively (Seq1 for SM1, Seq2 for SM2, Seq3 for SM3), the relationship of which varied in three ways (Baseline, A-Sync, F-Sync). Noting that two separate subject groups participated in Experiment 3 (Baseline and A-Sync conditions) and Experiment 4 (Baseline and F-Sync conditions) respectively. (B) Left: Experiment 3 results (N = 26). Grand averaged (mean±SEM) normalized target probability for the 1st (black), 2nd (light grey), and 3nd (dark grey) orientation for Baseline and A-Sync conditions. Right: Experiment 4 results (N = 28). Grand averaged (mean±SEM) normalized target probability for the 1st (black), 2nd (light grey), and 3nd (dark grey) orientation for Baseline and F-Sync conditions. A two-sample two-way repeated ANOVA was performed across Experiment 3 and 4. (C) Grand averaged (mean±SEM) primacy effect (1st - 2nd, white box) and recency effect (3nd -2nd, checked box) for Baseline and Synchronization (A-Sync and F-Sync) conditions in Experiment 3 and Experiment 4. Noting the synchronization-induced disruption in both primary and recency effect in Experiment 3 and 4. (**: p<=0.05, *: p<=0.1).

Figure 4

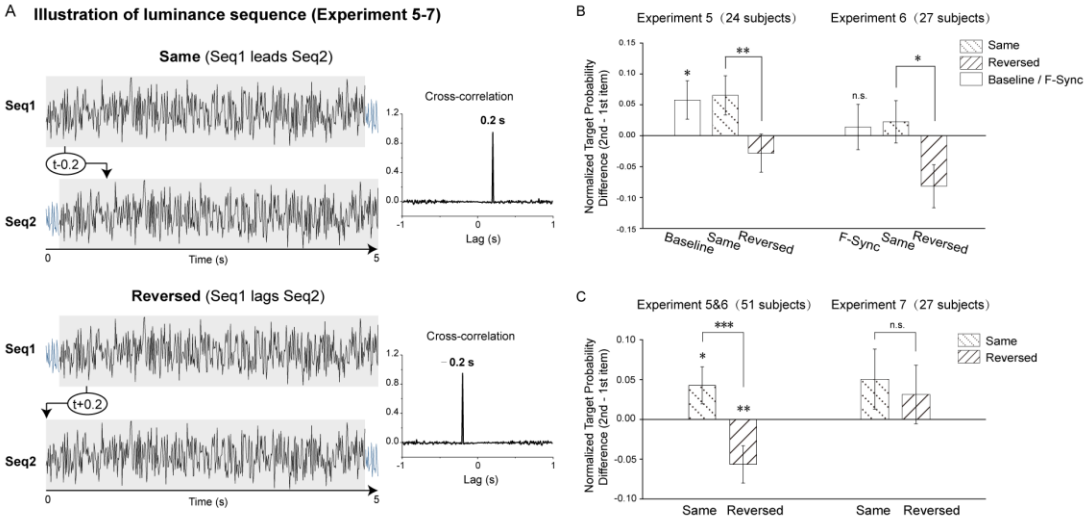


Figure 4

Reversing replay order disrupts serial position effect (Experiment 5-7)

(A) Experiment 5-7 (two-item SM task) employed the same experimental paradigm as Experiment 1 (see Figure 1A), except that the luminance sequences (Seq1 and Seq2) were modulated differently. Specifically, Seq1 was a temporally shifted version of Seq2, either leading (“Same”, upper panel) or lagging Seq2 (“Reversed”, lower panel) by 200 ms. Right: the Seq1-Seq2 cross-correlation coefficients as a function of temporal lag (-1 to 1 s). The Same condition (Seq1 leads Seq2) showed a cross-correlation at positive temporal lag of 200 ms, and the Reversed condition (Seq1 lags Seq2) showed the cross-correlation at -200 ms. (B) Two separate subject groups participated in Experiment 5 (N=24; Baseline, Same, Reversed) and Experiment 6 (N=27; F-Sync, Same, Reversed) respectively. Grand averaged (mean±SEM) recency effect (2nd -1st) for all conditions in Experiment 5 and Experiment 6. Noting the significant decrease in recency effect for Reversed than Same conditions in both experiments. A two-sample two-way repeated ANOVA was performed across Experiment 5 and 6. (C) Left: pooled recency effects (N=51; mean±SEM) for Same and Reversed conditions by combining Experient 5 and 6. Right: Grand average recency effects (N= 27; mean±SEM) for Same and Reversed conditions in Experiment 7 (500 ms temporal shift). (***: p<=0.01, **: p<=0.05, *: p<=0.1.)

Figure 5

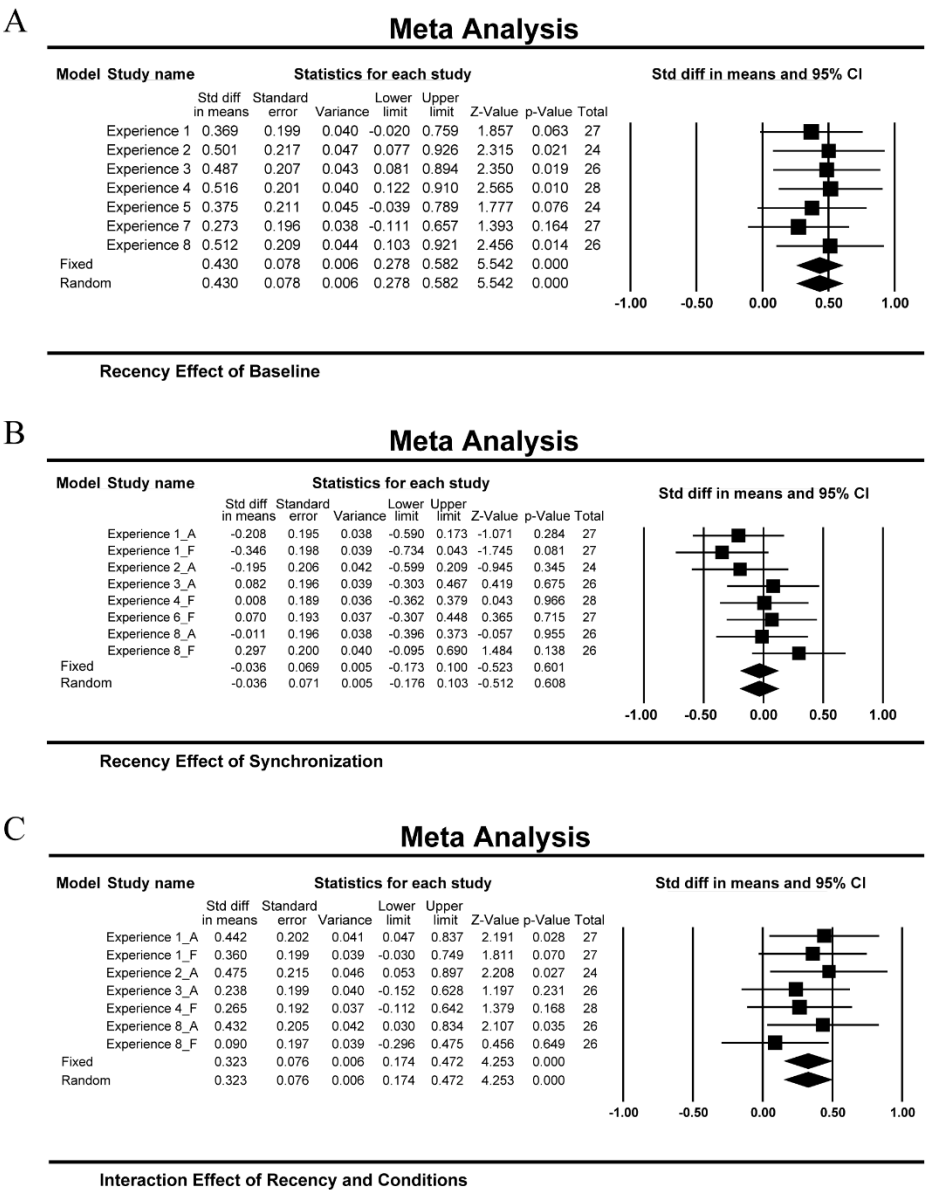


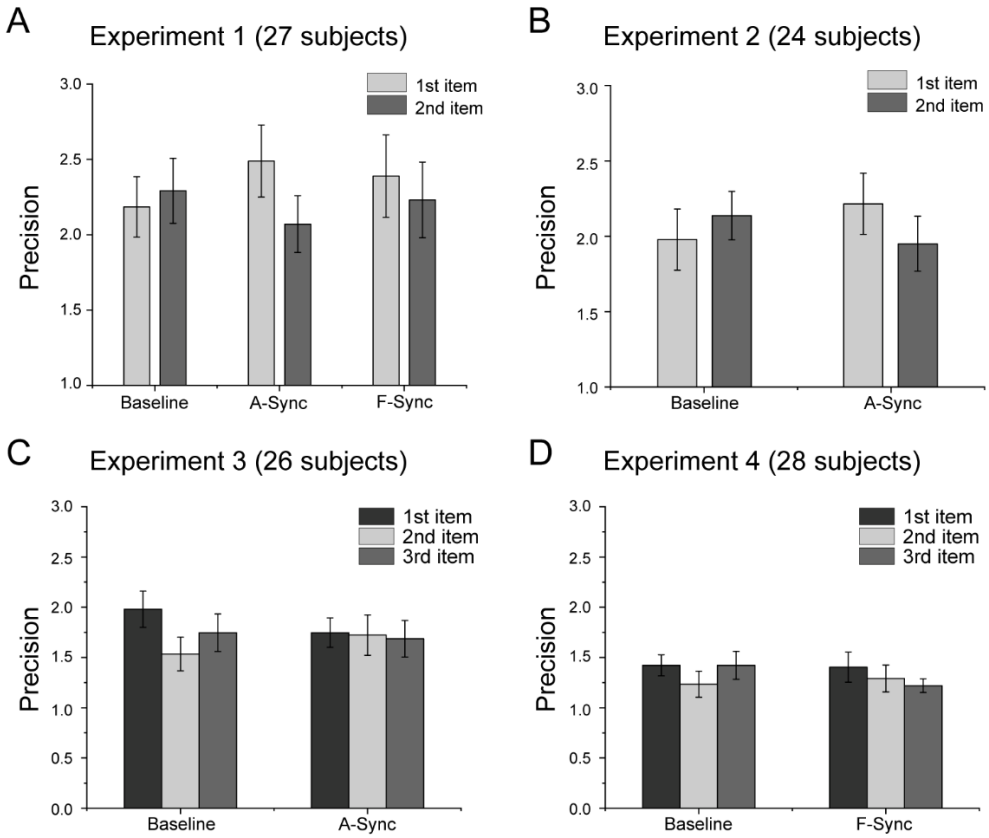
Figure 5

Meta-analysis across experiments

Meta-analysis (Comprehensive Meta-Analysis Software, CMA) on recency effect (given its presence for both 2-item and 3-item SM tasks) across all the 8 experiments (Expt. 1-7 and the spatial experiment). (A) Significant recency effect for Baseline condition (across Experiment 1, 2, 3, 4, 5, 7, 8). (B) Non-significant recency effect for Synchronization condition (across Experiment 1, 2, 3, 4, 6, 8). (C) Significant

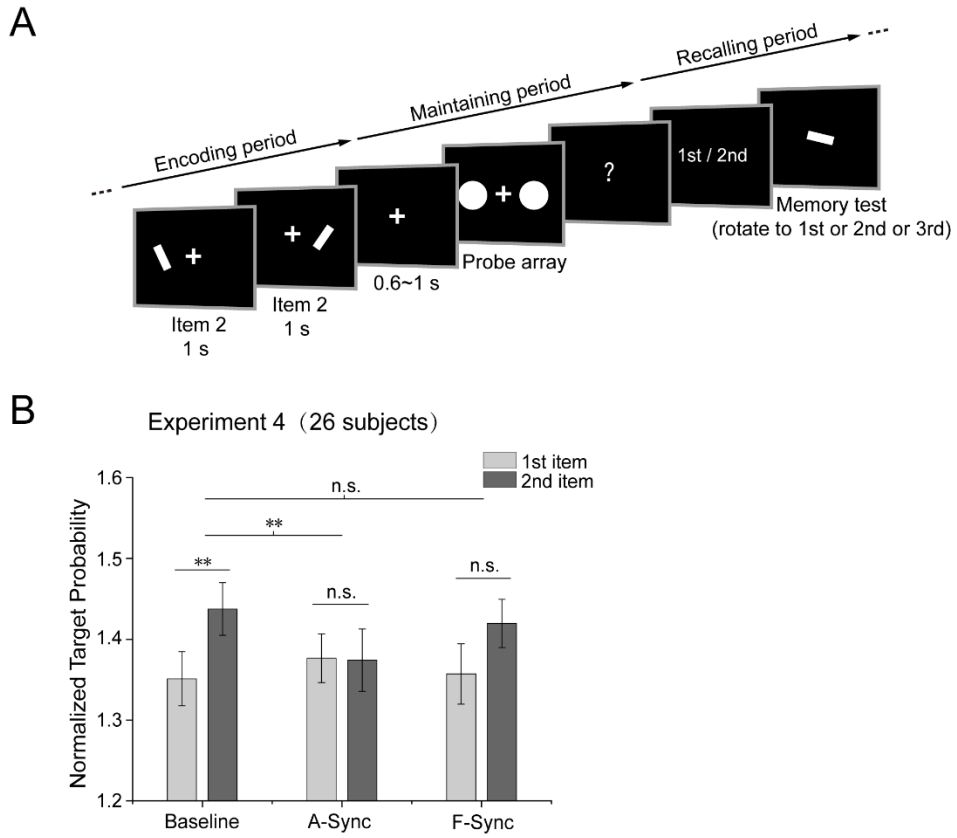
difference in recency effect between Baseline and Synchronization conditions (across Experiment 1, 2, 3, 4, 8).

Supplementary Figure 1



Precision results (Experiment 1-4)

Supplementary Figure 2

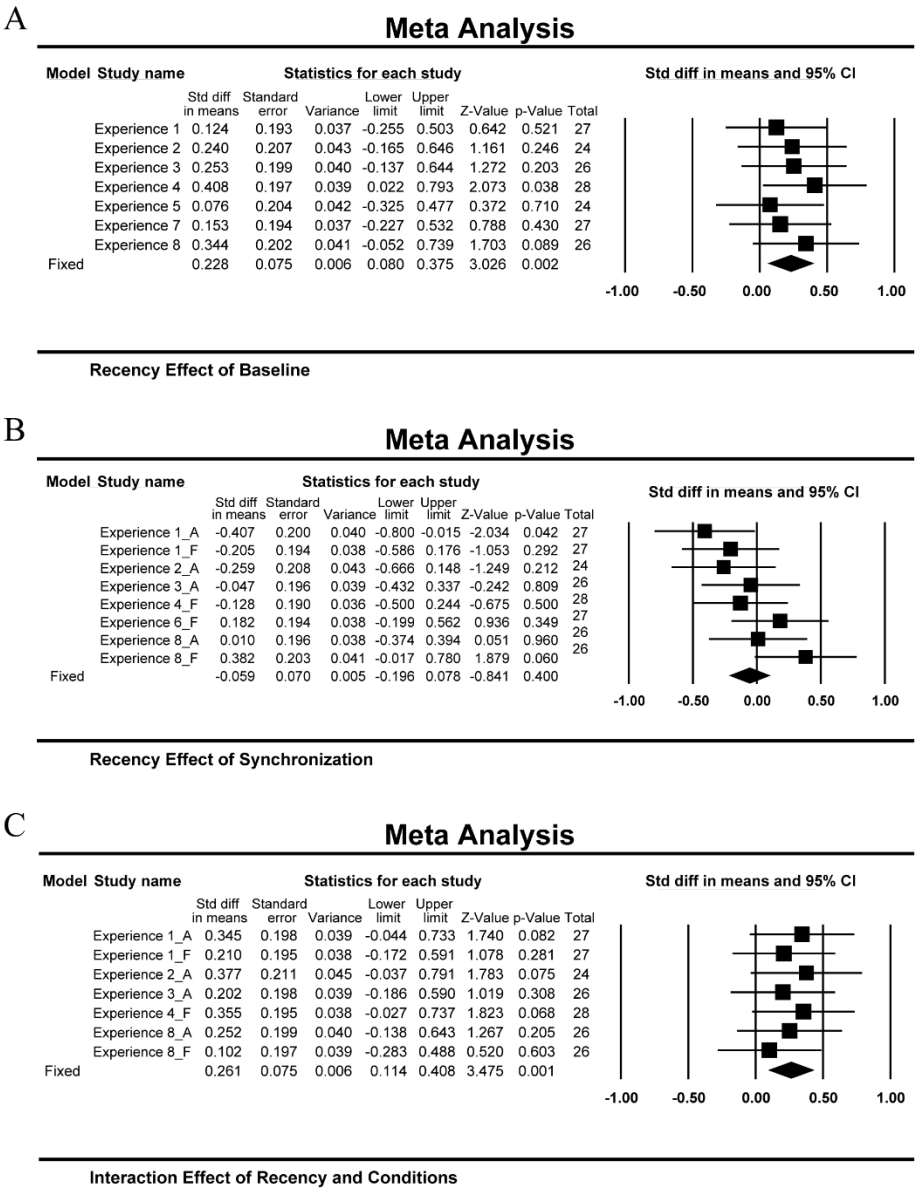


Temporal synchronization of spatial locations (Experiment 8)

(A) Twenty-six subjects participated in Experiment 8. In each trial, during the "Encoding period", two white bar stimuli were presented at two different spatial locations serially, and subjects needed to memorize the orientations of two bar stimuli as well as their temporal order (two-item sequence memory). During the "maintaining period", subjects performed a central fixation task, while at the same time, two white discs were displayed at the spatial locations of either the 1st or the 2nd memorized bars (SM1, SM2). Similar to previous manipulation, the luminance of the two discs were either nonrelated with each other (Baseline) or shared the same time series (A-Sync and F-Sync), and all the luminance sequences were generated anew in each trial. (B) Grand average (mean ± SEM) memory performance for the 1st (grey) and 2nd (black) items in the sequence memory for Baseline, A-Sync, and F-Sync conditions. Temporal synchronization of spatial locations showed a similar trend of synchronization-induced decreased recency effect, but not as strong as

manipulation on color probes. The baseline condition showed typical recency effect (paired t-test, $t_{(25)} = -2.613$, $p = 0.015$, Cohen's d = -0.513), whereas the synchronization conditions (A-Sync and F-Sync) did not (paired t-test; A-Sync: $t_{(25)} = 0.057$, $p = 0.955$, Cohen's d = 0.011; F-Sync: $t_{(25)} = -1.518$, $p = 0.142$, Cohen's d = -0.298). The interaction effect did not reach significance (two-way repeated ANOVA, interaction effect: $F_{(2,50)} = 1.515$, $p = 0.230$, $\eta_p^2 = 0.057$; position main effect: $F_{(1,25)} = 3.787$, $p = 0.063$, $\eta_p^2 = 0.132$; sync condition main effect: $F_{(2,50)} = 0.207$, $p = 0.813$, $\eta_p^2 = 0.008$). (***: p<=0.01, **: p<=0.05, *: p<=0.1.)

Supplementary Figure 3



Meta-analysis on precision results (Experiment 1-8)

Meta-analysis (Comprehensive Meta-Analysis Software, CMA) on recency effect estimated by precision results across all the 8 experiments (Expt. 1-7 and the spatial experiment). Noting the similar synchronization-induced recency disruption effect. (A) Significant recency effect for Baseline condition (across Experiment 1, 2, 3, 4, 5, 7, 8). (B) Non-significant recency effect for Synchronization condition (across Experiment 1, 2, 3, 4, 6, 8). (C) Significant difference in recency effect between Baseline and Synchronization conditions (across Experiment 1, 2, 3, 4, 8).