Differential processing of immediately repeated verbal and non-verbal stimuli: an evoked-potential study

MANUEL, Aurélie L., SCHNIDER, Armin

Abstract

Stimuli are better retained in memory if they are repeated after a delay than if they are immediately repeated. This effect is called the spacing effect. Recent EEG studies showed that delayed repetition of meaningful designs in a continuous recognition task induces an evoked response very similar to new presentations. In contrast, immediately repeated designs induced circumscribed, stronger activation of the left medial temporal lobe (MTL) at 200-300ms. In amnesic subjects, this signal was missing, indicating that it has a memory-protective effect. Here, we used high-density EEG in humans to explore whether meaningless verbal (non-words) and non-verbal (meaningless geometric designs) stimuli also have a spacing effect associated with such lateralized, temporally limited activation of the left MTL upon immediate repetition. Our results revealed a spacing effect for both materials. Timing and localization of brain activity differed as a function of stimulus material. Specific responses to immediate repetitions occurred at 200-285ms for non-verbal stimuli and at 285-380ms for verbal material. Source estimations revealed [...]
Differential processing of immediately repeated verbal and non-verbal stimuli: an evoked-potential study

Aurélie L. Manuel, PhD 1, Armin Schnider, MD 1

1 Laboratory of Cognitive Neurorehabilitation, Division of Neurorehabilitation, Department of Clinical Neurosciences, University of Geneva and University Hospital of Geneva, Switzerland

Abbreviated title: Spacing effect

Correspondence:
Dr. Aurélie Manuel
Service de Neurorééducation
Hôpitaux Universitaires de Genève
26, av. de Beau-Séjour
CH-1211 Geneva 14 / Switzerland
Tel: +41-22-372 3647; Fax: +41-22-372 3705; e-mail: aurelie.manuelstocker@hcuge.ch

Keywords:
Episodic memory, consolidation, EEG, spatio-temporal analysis, source localization
Abstract

Stimuli are better retained in memory if they are repeated after a delay than if they are immediately repeated. This effect is called the spacing effect. Recent EEG studies showed that delayed repetition of meaningful designs in a continuous recognition task induces an evoked response very similar to new presentations. In contrast, immediately repeated designs induced circumscribed, stronger activation of the left medial temporal lobe (MTL) at 200-300ms. In amnesic subjects, this signal was missing, indicating that it has a memory-protective effect. Here, we used high-density EEG in humans to explore whether meaningless verbal (non-words) and non-verbal (meaningless geometric designs) stimuli also have a spacing effect associated with such lateralized, temporally limited activation of the left MTL upon immediate repetition. Our results revealed a spacing effect for both materials. Timing and localization of brain activity differed as a function of stimulus material. Specific responses to immediate repetitions occurred at 200-285ms for non-verbal stimuli and at 285-380ms for verbal material. Source estimations revealed increased activity in right inferior frontal areas for immediate non-verbal repetitions and in left fronto-parietal areas for immediate verbal repetition in comparison to new presentations. These findings show that, while the spacing effect is a ubiquitous phenomenon, the neural processes underlying it vary according to the type of stimulus material.

Introduction

Information repeated after some intervening items (spaced learning) is usually better remembered than information repeated immediately (massed learning). This phenomenon, already described by Ebbinghaus (1885/1992), is known as the spacing effect (SE). It has been shown to enhance memory in recognition and free recall paradigms, with intentional or incidental learning and with meaningful or meaningless verbal or non-verbal materials (Challis, 1993; Mammarella et al., 2002; Russo & Mammarella, 2002; Russo et al., 2002). Most electrophysiological studies focused on differences in processing new vs old stimuli (Mecklinger, 2002).
This article is protected by copyright. All rights reserved.

2000; Rugg & Curran, 2007) or immediate vs delayed repetitions (Nielsen-Bohlman & Knight, 1994; Chao et al., 1995; Kim et al., 2001; Kim et al., 2008) without considering the impact on long-term retention and measuring the spacing effect. The neural correlates distinguishing spaced from massed learning have rarely been examined. EEG studies showed that immediate repetition of words or pictures was associated with an increased early P300 and N400 compared to spaced repetitions (Kim et al., 2001; Henson et al., 2004; Van Strien et al., 2007). However, the brain sources active during immediate repetition varied among studies between left and right temporal, parietal, occipital and cingulate cortex (Kim et al., 2008; James et al., 2009; Zhao et al., 2015).

fMRI and PET studies on episodic memory demonstrated left prefrontal (PFC) and medio-temporal (MTL) activation when encoding verbal stimuli (words, non-words) and a right-lateralization for non-verbal material (non-famous faces, pictures) (Kelley et al., 1998; Wagner et al., 1998). The type of processing also influences lateralization: the left PFC appears critical for encoding, the right PFC for retrieval, both non-verbal (Tulving et al., 1994) and verbal material (Shallice et al., 1994).

Recent high-resolution EEG studies reported left MTL activation at 200-300ms, inducing a frontal positivity, when meaningful pictures were immediately repeated in a continuous recognition task (James et al., 2009). Thirty minutes later, these pictures were less well recognized than pictures repeated after nine intervening items, compatible with the spacing effect (James et al., 2009; Nahum et al., 2011; Nahum et al., 2014). The left MTL provenance of this signal was shown with source estimations (James et al., 2009; Nahum et al., 2014) and confirmed with depth electrode (Nahum et al., 2011). It is unclear whether this lateralized, temporally limited activation of the left MTL is specific for the processing of meaningful designs or whether it constitutes a general processing step of immediately repeated stimuli. Thus, would it also occur upon immediate repetition of meaningless verbal (non-words) and non-verbal (geometric designs) information?
To address this question, we used a continuous recognition paradigm with meaningless verbal and non-verbal stimuli which were immediately repeated or after intervening items. Brain activity was recorded using high-density evoked potentials undergoing waveform, topographic and source analyses. We hypothesized a material-specific lateralization of electrocortical responses to immediate repetitions around 200-300ms.

Methods

Participants

Eighteen healthy, French-speaking, right-handed, participants aged 23 ± 3.2 years (18–29 years; eight men) participated in the study. Participants provided written, informed consent and were paid to participate. No participant had a history of neurological or psychiatric illness. All procedures were approved by the Ethics Committee of the University Hospital of Geneva and conducted according to the Declaration of Helsinki.

Stimuli and Procedure

Stimuli

Stimuli were 8.7*5.3cm non-words (verbal material) and meaningless designs (non-verbal material). Non-words were meaningless five-letter-words created from French words (www.lexique.org). Non-verbal items were black and white meaningless geometric designs constructed with a program created to generate random geometric figures. We ensured that all stimuli were composed of at least 4 elements displayed throughout the frame and touching the 4 borders of a 8.7x5.3cm frame. To assess whether there were differences in visual complexity of these geometric designs, we asked a control group of 11 participants to rate visual complexity with a 7-point scale ranging from "very simple" to "very complex". Mean visual complexity rating
was 3.56 ± 0.78 for the learning task block 1, 3.70 ± 0.93 for block 2 and 3.61 ± 1.03 for the delayed recognition task. There were no statistical differences between visual complexity for the items presented in the first block, the second block and the delayed recognition task (F(2,209) = 0.312, p = 0.73). None of the participants reported using verbal strategies to memorize meaningless non-verbal items.

**Learning task**

Participants performed two blocks of a continuous recognition task (Figure 1A). Each block was composed of 140 visual stimuli representing meaningless geometric designs (Non-verbal items, NV, N=70) and non-words (Verbal items, V, N=70). All the stimuli were repeated either immediately following the initial stimulation (One-back items, N=70, of which 35 were verbal, 35 non-verbal) or repeated after nine intervening items (Ten-back items, N=70, of which 35 were verbal, 35 non-verbal). The procedure was the same for the second block but with other non-verbal and verbal stimuli. Overall, there were 140 trials for the first presentation of non-verbal stimuli (NewNV), 140 trials for the first presentation of verbal stimuli (NewV), and 70 trials per condition for the repetitions; i.e. immediate repetition of non-verbal stimuli (One-backNV), immediate repetition of verbal stimuli (One-backV), delayed repetition of non-verbal stimuli (Ten-backNV) and delayed repetition of verbal stimuli (Ten-backV).

Stimuli were presented for 1000 ms on a 17-inch monitor, positioned at eye-level and at 70 cm viewing distance from the participant (visual angle ~ 2°). There was a fixed inter-stimulus interval of 2000 ms filled with a fixation cross. Participants had to indicate picture recurrences by pressing the response box button with the right index finger and new pictures by pressing the response box button with the right middle finger. The two blocks were performed consecutively, separated by a 2 min break. Each block lasted about 14 minutes.
**Delayed recognition task**

Thirty minutes after the completion of the learning task, participants performed a delayed recognition task to test for long-term retention of items learned during the learning task and test the presence of a spacing effect (Figure 1B). The delayed recognition task comprised all the 280 stimuli from the learning task plus 140 new items (70 new meaningless designs, \(p_{\text{NewNV}}\); 70 new non-words, \(p_{\text{NewV}}\)) presented in a random order and lasted 20 minutes. Participants had to indicate pictures that had appeared in the learning task.

The Spacing Effect index (SE), which reflects the advantage for remembering stimuli previously presented with a delay compared to those presented in immediate repetitions, was calculated for each material as follows: “\(\%\text{Hits Ten-back} - \%\text{Hits One-back}\)”, where \(\%\text{Hits Ten-back}\) describes the percentage of correct recognitions in the delayed recognition task of items that had been previously (\(p\)) repeated after nine intervening items in the learning task (\(p_{\text{Ten}}\); \(p_{\text{TenNV}}\), \(p_{\text{TenV}}\)); \(\%\text{Hits One-back}\) describes the percentage of correct recognitions in the delayed recognition task of items that had been previously repeated in immediate succession in the learning task (\(p_{\text{One}}\); \(p_{\text{OneNV}}\), \(p_{\text{OneV}}\)). To statistically assess the advantage for remembering \(p_{\text{Ten}}\) over \(p_{\text{One}}\), paired t-tests comparing \(p_{\text{TenNV}}\) vs \(p_{\text{OneNV}}\) and \(p_{\text{TenV}}\) vs \(p_{\text{OneV}}\) were performed.

Following the delayed recognition task, participants were asked to judge task difficulty and their confidence in judgment for both tasks using 10-point scale.

**Statistical analyses**

Percentage of correct responses and response times were analyzed with 2 x 3 repeated measures ANOVAs using Material (V, NV) and Condition (New, One-back, Ten-back) as the within subjects factors. Where significant interactions were found, follow-up one-way ANOVA
were conducted and when appropriate, post-hoc analyses with Fisher's least significant difference were performed. The same procedure was applied for repeated measure ANOVAs on the EEG and source estimations analyses. When necessary, Greenhouse-Geisser correction was used in cases of violation of sphericity. Effect sizes are reported with the partial eta square ($\eta^2$).

**EEG acquisition and pre-processing**

Continuous electroencephalography (EEG) was recorded during the learning task at 500Hz using a 156-channel Brainvision PyCorder system with actiCAP active electrode (Brain Products GmbH, Germany). Electrode impedance was kept low (<25 kΩ). EEG data pre-processing and analyses were performed using the Cartool software (https://sites.google.com/site/fbmlab/cartool) developed by Bruenet et al. (2011). Event-related potentials (ERPs) were computed by averaging EEG epochs from -100 to 800 ms post-stimulus onset for each participant and condition. Data were band-pass filtered (0.18-30 Hz), baseline corrected using the 100ms pre-stimulus period and recalculated against the average reference. In addition to an automatic ±80 μV artifact rejection criterion, EEG epochs containing eye blinks or other noise transients were removed by trial-to-trial inspection of the data. Only correct trials were retained for the analyses. Prior to group averaging, data at artifact electrodes from each participant were interpolated using 3D splines (mean 3.6% interpolated electrodes) (Perrin et al, 1987) and EEG epochs were averaged as a function of Condition (New, One-back, Ten-back) and Material (NV, V). The average number (±SD) of accepted epochs was 50±12 for the NewNV, 48±14 for the NewV, 50±13 for the One-backNV, 50±15 for the One-backV, 47±16 for Ten-backNV and 46±17 for Ten-backV. 2 x 3 repeated measure ANOVA on these values indicate no statistical differences (p > 0.05).
**EEG analyses and source estimation**

**General Analysis Strategy**

Electrical neuroimaging analyses were applied to ERPs to test the effect of stimulus material on memory processing (Murray et al., 2008; Michel, 2009; Brunet et al., 2011; Koenig et al., 2011; Tzovara et al., 2012). In addition to voltage waveform analyses, we performed topographic analyses on global dissimilarity and topographic clustering. The major benefit of analyzing the configuration of the electric field at scalp, i.e. topography, is the ability to circumvent interpretational issues attributable to the reference-dependent nature of ERPs (Michel et al., 2004). Therefore, topographic analyses are not biased by a priori hypotheses about electrode location or periods at which effects might be expected (Tzovara et al., 2012). Topographic analyses are useful in terms of neurophysiologic interpretation because changes in the topography of the electric field necessarily follows from changes in the configuration of the brain’s underlying active sources; they can, therefore, directly be interpreted as the engagement of distinct brain networks (Lehmann & Skrandies, 1980; Murray et al., 2008).

**Waveform Analyses**

We first computed an electrode- and time-wise 2 x 3 ANOVA on the ERP waveform for each of the 156 electrodes with the factors Material (V, NV) and Condition (New, One-back, Ten-back). This statistical analysis was conducted using the Statistical Toolbox for Electrical Neuroimaging (STEN) developed by Jean-François Knebel (http://www.unil.ch/line/en/home/menuinst/about-the-line/software--analysis-tools.html#standard_412). Temporal autocorrelation was controlled for by considering only effects meeting the statistical threshold of p < 0.05 for more than 10 contiguous data sampling points (i.e. ke 20 ms). We also applied a spatial criterion of at least 8 electrodes (5% of the...
electrode montage) for the voltage waveform ERP analysis (Guthrie & Buchwald, 1991; Murray et al., 2006; Toepel et al., 2014).

**Topographic Patterns Analyses**

A time-wise 2 x 3 topographic ANOVA (TANOVA) on global dissimilarity measures (DISS, Lehmann & Skrandies, 1980) was performed to identify statistical differences between the electric fields independently of their strength. This analysis is based on a non-parametric randomization procedure (5000 randomizations per time point) and is implemented in the RAGU software (Koenig et al., 2011).

To further determine periods of stable ERP topography that differed between conditions, we submitted the group-averaged ERPs to a topographic clustering based on a modified hierarchical cluster analysis (Topographic Atomize and Agglomerate Hierarchical Clustering, Murray et al., 2008; Brunet et al., 2011). This analysis is based on the evidence that the ERP topography does not vary randomly across time, but remains quasi-stable over 20–100ms functional microstates before rapidly switching to another period of stable topography. As a result, this analysis summarizes ERP data into a limited number of topographic maps and identifies time periods during which conditions elicit different electric field configurations (Lehmann & Skrandies, 1980; Murray et al., 2008; Michel, 2009). The optimal number of clusters to describe the dataset is determined by cross validation and the Krzanowski-Lai criterion (Pascual-Marqui et al., 1995). Periods over which different topographic maps explained the data at the group-averaged level were then statistically evaluated using single subject data. This fitting procedure calculated the spatial correlation between these "template" maps and each single subject ERP and labeled the map accordingly. The output is a measure of global explained variance (GEV), which indicates how well a given template map accounts for a given experimental condition over a specific time interval (Murray et al., 2008; Michel, 2009). These values were then submitted to
repeated measure ANOVAs with factors of condition (New, One-back, Ten-back), material (NV, V) and topographic map. Because topographic changes necessarily follow from changes in the configuration of the brain's underlying active sources, this analysis is useful to identify stable periods over which neural sources differ (Lehmann & Skrandies, 1980; Murray et al., 2008).

**Electrical source estimations**

We estimated the sources in the brain using a distributed linear inverse solution and the local autoregressive average (LAURA, Grave de Peralta Menendez et al., 2001; Grave-de Peralta et al., 2004). The solution space is based on a realistic head model and comprises 4147 nodes homogeneously distributed within the gray matter of the average brain of the Montreal Neurological Institute. LAURA provides a current density value (µA/mm3) for each node. The time periods for which intracranial sources were estimated and statistically compared between conditions was defined by the results of the topographic cluster analysis. Statistics on intracranial source estimations were performed by first averaging the signal over the period of interest (i.e. period of topographic modulation) to generate one single data point per participant and increase the signal to noise ratio. Then sources were statistically compared at each node with a 2 x 3 repeated measures ANOVA. Only periods for which p<0.05 with clusters of at least <15 nodes were retained (see Toepel et al., 2014, for a similar procedure).
Results

Behavioral results

Learning task

Participants performed well on this task, with differences between the conditions (Table 1A). Immediate repetitions (One-back) were processed more accurately ($F_{(2,34)} = 8.52$, $p = 0.001$, $\eta^2_p = 0.334$) and more rapidly ($F_{(2,34)} = 53.14$, $p < 0.001$, $\eta^2_p = 0.758$) than new items or delayed repetitions (Ten-back) items. There were no differences in response time between verbal and non-verbal items ($F_{(1,17)} = 1.05$, $p = 0.318$, $\eta^2_p = 0.059$). Non-verbal items were processed less accurately than verbal items ($F_{(1,17)} = 5.84$, $p = 0.027$, $\eta^2_p = 0.256$).

Delayed recognition task

In the delayed recognition task performed 30 minutes after the learning task, participants recognized new items more accurately ($F_{(2,34)} = 8.47$, $p = 0.009$, $\eta^2_p = 0.333$) than stimuli previously presented in immediate succession (pOne) and those presented with a delay (pTen; Table 1B). Our results indicate the presence of a spacing effect: Participants were more accurate at detecting pTenNV than pOneNV ($t_{(17)} = -2.99$, $p = 0.008$) and pTenV than pOneV ($t_{(17)} = -3.14$, $p = 0.006$). The spacing effect index ($\%$Hits Ten-back $- \%$Hits One-back) was 4.62 for verbal material and 6.48 for the non-verbal material. These values did not statistically differ ($p = 0.47$).

The delayed recognition task was perceived as more difficult ($t(16)=2.78$, $p < 0.01$) and resulted in less confidence in judgment than for the learning task ($t(16)=3.39$, $p < 0.01$; data from one participant is missing).

Accuracy was greater for the verbal stimuli than for the non-verbal stimuli ($F_{(1,17)} = 8.76$, $p = 0.009$, $\eta^2_p = 0.340$). There were no differences between conditions or materials for the response time ($p \geq 0.2$).

This article is protected by copyright. All rights reserved.
EEG results

Waveform analysis

An examplar waveform is shown in Figure 2A for electrode Fz. The time-wise 2 x 3 ANOVA on ERP waveforms revealed statistically significant interactions between material and condition (p<0.05, >20 consecutive milliseconds) at 234-343ms (with two periods slightly overlapping), 476-546ms and 566-628ms (Figure 2B). Furthermore, we observed a main effect of condition at 144-198ms, 208-326ms, 342-676ms and 702-800ms and a main effect of material starting slightly earlier, around 82-152ms, 180-520ms and from 746-800ms.

Global dissimilarity analyses

2 x 3 repeated measures ANOVA on DISS evidenced three periods of significant material x condition interactions (p<0.05; >20 consecutive milliseconds): 260-284ms, 504-522ms and 580-624ms (Figure 2C). These periods correspond to the periods with significant interactions in the waveform analysis (Figure 2B). Main effects of condition and material were respectively observed at 232-324ms, 340-678ms, 704-742 and 756-800ms and at 82-514ms and 678-700ms.

Topographic cluster analysis

The topographic cluster analysis identified a series of distinct maps over the 800ms (Figure 2D). The global explained variance was 91.49%, which indicates how well the maps identified at the group level describe the whole dataset. This analysis identified four distinct periods of topographic modulations: 70-125ms, 125-200ms, 200-285ms and 285-380ms post-stimulus onset.

This article is protected by copyright. All rights reserved.
Differences between verbal and non-verbal stimuli were evidenced from 70ms on. Between 70 and 125ms, the map identified during the topographic cluster analysis was differently expressed for non-verbal than verbal material ($F_{(1,17)} = 17.72, p = 0.001, \eta^2_p=0.51$). Differences between material continued between 125-200ms, where the map identified was differently expressed according to material ($F_{(1,17)} = 6.45, p = 0.021, \eta^2_p=0.28$).

From 200ms on, specific differences in processing immediate repetitions occurred. Over the 200-285ms interval, there was a significant interaction between Material, Condition and Map ($F_{(4,68)} = 4.94, p = 0.001, \eta^2_p=0.23$) driven by differences for non-verbal material ($F_{(4,68)} = 10.07, p < 0.001, \eta^2_p=0.37$) but not for verbal material ($F_{(4,68)} = 0.731, p = 0.574, \eta^2_p=0.04$). Post hoc analysis revealed that the maps framed in dark and medium orange were significantly more present for One-backNV, whereas the map framed in light orange was less present in One-backNV than for the other conditions ($p<0.05$; Figure 2E).

Over the 285-380ms interval, there was again a significant Material x Condition x Map interaction ($F_{(4,68)} = 2.44, p = 0.05, \eta^2_p=0.13$) driven by differences between condition and map for the verbal stimuli ($F_{(4,68)} = 3.54, p = 0.01, \eta^2_p=0.17$) but not for the non-verbal stimuli ($F_{(4,68)} = 1.48, p = 0.22, \eta^2_p=0.08$). Post hoc analysis revealed that the map framed in dark purple was less present for One-backV than for NewV or Ten-backV ($p<0.05$; Figure 2F).

The temporal course of the above reported topographic modulations confirm the results of the ANOVA on waveform and global dissimilarity analyses and suggests that stimuli are differentially processed as a function of material (approx 70 ms post-stimulus onset) and then differed specifically for immediate repetitions, but with a latency shift between non-verbal and verbal immediate repetitions from 200ms on.
Source estimations

2 x 3 repeated measure ANOVAs on sources were performed during the two periods showing significant condition x material interactions in the topographic cluster analysis, respectively 200-285ms and 285-380ms. The topographic cluster analysis defined when stable topographies differed between conditions and served as a basis for computing statistics on source estimations as stable topographies indicate the presence of distinct underlying brain generators (Lehmann & Skrandies, 1980; Murray et al., 2008). We did not perform source analysis on the later periods of interaction revealed by the ANOVA on waveforms and global dissimilarity (around 500-600ms), as there were no differences in the topographic cluster analysis.

Regarding the 200-285ms period, the analyses revealed a Material x Condition interaction in a bilateral temporo-parietal cluster, comprising bilateral temporal, medio-temporal and parietal areas. Follow-up ANOVAs across all solution points during this time interval and for each material revealed a main effect of condition for the Non-verbal stimuli, but not for the verbal stimuli. Post-hoc paired t-tests on source estimations revealed that the difference emanated from specific neural responses for One-backNV in comparison to NewNV and Ten-backNV. Immediate repetitions of non-verbal stimuli induced an increased activation in right inferior frontal cortex in comparison to new presentations and bilateral activations in the same regions in comparison to delayed repetitions. There were no differences between NewNV and Ten-backNV (Figure 3A).

During the 285-380ms period, there was a Material x Condition interaction in a bilateral medio-temporo-parietal cluster. Follow-up ANOVAs across all solutions points during this time interval and for each material revealed a main effect of condition for the verbal, but not for the non-verbal condition. Post-hoc paired t-tests on source estimations revealed that the difference followed from specific neural responses for OneV in comparison to other verbal stimuli. OneV induced greater activation in the left inferior parietal cortex in comparison to new presentations.
and bilateral activations in the middle frontal cortices compared to delayed repetitions. Again, there were no differences between NewV and Ten-backV (Figure 3B).

**Discussion**

This study has three main results: First, it confirms the presence of a spacing effect for meaningless verbal and non-verbal stimuli. Second, the timing of the processing of immediate repetitions, as revealed by evoked potentials, differs according to stimulus material: processing of meaningless designs was faster than processing of non-words. The specific timing was confirmed with different types of analyses. Third, in contrast to immediate repetition of meaningful designs, which had activated the left medial temporal lobe (MTL) in earlier studies (James et al., 2009; Nahum et al., 2011; Nahum et al., 2014), the meaningless stimuli used in the present study evoked prefrontal and parietal activation.

The behavioral result confirms the spacing effect's standing as a ubiquitous memory effect. It extends previous studies demonstrating a spacing effect for meaningful words (Challis, 1993; Braun & Rubin, 1998), pictures (James et al., 2009), meaningless shapes (Cornoldi & Longoni, 1977), faces (Russo et al., 1998; Mammarella et al., 2002), and nonwords (Mammarella et al., 2002; Russo et al., 2002).

Electrophysiologically, first differences between verbal and non-verbal stimuli emerged from 70ms on, irrespective of the type of processing. This latency corresponds to the perceptual stage of stimulus processing (VanRullen & Thorpe, 2001; Di Russo et al., 2002). Differences relating to the type of processing appeared around 200 to 400ms, when immediate repetitions induced signals markedly different from new stimuli and delayed repetitions. The finding corroborates previously reported dissociations at this latency between immediate and delayed repetition of pictures (Nielsen-Bohlman & Knight, 1994; James et al., 2009; Nahum et al., 2011), faces (Xue et al., 2011), environmental sounds (Chao et al., 1995), or words (Kim et al., 2001; Kim et al., 2008;
Zhao et al., 2015). However none of them have directly compared in the same paradigm how repetition of verbal and non-verbal differ on their way to consolidation.

Our study suggests important dissociations in the processing of immediate repetitions: latency and source depended on the stimulus material. While immediate repetitions of non-verbal stimuli were processed around 200-285ms, immediate repetitions of verbal stimuli were processed later, at 285-380ms. This timing difference between materials is in accordance with previous ERP studies using continuous recognition tasks. James et al. (2009) reported specific electrophysiological differences between immediate and new presentations or delayed repetitions of meaningful designs at 200-300ms. Kim et al. (2001) reported a dissociation between immediate and delayed repetitions of words presented in a continuous recognition task, which started at 310ms. No study has directly compared how repetition lag is influenced by material within a combined paradigm. The same temporal sequence in evoked potentials as in the present study (first non-verbal, then verbal) was observed in an old/new recognition task, in which there was one intervening item between repetitions; no comparison between immediate and delayed repetitions was made (Beisteiner et al., 1996). Our results show, in a comparable paradigm, that immediate repetition of visually presented non-words is processed later than meaningless pictures. A potential explanation for this difference could be that non-words automatically evoke semantic processing, the search for meaning. Indeed, the N400, which is associated with semantic processing or orthographic/phonological processing (Kutas & Hillyard, 1980; Bentin et al., 1985; Holcomb, 1993; Deacon et al., 2004) peaks at the same latency (≥ 300ms).

Source estimations further support the material-specific differential neural pattern in response to immediate repetitions. Immediate repetitions of meaningless designs (non-verbal material) induced right-lateralized activation in the inferior frontal cortex in comparison to new presentations, and bilateral activation in comparison to delayed repetitions. Conversely, immediate repetitions of verbal stimuli showed left-lateralized activation of inferior parietal...
cortex in comparison to new presentations and bilateral activation of the middle frontal cortices when compared to delayed repetitions. These results are compatible with previous fMRI studies showing increased activation in prefrontal cortex for massed repetition of non-verbal stimuli (pictures of natural scenes) in contrast to distributed repetitions (Bradley et al., 2015) and with this area’s involvement in memory encoding and monitoring (Buckner et al., 1999). Regarding verbal material, a previous magnetoencephalography (MEG) study showed an increased early P300 at centro-parietal sites upon immediate word repetition and attributed this effect to cingulate cortex activity (Kim et al., 2001; Kim et al., 2008). The present study suggests that the source of this potential is the left inferior parietal cortex, which was activated when processing immediate repetitions of non-words. This area is also involved in lexical processing (Price & Mechelli, 2005; Vigneau et al., 2006; Cao et al., 2008) and probably in memory processing (Wagner et al., 2005; Cabeza et al., 2008). Irrespective of the precise role of these areas, our results show that different types of immediately repeated stimuli undergo lateralized processing that is not only distinct in time but also in terms of brain areas involved.

Although verbal and non-verbal stimuli differ at the temporal and spatial level, they share some common processes. For both types of material, delayed repetitions have a very similar expression as new stimuli in evoked potentials. One may, therefore, speculate that the spacing effect indeed emanates from a treatment of delayed repetitions as if they represented new events. In contrast, the strong electrophysiological reaction to immediate repetitions indicates an interference with an ongoing (consolidation) process, for the good or the bad.

The present results differ in a very important way from those obtained with meaningful, concrete designs, where immediate repetitions, in comparison to new pictures and delayed repetitions, induced strong activation of the left medial temporal lobe (James et al., 2009; Nahum et al., 2014). There are diverse explanations for the absence of MTL activation in the present study. First, the stimulus material may simply not have activated the MTL. Functional imaging studies are inconsistent with regards to the lateralization of MTL activity. Kelley et al. (1998)
demonstrated right MTL activation for non-verbal material (faces), whereas the left MTL was activated by verbal (words) as well as non-verbal processing (pictures, faces). Golby (2001) showed left MTL lateralization with words, right MTL lateralization with abstracts patterns, and bilateral activation with faces.

A related explanation refers to the complexity of the stimuli. Using fMRI to compare spaced versus massed learning of complex word pairs, Callan and Schweighofer (2010) observed differential activation between spaced and massed presentations in the PFC, but not the MTL. The stimuli used in the present study are probably more challenging than the meaningful, concrete designs used in the previous studies (James et al., 2009; Nahum et al., 2011; Nahum et al., 2014): accuracy was markedly lower in the present study compared to James et al. (2009) (88% correct responses vs 96% for the continuous recognition task; 57% vs 86% for delayed recognition task). The fact that, conversely, response times were shorter in the present study, suggests that subjects’ strategy in responding to the present stimuli was different from the previous studies, be this a characteristic of the present subject group or an effect of stimulus type. Nonetheless, the performance drop in the delayed recognition task was so important that higher stimulus complexity appears to be a plausible explanation for the prefrontal activations observed in the present study. Guerin et al (2009) reported complexity-dependant activity in the prefrontal cortex during an old/new recognition paradigm. Binder et al (2005) reported greater activation for abstract than concrete words in left prefrontal areas. One may speculate that the elaborate processing of stimuli with higher complexity induces a temporal dispersion, varying between individual stimuli, so that, even if the MTL were involved in the process, no temporally circumscribed evoked potential would be produced. Concrete designs are typically unequivocal and may, therefore, induce a clear-cut evoked potential.
From a clinical point of view, the present study shows that non-words and meaningless geometric designs are inapt stimuli to probe the functional intactness of the medial temporal lobes with high-density EEG. Nevertheless, the results highlight how material-specific processing of spaced and massed repetitions may influence subsequent recognition.

Acknowledgment

This work was supported by the Swiss National Science Foundation, grant no. 32003B-155947. The STEN toolbox (http://www.unil.ch/line/en/home/menuinst/about-the-line/software--analysis-tools.html#standard_412) has been programmed by Jean-François Knebel, from the Laboratory for Investigative Neurophysiology (the LINE), Lausanne, Switzerland, and is supported by the Center for Biomedical Imaging (CIBM) of Geneva and Lausanne and by National Center of Competence in Research project “SYNAPSY – The Synaptic Bases of Mental Disease”; project no. 51AU40_125759. The authors declare no conflict of interests. We thank Rémi Bloch for programming the non-verbal geometric design generator tool.

Abbreviations

MTL: medio-temporal lobe
PFC: prefrontal cortex
SE: spacing effect
EEG: electroencephalogram
ERP: event-related potential
DISS: global dissimilarity
GEV: global explained variance

This article is protected by copyright. All rights reserved.
MEG: magnetoencephalography

References


This article is protected by copyright. All rights reserved.


This article is protected by copyright. All rights reserved.


This article is protected by copyright. All rights reserved.


### Table 1: Behavioral results

<table>
<thead>
<tr>
<th></th>
<th>Accuracy (% correct)</th>
<th>RT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>New</td>
<td>One-back</td>
</tr>
<tr>
<td>A Learning task</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-verbal</td>
<td>89 ± 6</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>Verbal</td>
<td>89 ± 8</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>B Delayed recognition task</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Figure and table legends

**Figure 1:** Task design

**A.** Participants performed two blocks of a continuous recognition task containing verbal (non-words, V) and non-verbal (meaningless geometric designs, NV) stimuli. Stimuli were presented (New) and repeated either after immediately after initial presentation (One-back; One-backNV, One-backV) or after a delay (Ten-back; Ten-backNV, Ten-backV). The two blocks were composed of different stimuli and separated by a 2 min break. **B.** Thirty minutes after the completion of task A, participants performed a delayed recognition task comprising all the stimuli previously presented (pOneNV, pOneV, pTenNV, pTenV) plus new non-words (pNewV) and new meaningless designs (pNewNV).
**Figure 2:** Electrical neuroimaging results

**A.** Exemplar ERP waveform from a frontal electrode (Fz) in response to NewNV (pink trace), One-backNV (blue trace), Ten-backNV (red trace), NewV (turquoise), One-backV (green trace) and Ten-backV (black trace). Traces are displayed in microvolts as a function of time relative to onset of the stimulus. **B.** Electrode- and time-wise ERP waveform analysis. Results of 2 x 3 repeated measure ANOVA on waveforms with the factors material (Verbal, Non-verbal) and condition (New, One-back, Ten-back) revealing significant interactions between both factors (only p<0.05 for at least 20 consecutive milliseconds). **C.** Time-wise topographic analysis. Results of the millisecond-by-millisecond topographic ANOVA on the global dissimilarity (TANOVA). The dotted line indicates p<0.05. Periods of significant (p<0.05 for at least 20ms) topographic interaction are marked in black. **D.** Topographic cluster analysis identified six periods of stable electric field topography across the 800ms post-stimulus period. Red indicates positivity, and blue indicates negativity. Multiple maps were identified for two time windows. **E, F.** Results of the single-subject fitting. The y-axis displays the GEV (global explained variance) of each map which provides a measure across subjects of how well a given template map accounts for a given condition over this time period. Results of the follow-up ANOVA over the 200-285ms time period indicate that the material x condition x map interaction was driven by specific responses to One-backNV (**E**) Over the 285-2380ms time period, the interaction was driven by specific responses to One-backV (**F**).

**Figure 3:** Source estimations

Results of neural source estimation analyses on the two stable topographic clustering periods showing significant material x condition interaction. The figure displays the results of the t-tests comparing One-back vs Ten-back, One-back vs New and New vs Ten-back during the 200-285ms.
period for the non-verbal material (A) and during the 285-380ms interval for the verbal material (B). The scale indicates the color meaning.

Table 1: Behavioral results

Behavioral results for the continuous recognition task (A) and the delayed recognition task (B). The results indicate the presence of a spacing effect (Accuracy Ten-back > Accuracy One-back; p < 0.01) for the non-verbal\(^1\) and verbal materials\(^2\).

--- 30 min ---