Event-Related potentials in an associative word pair learning paradigm

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Abstract

The study investigated the effect of unintentional learning of semantically unrelated word pairs on event-related brain potentials. Two experiments were conducted, in whose acquisition phase participants listened to five pairs of semantically unrelated words, each pair being repeated twenty times. In the test phase of Experiment 1, these "old" pairs were presented mixed with "new" pairs containing other words. In the test phase of Experiment 2, a third condition was added in which the first word in a pair was one of the words presented during acquisition but the second word was new. In both experiments, the second word in new word pairs elicited an N400 and a late (550-1000 ms) frontal positivity. The amplitude of the N400 to new second words in Experiment 2 was significantly larger when the first word in the pair was an old (previously learnt) word, as compared with the condition in which both first and second words were new. The results indicate that, in addition to a repetition effect, unintentional learning of word pairs results in building new associations between previously unrelated words.

Keywords: Associative learning, ERP, Late Positive Complex (LPC), N400, Priming

Introduction

Event-related potentials (ERPs) provide a technique of extracting information in the order of milliseconds on the time course of cognitive processes. The averaged ERP waveforms consist of a sequence of positive and negative voltage deflections (Luck, 2005). Particularly, a negative deflection peaking around 400 ms post-stimulus was labeled N400 (Kutas & Hillyard, 1980, Kutas & Federmeier, 2011). It is a characteristic part of the normal brain response to words and other meaningful (or potentially meaningful) stimuli but is typically suppressed whenever the word is strongly prepared by its context (e.g., "I drink my coffee with cream and *sugar*"). A large N400 is elicited by the last word of the sentence when this word violates the expectancy created during the sentence ("incongruent ending" e.g., "I drink my coffee with cream and *dog*"), as compared with the word that agrees with the expectancy ("congruent ending") (Balass, Nelson and Perfetti, 2010; Kutas & Federmeier, 2011).

In addition to the above-depicted sentence paradigm with congruent versus incongruent endings, similar results were obtained in experiments with word pairs. Overall, the corresponding conditions are covered by a general name of priming paradigm (Weingarten et al., 2016). In word pair tasks, therefore, the first word in a pair is referred to as a "prime", and the second word, as a "target". When these terms are used in sentence experiments, the prime is the whole sentence context; and the end word of the sentence, which can be either congruent or incongruent with the context, is the target. N400 amplitude is typically larger for targets that are incongruent with their primes than for congruent targets (Kutas & Hillyard, 1984). Its amplitude is inversely related to the degree of semantic expectancy and the modulation of the N400 amplitude is referred to as the semantic priming effect (Brualla, Romero, Serrano, & Valdizán, 1998).

In contrast to the semantic relationship, an association between two words is due to their spatial and/or temporal co-occurrence in the real world and/or in a language (McRae, Khalkhali &

Hare, 2012). N400 effect is sensitive to how often two concepts co-occurred within an individual's past experience. An example of such co-occurring concepts is the pair rock and roll (Lancia, 2007; Buchanan, Holmes, Teasley & Hutchison, 2013). One word can remind us of the other one because of their main association with the music style, although there is no apparent semantic relationship. Moreover, Ortu, Allan and Donaldson (2013) employed three classes of word-pairs including pairs with a moderate association, high association and without association. Both moderately and highly associated word pairs elicited a suppressed N400 as compared to unrelated word pairs.

Semantic learning can affect N400 in word pairs (Balass et al., 2010). In the acquisition phase of this experiment participants learned the meaning of rare words. In the test phase word pairs were presented, whose first word was either one of the just learnt rare words, or a new rare word, or a highly familiar word. The N400 to the second word was more positive to both learnt words and familiar words than to new words. The suppression of the N400 to semantically related words was observed only for pairs in which the first word was either learnt or familiar. This effect of relatedness was not observed for new words. Thus, the results indicated that word experience affects N400.

Repetition of words in lists enhanced the late positive component (LPC) – a widely distributed, long-latency, positive-going shift in the ERP – either along with a reduction of N400 or without it (Rugg, 1985; Van Petten, Kutas, Kluender, Mitchiner and McIsaac, 1991; Rugg, Pearl, Walker, Roberts, and Holdstock, 1994; Rugg, Doyle, & Holdstock, 1994). A comparison between N400 effects elicited by word repetition and semantic congruence shows that the former peaks later and lasts longer (e.g., Besson et al., 1992). In the current study two repetition effects can be dissociated: (1) single word level repetition effect that may cause similar effects of N400 reduction with an increase of the LPC, and (2) the repetition of the association within word pairs, that has not been studied by previous research.

In two experiments we tested whether unintentional learning of semantically not associated word pairs would result in N400 suppression similar to that in semantically associated pairs (Balass et al., 2010). We compared ERPs to unrelated word pairs that have been recently learnt ("old" pairs) and to other unrelated word pairs that were presented for the first time ("new pairs"). In the first experiment, we expected that the N400 to the second word in a pair to be smaller in learnt word pairs than in new word pairs because consistent repetition of semantically unrelated word pairs may make them acting like related word pairs. Then we aimed to further clarify whether the N400 effect is to be explained by increased familiarity of repeatedly presented words or is based on building new associations between the members of a pair. In the second experiment we introduced a novel element in the paradigm: in addition to old and new word pairs, we had pairs (old-new) where the first word is old (learnt) and the second word is new (unlearnt). We hypothesized that the association between words in pairs was built by repetition of the pairs, thus, the association is learnt, not just single word repetition drives the effect. In the second experiment N400 to the second word in the old-new pairs was expected to have larger amplitude as compared to the new-new pairs because of stronger deviation from the expected association.

Methods

Participants

Twenty-two subjects participated in this study. The group comprised thirteen females and nine males with a mean age of 25.23 (range: 19-33, standard deviation: 12.45) recruited from the University of Tuebingen student population. None of them reported visual, auditory or neurological deficits. According to the Edinburgh Handedness Scale (Oldfield, 1971), seventeen participants were right-handed, four left-handed and one participant was ambidextrous. They were either native German speakers or came to Germany before the age of 6 and started their primary school here. Informed consent was obtained from all subjects

included in the study. They received an expense allowance of 8 €/h for their participation. One participant's data were excluded from some of the analyses because of missing data in the acquisition phase of the second experiment due to EEG recording failure.

Stimuli and procedure

We carried out two learning experiments, each entailing two phases. We hypothesized that the result of learning in the first (acquisition) phase should be manifested in the second (test) phase.

Experiment 1 contained an acquisition phase, in which five word pairs were presented 20 times each, ten times in one order (e.g., warm / Kilo) and ten times in the reverse order (e.g., Kilo / warm). In the test phase of Experiment 1 the same five word pairs from the acquisition phase were presented 100 times ("old" pairs) 20 times each, and there were 100 completely new pairs which had not been presented before.

The acquisition phase of Experiment 2 was identical to the acquisition phase of Experiment 1, but the five employed word pairs did not repeat any word from Experiment 1. The test phase included 300 word pairs subdivided into three categories: 100 "old" pairs presented at the acquisition phase and 100 new pairs were analogous to those in Experiment 1. An additional category involved 100 word pairs, whose first word was the same as one of the "old" words, learnt in the acquisition phase. However, the second word in the pair was new. These pairs were designated as "old-new pairs". There was no significant difference between "old" and "new" words in terms of word frequency, the average length (in ms) and the average number of syllables. There were two versions of the acquisition-test word pairs sets. One of them was used in the experiment 1 and the other in the experiment 2 (counter-balanced between participants).

The two above-described EEG experiments were preceded by a pilot paper-and-pencil experiment with fifteen students of medicine and psychology (nine females, all native German speakers). They were presented with about 1000 different word pairs and were required to judge the association strength within each pair using the scale from 0 (no association at all) to 8 (very strong association). Moreover, they were instructed that they should regard word pairs such as man/woman and cat/mouse as “8”. After this, only word pairs whose association strength was never assessed above 3 were employed in the two EEG experiments.

In both experiments, the participants were sitting in a comfortable chair with the eyes closed. The words were presented auditorily by a female voice. The loudness was normalized to be about 65 dB SPL. The word pairs were presented in a pseudorandom order, and one old pair was never presented twice in a row. Furthermore, in the test phases not more than four pairs of the same category (i.e., old, new, old-new) were presented in immediate succession. The interval between the offset of the first word and the onset of the second word within each pair was 100 ms. Due to the variable length of the words, the onset-to-onset interval varied from 500 to 1100 ms. The second word was followed by a motor response (see below), and the next word pair started 1 s after the motor response. In each experiment, the test phase started immediately after the end of the acquisition phase without a break. There was a break of about 30 minutes between the two experiments, during which participants were busy with unrelated to the experiments activities.

Participants were asked to compare the number of syllables in the two words of each pair. If both words had the equal number of syllables, participants had to press one of the keyboard buttons (left or right Ctrl buttons on a standard keyboard), and if they had a different number of syllables, the other button. The side of the response was counterbalanced among the participants. The task was irrelevant concerning the aim of the experiments. It was just used to increase attention to the words. Because the mean length of experiment 1 was around 19

minutes and experiment 2 was about 27 minutes, we were concerned that participants' attention would decline if no overt response is required.

EEG recording and ERP analysis

The EEG was recorded from 64 channels online referenced to Cz. The EEG was acquired with 1000 Hz sampling rate with a low-pass 280 Hz filter. The active electrodes (EasyCap) were placed using the 10-10 system. BrainVision Analyzer Version 2.1.2.327 was used for preprocessing of EEG. First, the data were downsampled off-line to 256 Hz and then re-referenced to a common average reference. After that, offline low pass 30 Hz and 0.1 Hz high-pass filters were applied. Bad channels were replaced using spherical interpolation. An Independent Component Analysis (ICA) was employed to separate and remove activity related to ocular artifacts. After artifact correction four channels (Fp1, Fp2, AF7, and AF8) were removed from further analyses. Finally, the data were again re-referenced to average mastoids to warrant the comparison with the published results, most of which have been reported with mastoid reference. ERPs were averaged in epochs started -200 ms before a stimulus and lasted for 1000 ms after it. The baseline was defined as the average amplitude between -200 ms and 0 ms.

The mean ERP amplitude was measured in the time windows of 250-550 ms (N400) and 550-1000 ms (LPC). We did not observe a clear peak in the grand average ERPs after N400 time window; hence we defined LPC from the end on N400 window to the end of the epoch. The statistical analysis was conducted using SPSS version 23 (IBM corp.) using Analysis of variances (ANOVAs) within the General Linear Model. Specifically, two ANOVAs were employed. The first ANOVA over midline electrodes included the factors Site (5 levels: Fz, FCz, Cz, CPz, Pz) and Condition. The factor Condition included three levels in Experiment 1

(acquisition phase; old pairs in the test phase; new pairs in the test phase) and four levels in Experiment 2 (acquisition phase; old pairs, new pairs, and old-new pairs in the test phase), see Figures 1 and 3 for traces of the ERP signals at midline electrodes.

The second ANOVA over lateral electrodes involved the factors Condition (identical to the first ANOVA), Region of Interest (ROI: 4 levels) and Side (2 levels, i.e. left versus right). For this sake we built average values for eight ROIs (4 ROIs on each side) including: left anterior ROI: F1, F3, FC1, FC3, FC5, FT7, left central ROI: C1, C3, C5, T7, left temporo-parietal ROI: CP1, CP3, CP5, TP7, P5, P7, left posterior ROI: P1, P3, PO3, PO7, O1, right anterior ROI: F2, F4, FC2, FC4, FC6, FT8, right central ROI: C2, C4, C6, T8, right temporo-parietal ROI: CP2, CP4, CP6, TP8, P6, P8 and right posterior ROI: P2, P4, PO4, PO8, O2.

The significance level was set at .05, and for all effects with more than two levels degrees of freedom were corrected for non-sphericity using Greenhouse-Geisser epsilon. Below, corrected degrees of freedom will be reported.

Results

Performance

In the first experiment, median reaction time (RT) was faster after new word pairs as compared to old pairs in the acquisition and test phases ($F(2, 40) = 21.26$, $p < .001$, $\eta^2 = .55$), with means (averaged over individual *medians*) ± standard error (SE) being 937 ± 63.3 ms, 867 ± 62.7 ms, and 1120 ± 75.1 ms, for old (acquisition), old (test), and new pairs, resp. Pairwise comparisons confirmed the significance of the differences: old (acquisition) vs new, $p < 0.001$; old (test) vs new, $p < 0.001$; old (acquisition) vs old (test), $p = 0.08$.

The results of the second experiment were similar: old pairs in the acquisition and test phases were characterized by shorter RT than new and old-new pairs (F(2, 35) = 28.2, $p < .001$, η^2 =.57, means ± SE being 874 ± 59.7 ms (acquisition), 948 ± 49.3 ms (old pairs, test), 1090 ± 62 ms (new pairs), and 1140 ± 54.4 ms (old-new pairs). Pairwise comparisons revealed significant (all *p*s < .001) differences between old pairs (both acquisition and test), on the one hand, and new and old-new pairs, on the other hand. All other comparisons yielded *p*s > 0.4.

ERP

Experiment I, First word

N400 (250-550 ms time window)

Midline electrodes. As ERP waveforms in Figure 1 displays (see also Figure 2), a significant main effect of Condition ($F(1, 30) = 6.24$, $p = .010$, η^2 =.22) resulted from the N400 amplitude for old pairs in the acquisition phase being significantly smaller than for the same words in the test phase ($p = .044$) and for new pairs $p = .009$). A significant main effect of Site at the midline electrodes ($F(1, 27) = 12.44$, $p = .001$, η^2 =.37) resulted from the fact that the amplitude at Fz was larger than at CPz ($p = .006$) and Pz ($p = .001$). These two changes led to a significant interaction between Condition and Site ($F(3, 69) = 3.44$, $p = .018$, η^2 =.14).

Although the N400 was largest at Fz and FCz, between-condition differences at these sites did not reach significance (all *ps* > .05). At Cz, the N400 for new pairs was larger than in the acquisition phase ($p = .008$), and at Pz, it was larger for new pairs than for old pairs in both acquisition ($p = .001$) and test phase ($p = .002$). Finally, at CPz all three conditions differed significantly from each other: the N400 for old pairs was larger in the test phase than in the acquisition phase ($p = .047$), and for new pairs it was larger than for old pairs in both acquisition phase ($p = .002$) and test phase ($p = .008$).

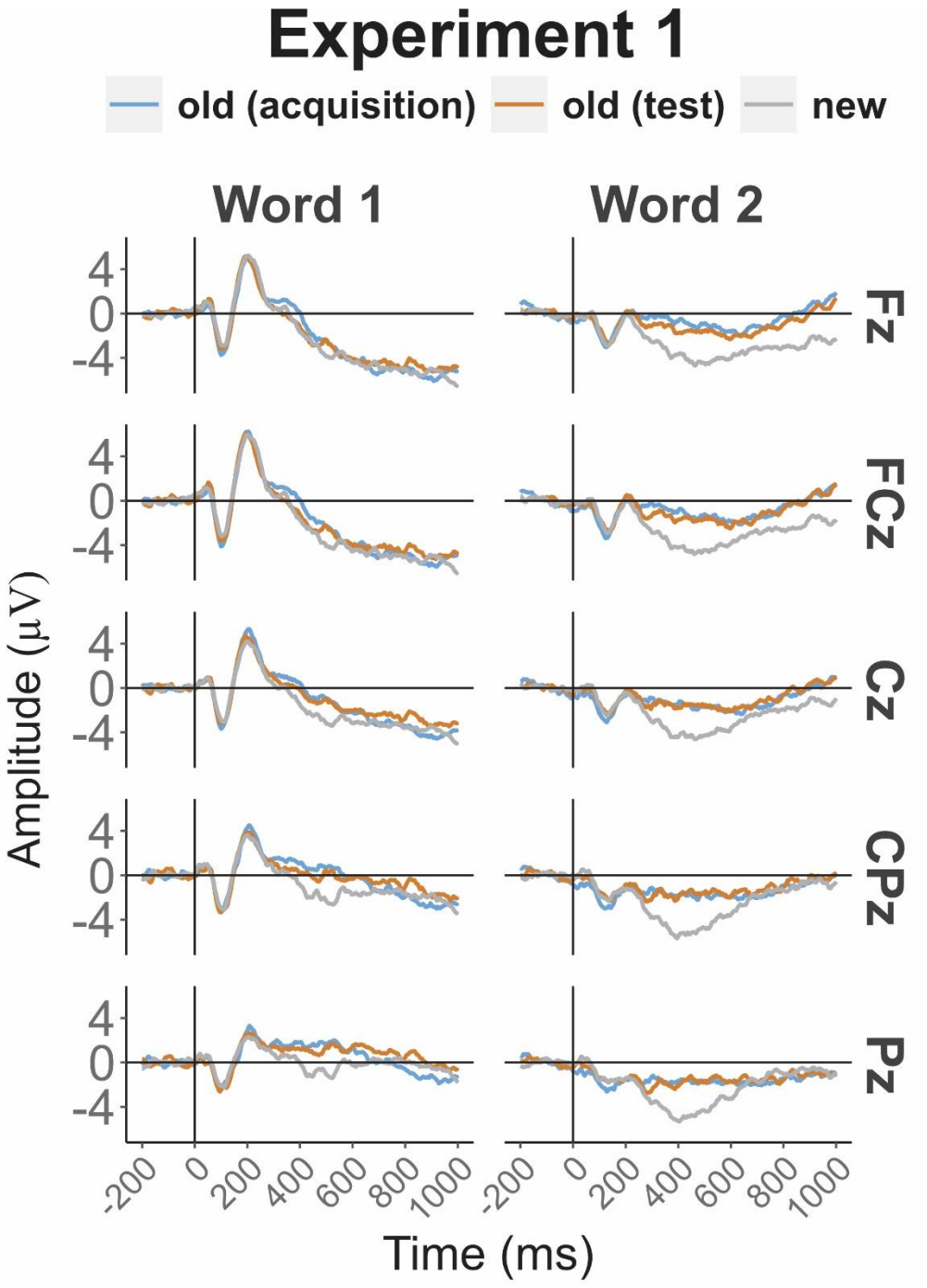

Figure 1 – Midline channels ERP waveforms to the first and second words in pair in the first experiment.

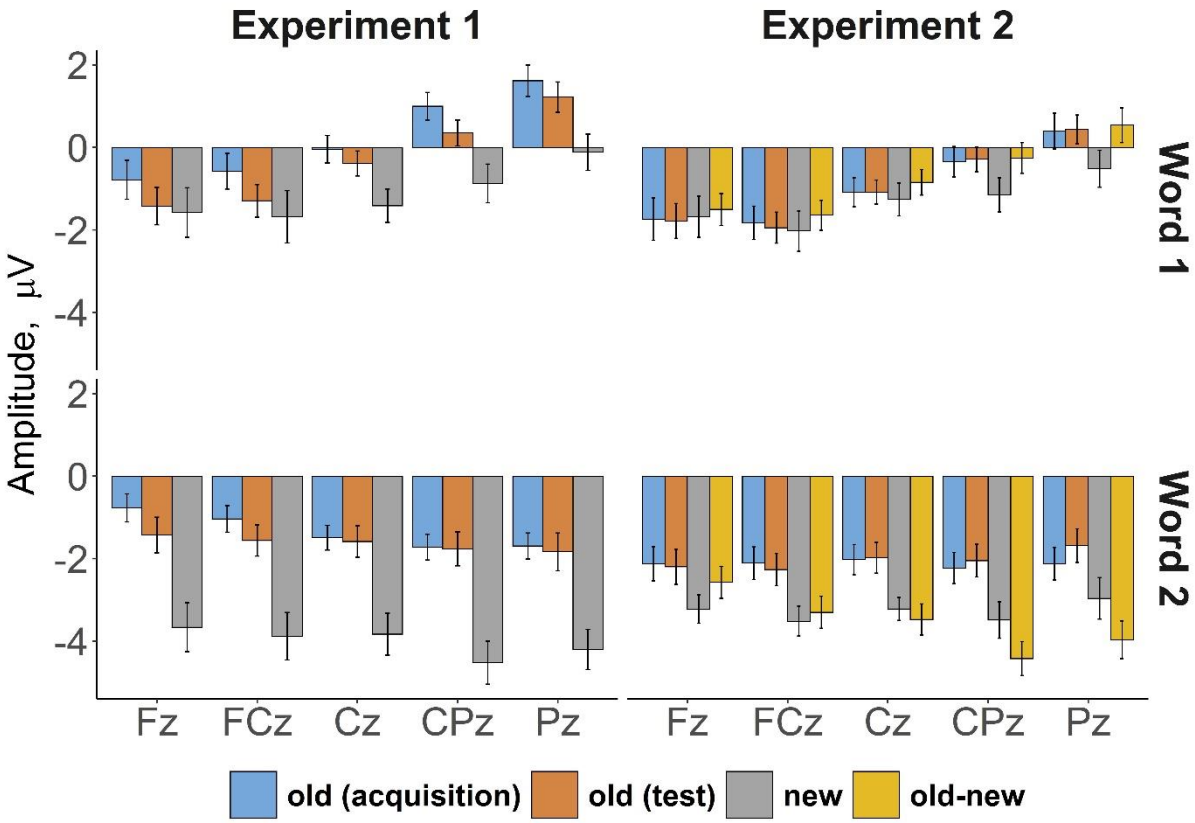

Figure 2 – Averaged in N400 time window (250-500 ms) ERP amplitudes at midline channels. Error bars represent the standard error of the mean.

Lateral electrodes. Like at the midline, the main effect of Condition ($F(1,30) = 7.06$, $p = .002$, $\eta^2 = .25$) indicated that the N400 for new pairs in the test phase was significantly ($p = .006$) larger than the amplitude in the acquisition phase, and also larger than the amplitude for old pairs in the test phase ($p = .044$) (see Figure 4 for the spatial distribution of the effect). The amplitude for old pairs in the test phase was significantly larger than in the acquisition phase ($p = .030$). In addition, a significant main effect of ROI ($F(1,23) = 6.08$, $p = .019$, $\eta^2 = .23$) indicated that the amplitude over temporo-parietal ROIs was larger than the amplitude over central ($p = .009$) and posterior ROIs ($p = .028$).

LPC (550-1000 ms)

No interactions or main effects of Condition were found neither in the midline nor in the lateral electrodes.

Experiment I, Second word

N400

Midline electrodes. As shown in Figures 1, the ERP waveforms at midline electrodes displays a significant main effect of Condition ($F(1, 39) = 15.11$, $p < .001$, $\eta^2 = .41$) resulting from the amplitude for new pairs being significantly (*ps* < .001) larger than the amplitude for old pairs in the test phase and the acquisition phase difference between old pairs in the acquisition phase and in the test phase was not significant ($p = .538$).

Besides this, a significant main effect of Site ($F(1, 37) = 5.32$, $p = .012$, $\eta^2 = .20$) indicated that the amplitude at CPz was larger than at Fz, FCz and Cz (all $p < .05$) and the amplitude at Pz was larger than at Fz ($p = .028$).

Lateral electrodes. A significant main effect of Condition ($F(1, 39) = 19.07$, $p < .001$, $\eta^2 = .47$) indicated that the amplitude for new pairs in the test phase was significantly larger than the amplitude for old pairs and in the acquisition phase (both $p < .001$). On the other hand, although the amplitude for old pairs in the test phase was larger than in the acquisition phase, the corresponding difference was not significant ($p > .5$).

The amplitude over anterior ROIs was significantly ($p < .001$) larger than over central, temporo-parietal and posterior ROIs, which gave rise to a significant main effect of ROI ($F(1, 29) = 5.56$, $p = .017$, $\eta^2 = .21$). An interaction between ROI and Condition ($F(3, 60) = 4.49$, $p = .007$, $\eta^2 = .18$) indicated that the increased amplitude in the new pairs condition, although

significant in every ROI (smallest p = 0.003), was largest in the temporo-parietal areas and smallest in the anterior areas.

LPC

Midline electrodes. The amplitude for new pairs in the test phase was more negative than the amplitude for old pairs in the test phase and in the acquisition phase creating a significant main effect of Condition ($F(2, 41) = 4.19$, $p = .023$, $\eta^2 = .17$). The effect of Condition was strongly affected by the location of the electrode (Condition by Site interaction: $F(3, 55) = 12.65$, $p < .001$, $\eta^2 = .38$). ANOVAs at the individual channels showed a significant main effect of Condition in Fz, FCz and Cz ($F(3, 55) = 10.8$, $p < .001$, $\eta^2 = .34$; $F(3, 55) = 7.3$, $p = .002$, $\eta^2 = .26$; $F(3, 55) = 4.6$, $p = .017$, $\eta^2 = .18$ at each channel respectively). The effect was driven by a significantly larger LPC in the old conditions as opposed to the new one (old (test) vs new: p = .001, .006, .016 at Fz, FCz and Cz, respectively; old (acquisition) vs new: p = .001, .003, .029 at Fz, FCz and Cz, respectively).

Lateral electrodes. Like at the midline, the amplitude for new pairs in the test phase was more negative than old pairs in the acquisition phase ($p = .023$) giving rise to a significant main effect of Condition ($F(2, 40) = 3.45$, $p = .044$, $\eta^2 = .14$). The effect of Condition was significant over anterior ($F(2, 41) = 8.6$, $p < .001$, $\eta^2 = .29$) and central ($F(2,36) = 5.8$, $p = .009$, $\eta^2 = .22$) but not in more posterior areas (Condition x ROI: $F(2, 47) = 10.72$, $p < .001$, $\eta^2 = .34$). Pairwise comparisons within the ROIs revealed a pattern similar to the midline: the LPC was larger in old pairs than in new pairs (old (acquisition) vs new: p = .002, .009 in anterior and central ROIs, respectively; old (test) vs new p = .003, .016 in anterior and central ROIs, respectively). Thus, the second word elicited a larger LPC in old pairs than in new pairs primarily at the anterior leads.

Experiment II: First word

N400

Midline electrodes. ERP waveforms at midline electrodes (Fz, FCz, Cz, CPz, and Pz) to the first word in the second experiment are shown in Figure 3. A significant main effect of Site ($F(1, 27) = 15.48$, $p < .001$, $\eta^2 = .42$) was due to the fact that the amplitude at Fz and FCz was significantly larger than at Cz ($p = .040$), CPz ($p = .006$) and Pz ($p = .001$). While the main effects of Condition was not significant ($F(2, 49) = 1.2$, $p = .3$, $\eta^2 = .06$), its interaction with Site was ($F(4, 83) = 4.3$, $p = .003$, $\eta^2 = .18$). Pairwise comparisons in each channel revealed that the effect was driven by the larger N400 to new first words as compared to old first words in Pz and CPz (both ps < .05).

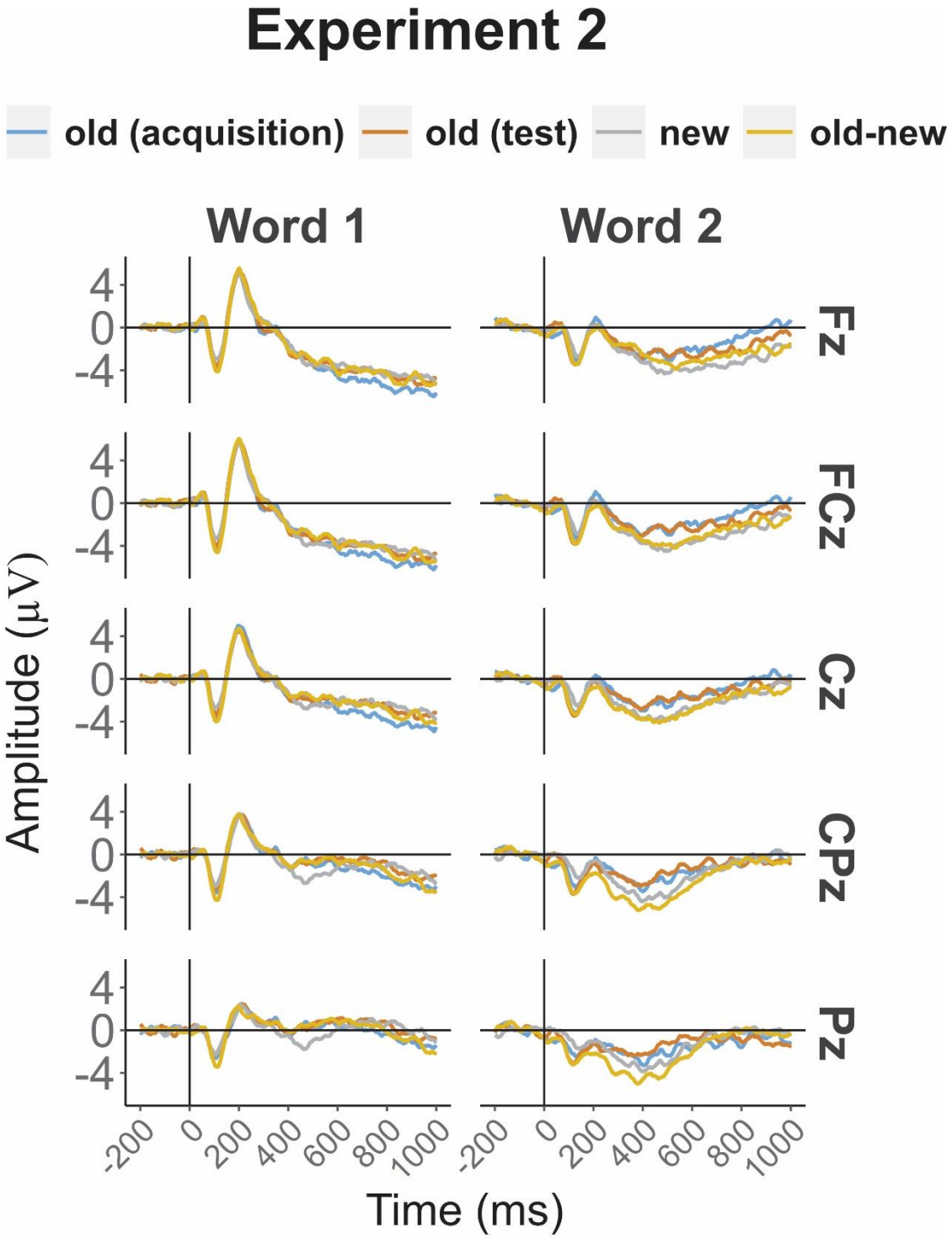

Figure 3 – Midline channels ERP waveforms to the first and second words in pair in the second experiment.

Lateral electrodes. The main effect of Condition and its interactions with other factors were not significant. Generally, the amplitude of N400 over anterior ROI was larger than over central (p = .032), temporo-parietal (p = .016) and posterior ROIs (p = .017); also the amplitude over both central ROIs was significantly larger than over temporo-parietal (p = .013) and parietal (p = .02) ROIs, which led to a significant main effect of ROI (F(1, 23) = 6.47, p =.015, η^2 = .24). In addition, the main effect of Side (F(1, 20) = 6.94, p = .016, η^2 = .26) indicated that the amplitude of N400 on the right side was larger than on the left side.

LPC (550-1000 ms)

No interactions or main effects of Condition were found neither in the midline nor in the lateral electrodes.

Experiment II, Second word

N400

Midline electrodes. As the ERP waveforms at midline electrodes to the second word in Experiment II in Figure 3 display (see also Figure 2), a significant main effect of Condition (F(3, 53) = 7.37, p < .001, η^2 = .27) resulted from the fact that the amplitude for old-new pairs was significantly larger than for old pairs in the test phase (p < .001) and in the acquisition phase (p = .01). Similarly, the amplitude for new pairs was significantly larger than for old pairs in the test phase (p = .002) and in the acquisition phase (p = .017). The interaction between Condition and Site was highly significant: F(3, 60) = 6.38, p < .001, η^2 = .24). Post-hoc t-tests revealed that the N400 was significantly larger in the old-new than in the new-new condition at

Pz (p = .01) and CPz (p = .006). The difference between the test phase old-old and both new-new and old-new conditions was significant at all electrodes but Fz, whereas at Fz only old-old and new-new pairs were significantly different (ps <. 05).

Lateral electrodes. Like at the midline, a significant main effect of Condition ($F(3, 53) = 7.54$, $p < .001$, $\eta^2 = .27$) emerged from the fact that the amplitude for old-new pairs was significantly larger than for old pairs in the test phase ($p < .001$) and the acquisition phase ($p = .007$). The amplitude for new pairs was also significantly larger than for old pairs in the test phase ($p = .001$) and the acquisition phase ($p = .007$). The difference between old pairs in the acquisition phase and the test phase was not significant.

The amplitude of N400 was left lateralized ($F(1, 20) = 14.4$, $p = .001$, $\eta^2 = .42$). The difference between both old and both new second words was smaller over anterior ROI than in all other ROIs. The effect was stronger on the left side than on the right side. These resulted in the significant interactions between Condition and ROI ($F(3, 52) = 5.24$, $p = .005$, $\eta^2 = .21$), between ROI and Side ($F(2, 36) = 5.82$, $p = .008$, $\eta^2 = .23$), as well as in a triple interaction of Condition, ROI, and Side ($F(4, 89) = 3.31$, $p = .01$, $\eta^2 = .11$). Post-hoc t-tests indicated a significantly larger N400 amplitude in old-new than new-new word pairs in the posterior ROI (left side: $p = .028$; right side: $p = .008$). The differences between the acquisition phase and the same (i.e., old) stimuli in the test phase were not significant in any region. The effect of smaller N400 in the old-old pairs than in the old-new and new-new pairs was confirmed in all but anterior ROIs (ps <. 05, see Figures 2 and 3)

LPC

Midline electrodes. While the main effect of Condition was not significant, there was a significant Condition by Site interaction: $F(2,47) = 7.1$, $p < .001$, $\eta^2 = .27$. The effect was

significant in Fz ($F(2, 39) = 5.2$, $p = .01$, $\eta^2 = .21$), FCz ($F(2, 43) = 4.3$, $p = .016$, $\eta^2 = .19$) and Cz ($F(2, 43) = 3.3$, $p = .04$, $\eta^2 = .15$) channels. Pairwise comparisons at single electrodes showed that in both old conditions LPC was significantly larger than in new-new condition at Fz and FCz ($p < .05$). LPC was larger in old (acquisition) vs both new conditions at FCz, Fz and Cz; both old > old-new at Cz; old (test) > new at FCz (all ps<.05). These differences were not significant at CPz and Pz.

Lateral electrodes. At lateral electrodes there was no significant main effect of Condition, but the interactions Condition by ROI ($F(2,42) = 9.67$, $p < .001$, $\eta^2 = .34$) as well as Condition by ROI by Side ($F(4,75) = 7.3$, $p < .001$, $\eta^2 = .28$) were significant. A further analysis showed that the main effect of Condition attained significance only in the anterior ROIs ($F(2,42) = 5.73$, $p = .006$, $\eta^2 = .23$), where the LPC was larger in both old conditions as compared to new and old-new conditions (*p*s <. 05) without significant differences in the comparisons [old (acquisition) vs old (test)] and [new vs old-new]. Moreover, the anterior effect (larger LPC after old pairs) was particularly strong over the right anterior area resulting, specifically for anterior ROIs, in a significant Condition by Side interaction ($F(4,75) = 7.77$, $p < .001$, $\eta^2 = .28$) and a significant main effect of Side ($F(1,20) = 15.2$, $p < .001$, $\eta^2 = .43$).

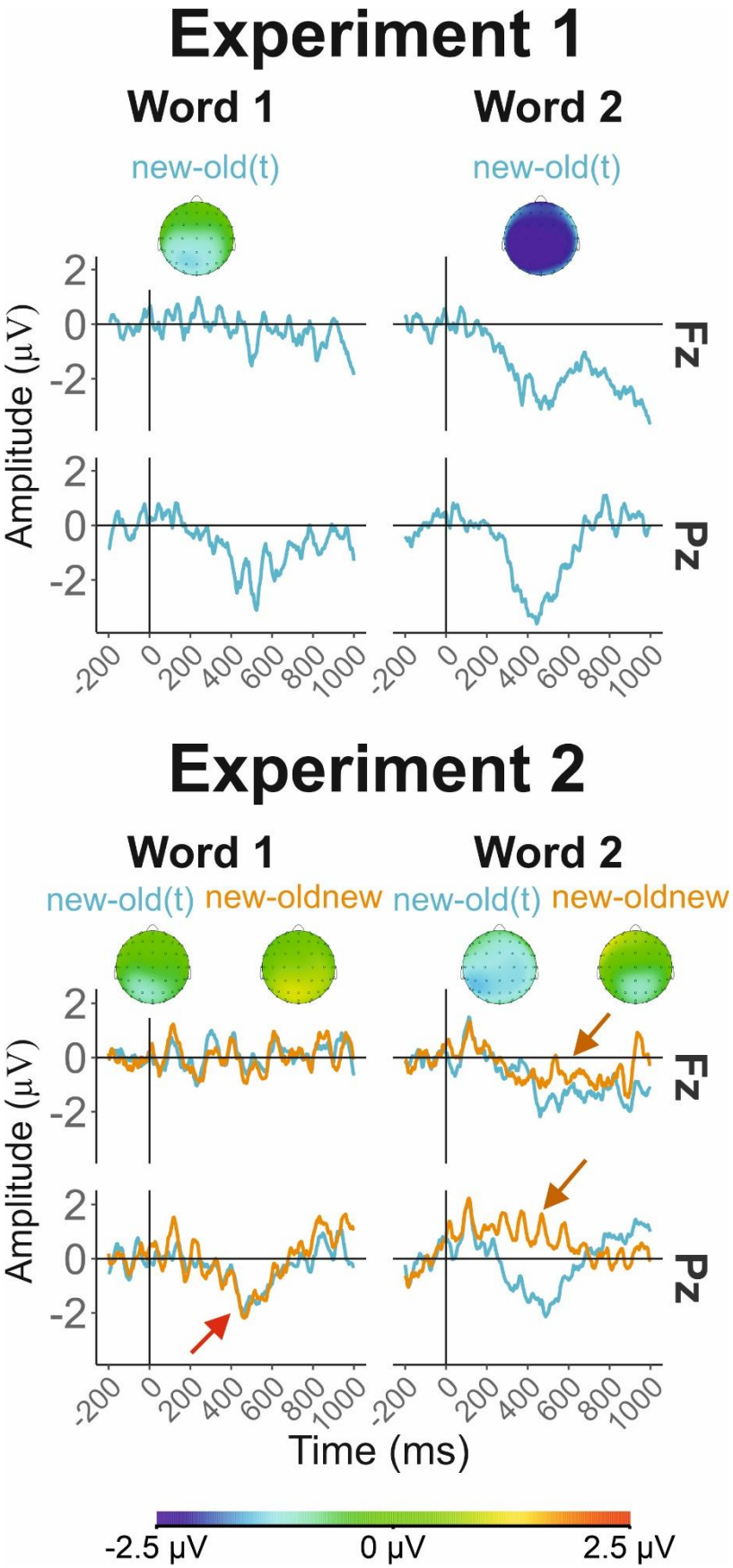

Figure 4 – Difference ERP waveforms in Fz and Pz electrodes and difference topographic maps for N400 effect to the first and second words in both experiments. Notes: new-old(t) – the difference between new and old (test phase) word pairs; oldnew-new – the difference between

old-new and new word pairs. Note that the difference between old and new FIRST words in Experiment 2 (**red arrow**) is a pure word repetition effect, because no effect of association within pairs can be expected. In contrast, the difference between SECOND words in old-new and new-new pairs in Experiment 2 (**yellow arrows**) is a pure association effect, because the effect of repetition is identical in both compared conditions.

Discussion

Summary of the data

The study aimed to investigate the effect of unintentional learning of semantically unrelated word pairs on semantic ERP components. The modulation of ERPs by word repetition was investigated in two experiments, both containing an acquisition phase and a test phase. In both experiments, healthy subjects heard a series of semantically unrelated word pairs. A portion of the words were repetitions of previously presented items.

In the test phase of the first experiment, "old" word pairs repeated word pairs presented during the acquisition phase, while "new" pairs contained other words that had not been presented before. In the test phase of the second experiment, a third condition was added in which the first word in a pair was one of the words presented during acquisition, but the second word was new.

The strongest effects were obtained the time window of 250-550 ms, which we identified as the N400. In the *first experiment*, the amplitude to the first words in old pairs in the test phase was significantly more negative than to the same stimuli in the acquisition phase. The amplitude for new pairs was also more negative than for old pairs. In response to the second word, in the test phase, N400 amplitude for new pairs was significantly more negative than for old pairs, which can be attributed either to the effect of learnt association or that of repetition, or both.

In the *second experiment*, the amplitude of the N400 was larger to new than old first words in posterior leads only (no main Condition effect, but Condition by Site interaction). The N400 was significantly larger to new second words than to old second words at all sites. The effect was most pronounced in the central, temporo-parietal and posterior regions. At these locations, furthermore, new second words elicited a larger N400 when the first word in the pair was old, as compared with the condition when the first word was also new. The relatively small N400 amplitude to old words did not differ between the test phase and the acquisition phase.

In *both experiments*, additionally, the LPC (time window 550 - 1000 ms[1]) at frontal leads had larger amplitude to old second words than to new second words. The effect was absent to first words.

ERP repetition effect

Some studies have examined the effects of repetition on the ERPs elicited by words in lists. Initially, Rugg (1985) compared the effects of repetition to those of associative semantic priming. He found that both repeated and semantically primed words elicited more positive ERPs than did new or unrelated words in the latency range of 300 to 500 msec post-stimulus. This was consistent with the conclusion that both repeated and related words elicit a smaller N400 in lists.

In the following study, Rugg (1987) investigated a combination of repetition and semantic effects. Unlike our study, the experiments included both words and pronounceable non-words. Subjects silently counted occasional non-words against a background of meaningful words, a

[1] Because of the lack of a clear LPC peak, we also tried other window definitions of this component. The result was always the same.

portion of which were either semantic associates or repetitions of a preceding word. Repeated words were distinguished by waveforms containing an initial transient negative-going deflection called N140. In contrast to the current study, the repetition effect in Rugg (1987) did not appear as a typical N400. Since that experiment included two types of stimuli (words and non-words), onset latency differed between the two studies. One may suppose (although we do not have a direct comparison) that the task to distinguish words from non-words is more difficult, or stronger interfere with semantic processing, than our task to count syllables, and that for this reason word/non-word discrimination took more time. In the light of the above, it seems reasonable to assume that the differences between word and non-word repetition effects on ERPs are attributable to the dissimilar properties of these two types of item, which is completely different from our experiment restricted to one kind of stimulus, unrelated word pairs.

Van Petten, Kutas, Kluender, Mitchiner and McIsaac (1991) additionally investigated the effects of repetition as a function of word frequency. Whereas repeated low-frequency words elicited a positive ERP component after 500 ms post-stimulus, the predominant effect for repeated high-frequency words was a reduction of N400 amplitude. The current result is in line with that finding because most words presented in our study belonged to highly to moderately frequent German words.

Several studies showed that the difference between ERPs to new and repeated words begins only after N400 and mainly covers the time window roughly corresponding to the LPC in the present study (e.g., Curran, 1999; Balass et al., 2010). In other studies, the effect starts about 300 ms post stimulus but attains its maximum at about 600 ms (e.g., Rugg et al., 1994, 1997; van Strien et al., 2007). Most frequently, however, the repetition effect involves both N400 and LPC time windows (e.g., Karayanidis et al., 1991; Kutas & Van Petten, 1988; Fischler & Raney, 1991; Race et al., 2010). The earlier effect, that can be regarded as N400 suppression to repeated

words, usually has a parietal maximum. The spatial distribution of the later effect (larger LPC positivity to repeated words) is more variable and has, to our best knowledge, not been an object of a special analysis. Most studies also clearly show the strongest effect in parietal areas (e.g., Rodriguez-Fornells et al 2002; Swick, 1998; Van Strien et al., 2007; Race et al., 2010; Olichney et al., 2006), which, therefore, appears as a direct continuation of the N400 effect. However, other publications indicate a maximal LPC effect at frontal leads (e.g., Bentin & Peled, 1990, nonword detection task; Besson et al., 1992); still others show a maximum at Cz decreasing in both frontal and parietal direction (Rugg et al., 1997).

Repetition effect versus learnt association effect

Contrary to the previous studies, we presented words not in lists (e.g., Rugg, 1985, 1987; Rugg et al., 1994, 1997) and not in sentences (e.g., Besson et al., 1992; Calloway & Perfetti, 2019) but in pairs. Nevertheless, our experiments involved massive repetition of the "old" words, and thus repetition effects were expected. To begin with the LPC effect, the larger positivity in response to old words than to new words was observed only in fronto-central areas and only to the second words in pairs. The former finding does not contradict the repetition effect interpretation; as stated above, most electrophysiological word repetition studies showed a posterior LPC effect, but a minority of studies found fronto-central effects as well. The latter finding appears more surprising. Both first and second "old" words were repeated with the same frequency, thus the repetition effect can be equally expected for both.

At present, most plausible explanation is the rather short and variable onset-to-onset interval within pairs. In about half of the trials, the second word was already presented while the LPC to the first word was being developed. This might substantially suppress the LPC effect but can hardly explain the fact that no LPC altogether can be seen in response to first words.

Turning now to the dynamics of the N400, we see a distinct scale of effects. The strongest increase of the N400 was elicited by new second words in Experiment 2 in the pairs in which the first word was old. This increase was significantly larger than the corresponding increase to the new second words in the pairs in which the first word was also new. Much weaker but still significant at most sites was N400 increment to new first words in Experiment 1. Finally, the weakest change was N400 increment to new (as compared with old) first words in Experiment 2, which was significant only at posterior electrodes.

This hierarchy of N400 increments can, from our point of view, be explained neither by repetition nor by semantic association alone, but only by the *combination* of the two. Particularly, the weakest significant N400 change (Experiment 2, new versus old first word) can be attributed to repetition solely. However, the same effect in Experiment 1 cannot. In Experiment 1, new first words were always related to new second words, and therefore, after a new first word, the subject already knew that a new pair was being presented. Since if the first word was new, the second word was also new, we cannot be sure whether the effect of the first word in Experiment 1 was because the first word was new or because the whole pair was new.

The situation was different in Experiment 2. When hearing the first old word, the participant was unable to know whether the pair is old or new. If N400 suppression to the first word were a pure repetition effect, this effect should be independent of the fate of the second word in a pair; therefore, there would be no reason to expect any difference between the responses in the two experiments. The obtained difference is probably due to the repetition of the whole pair being an additional factor distinct from just word repetition.

Moreover, if word repetition was the dominant effect in the N400 time window, not only the response to the first word would not depend on the second word, but also vice versa, the response to the second word would not depend on the first one. This was also not the case. First,

the increase of the N400 to new words was about twice as strong for second words as for the first words. Additionally, at posterior sites in Experiment 2, the increase of N400 amplitude to the new second word was significantly larger when the preceding word was old than when it was also new.

To summarize, the data indicate the presence of at least three different effects. Two of them may be attributed to the same factor of word repetition, but differ so much from each other (the parietal N400 effect to the first word and the fronto-central LPC effect to the second word) that they cannot be referred to as a single "repetition effect". The concept of several functionally distinct electrophysiological repetition effects has been suggested in the literature (Guillem et al., 1999; Penny et al., 2001; Race et al., 2010; Calloway & Perfetti, 2019), but none of the proposed models can be directly applied to the present data due to the large variety in experimental designs.

Over and above the two repetition effects, the third effect reflects the specifics of the presented stimulus material, i.e., the relations between words in pairs. This factor can explain the differences between the N400 effects in Experiments 1 and 2 and between the responses to old-new and new-new pairs in Experiment 2.

An additional finding that exceeded the explanatory power of the repetition effect is a small but significant difference between N400 responses to *old first words* in the acquisition and test phases of the first experiment. Because each word was already presented twenty times in the acquisition phase, we did not expect any difference as compared with the test phase. But if such a difference takes place, based on the repetition effect one might expect a smaller N400 in the test phase; however, the opposite difference was found.

Within-pair associations

Therefore, a considerable portion of the N400 effect distinguishing between the responses to learnt and new word pairs cannot be attributed to word repetition but is due to a relation emerging between the members of a repeated pair. There is a long lasting discussion in the N400 literature on whether, or to what extent, this component manifests automatic versus controlled processes involved in building semantic associations. The former processes are theorized on the basis of the Automatic Spreading Activation (*ASA)* model (Collins & Loftus, 1975). According to many authors (e.g., Neely, 1977; Neely & Keefe, 1989; Hill, Strube, Roesch-Ely & Weisbrod, 2002**)**, the mental lexicon is assumed to be a semantic network with related words in neighboring nodes. ASA occurs because the lexical access to a word activates the corresponding node and this activation spreads to the neighboring nodes corresponding to related words. When a prime is presented, a set of nodes corresponding to related words are pre-activated. The spreading activation lowers the threshold for the processing of all related nodes. Therefore, when a related (i.e., already pre-activated) word is presented, its processing requires *less* additional activation, thus resulting in a *smaller* amplitude of the N400 to this word. In accordance with this idea, the semantic facilitation effect in a lexical decision task is usually attributed to the spreading-activation process (e.g., Schvaneveldt & Meyer, 1973; Koriat, 1981). Note that the ASA model presumes the same basic underlying mechanism for N400 suppression to *semantically related* second words and to second words in word pairs that have been recently presented and learnt *in semantically unrelated sets*, as compared with semantically unrelated word pairs *presented for the first time*.

Esper (1973; cit. for Maki, McKinley, & Thompson, 2004) emphasized the principal difference between two kinds of associations: inner associations are based on semantic or logical relationships and not on empirical frequencies. Thus CAT and MAMMAL are internally associated even though these two words rarely occur close in most real sentences. In contrast,

CAT and DOG are, to a large extent, an outer association because the two words frequently co-occur.

The process of building such associations between previously unrelated words can be conceived of as a chain of mediated priming processes whose ERP correlates were studied by Chwilla et al. (2000) and Chwilla & Kolk (2002). The former study investigated mediated two-step priming with one intervening concept (e.g., the prime LION activates the target STRIPES via TIGER). The latter study showed that the N400 to the target can be suppressed even when the prime is separated by several mediators (e.g., a prime MANE is associated with the target STRIPES through mediators LION and TIGER).

Following these findings, we assume that, when an unrelated word pair (e.g., HORSE – FAST) is repeated, the brain does not just notice the empirical co-occurrence of the two stimuli. Rather, it is searching for an interpretation of this newly emerging connection (see Kotchoubey, 2006; Schlesewsky & Bornkessel-Schlesewski, 2019). The activation spreads to broader and broader networks of nodes related to the two words. Some of these activated nodes are related to *both* words in the pair and, under their activation, build a semantic bridge between them. In the above example, repeated presentation of HORSE may activate such neighboring nodes as LEGS, RIDING, and finally RACE, which should run FAST to win a match. Thus an association that is primarily built as a purely outer one (in the sense of Esper, 1973), can acquire inner properties.

The automaticity of the processes underlying the N400 effect can, however, be questioned. Several studies demonstrated the automatic N400 effect by using sensory masking, i.e. non-masked target words were presented after strongly masked (unrecognizable) primes (e.g., Dehaene et al., 2001; Kiefer, 2002; Misra & Holcomb, 2003). Daltrozzo et al. (2012) used the same masking approach but masked not only primes but also targets. At the strongest level of

masking, participants were unable to distinguish between congruent and incongruent sentences (i.e., the error rate about 50%), but still could correctly compare the completely masked target word with a subsequent probe word presented without a mask. No N400 to incongruent targets was found in this conditions, while the LPC disappeared at even lighter masking levels when the error rate was still very low (about 15%). These findings contradict the results of the experiments in which only primes were masked, indicating that the N400 effect is lacking when stimuli are not explicitly processed, even if their implicit (i.e., automatic) semantic processing can take place.

In the present study, a completely automatic activation would result in a decrease of the N400 to repeated (i.e., associated) words, thus explaining why the N400 effect was so much stronger to the second than to the first word. It cannot, however, explain an additional N400 increase to the second word in old-new pairs as compared with new-new pairs. Actually, the ASA model predicts only N400 decrement to primed words but not an increment to non-primed words, which is in line with the observation that an N400 is usually present to all non-primed words (e.g., Van Petten, 1995).

The increase of the N400 in old-new pairs can, rather, be accounted for by the view on the N400 reflection the processes of semantic integration (e.g., van Berkum et al., 1999; Sitnikova et al., 2003) or reallocation of resources necessary for such integration (e.g., Kotchoubey, 2006; Schlesewsky & Bornkessel-Schlesewski, 2019). After a new first word, the second word in a pair is also a new one, and there is no need to look for a meaningful relation between two novel, non-learnt, non-associated stimuli. After an old first word, on the other hand, a previously learnt association is activated. On the background of this activation, an unexpected second word can lead to redirection of resources to restore the “lost agreement” between the two members of the pair.

Limitations and further possibilities

Although numerous data of the literature suggest that the effects should be specific for meaningful words, or maybe for meaningful stimuli in general (Sitnikova et al., 2003), this supposed specificity is not warranted. Perhaps we need at least two additional experiments in which (i) meaningless non-words and (ii) equally complex (acoustically) but not word-like sounds would be presented in pairs like in the current experiments.

Another unclear issue concerns the role of attention. ASA theory presumes that the effects should be largely independent of attention. In accord with this idea, some N400 effects were even obtained independently of conscious awareness of stimuli (e.g., Kiefer, 2002). Other data indicate, however, that active instruction to respond to stimuli can increase the amplitude of the N400 as compared with more passive conditions (e.g., Erlbeck et al., 2014). Particularly, the process of semantic integration supposedly responsible for the increase of the parietal N400 in old-new pairs, can be attention-dependent. In the present study, we used an instruction that demanded participants' attention to stimuli. In some contexts, e.g., in clinical applications of the paradigm, the issue of attention may be very important. A replication of the present experiments without active instruction would help to clarify the issue.

Conclusions

We found that the acquisition of new word association in semantically unrelated word pairs results in a change of N400 amplitude very much similar to that observed in semantically related word pairs. The mere effect of word repetition, though present, does not explain all obtained

results. Consistent co-occurrence of words elicited additional effects more specifically related to associative learning. The relative role of automatic processes (particularly, the Automatic Spreading Activation) and controlled, potentially attention-dependent processes (e.g., semantic integration) should be investigated in further experiments.

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