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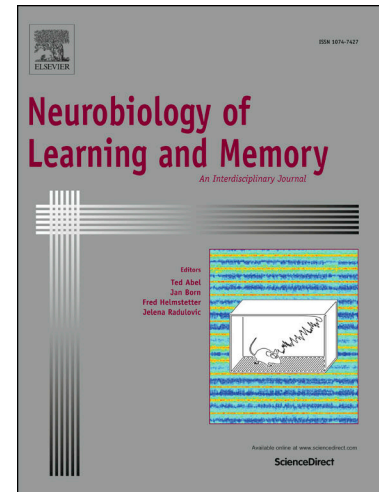
Differential neurophysiological correlates of retrieval of consolidated and reconsolidated memories in humans:

an ERP and pupillometry study

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Abstract

Consolidated memories can return to a labile state if they are reactivated by unpredictable reminders. To persist, active memories must be re-stabilized through a process known as reconsolidation. Although there is consistent behavioral evidence about this process in humans, the retrieval process of reconsolidated memories remains poorly understood. In this context, one fundamental question is whether the same or different neurophysiological mechanisms are involved in retrieval of consolidated and reconsolidated memories. Because it has been demonstrated that the exposure to the reconsolidation process may restructure and strengthen memories, we hypothesized distinct neurophysiological patterns during retrieval of reconsolidated memories. In addition, we hypothesized that interfering with the reconsolidation process using a new learning can prevent these neurophysiological changes. To test it, consolidated, reconsolidated and declarative memories whose reconsolidation process was interfered (i.e., picture-word pairs) were evaluated in humans in an old/new associative recall task while the brain activity and the pupillary response were recorded using electroencephalography and eyetracking. Our results showed that retrieval of reconsolidated memories elicits specific patterns of brain activation, characterized by an earlier peak latency and a smaller magnitude of the left parietal ERP old/new effect compared to memories that were only consolidated or whose reconsolidation process was interfered by a new learning. Moreover, our results demonstrated that only retrieval of reconsolidated memories is associated with a late reversed mid-frontal effect in a 600-690 time window. Complementarily, memories that were reactivated showed an earlier peak latency of the pupil old/new effect compared to non-reactivated memories. These findings support the idea that reconsolidation has an important impact in how memories are retrieved in the future, showing that retrieval of reconsolidated memories is partially supported by specific brain mechanisms.

Highlights

- We studied retrieval of declarative memories that were consolidated, reconsolidated or whose reconsolidation process was interfered by a new learning, in humans.
- Retrieval of reconsolidated memories were associated to an earlier and smaller left parietal old/new effect, followed by a reversed mid-frontal old/new effect.
- Retrieval of memories that were reactivated showed an earlier peak latency compared to retrieval of non-reactivated memories.
- Partially distinct brain mechanisms may support retrieval of reconsolidated memories.

1. Introduction

How memory changes over time is a fundamental question for the neuroscience of memory. Answering this question has important implications not only for the basic understanding of memory but also for the development of applications in the clinical and educational fields. For instance, knowing how memories change could allow the design of techniques to modulate existing memories in patients with memory disorders (Sandrini et al., 2015; Schwabe et al., 2014) as well as the development of new procedures to improve learning in educational contexts (Karpicke & Roediger, 2008). One significant step towards a better understanding of the dynamic nature of memory was the discovery that once a memory is stored in the brain, it does not remain permanently fixed or unmodifiable. In fact, it has been demonstrated that previously consolidated memories can return to an unstable state if they are reactivated under specific circumstances. In order to persist, these memories must be re-stabilized by a process known as memory reconsolidation (Nader & Hardt, 2009; Nader, Schafe, & Le Doux, 2000). During this process, reactivated memories may become vulnerable to interference by pharmacological agents (Schwabe et al., 2012) or behavioral manipulations (Forcato et al., 2007; Hupbach et al., 2007; Walker et al., 2003), giving us a unique opportunity to modulate memories (Sandrini et al., 2015). However, little is known about the neural processes by which these memories, that were successfully reconsolidated or whose reconsolidation process was interfered, are retrieved in the future. In this context, the main purpose of the current study was to investigate the neurophysiological correlates (i.e., neural activity and pupillary response) underlying retrieval of declarative memories that were successfully reconsolidated or interfered during the reconsolidation process.

Taking into account the evidence that the reconsolidation process allows restructuring of memories through modification of their strength and content (Dudai, 2012; Forcato et al., 2011; Sandrini et al., 2015), it seems plausible that retrieval of consolidated, reconsolidated and interfered memories during reconsolidation is supported, at least partially, by distinct brain mechanisms. Therefore, we hypothesized that consolidated, reconsolidated and interfered memories elicit different neurophysiological patterns during retrieval. Specifically, because it has been demonstrated that the exposure to the reconsolidation process may lead to memory strengthening, we expected patterns of neural activity reflecting facilitated recollective processes during retrieval of reconsolidated memories. Accordingly, we also expected that the interference of reconsolidation by a new learning would prevent these neural changes. To test it, we implemented an adapted version of the three-day protocol used in a previous study (Forcato et al., 2016). Memory performance was evaluated using an associative recall old/new task (Donaldson & Rugg, 1999) in which participants had to classify pictures as "old" or "new" (previously studied or not) and to recall a word associated to old items. In order to examine the neurophysiological correlates of retrieval, we measured simultaneously event-related potentials (ERPs) and pupillary responses using electroencephalography (EEG) and eyetracking, respectively. We mainly focused on two known biological markers associated with memory retrieval: the left parietal ERP old/new

effect (Donaldson & Rugg, 1998, 1999; Rugg & Curran, 2007; Wilding, 2000; Wilding & Evans, 2012) and the pupil old/new effect (Heaver & Hutton, 2011; Kafkas & Montaldi, 2015; Montefinese et al., 2013; Otero et al., 2011; Vo et al., 2008). The left parietal old/new effect, that has been observed in different retrieval tasks such as source retrieval, cued recall and old/new recognition, refers to the positive difference in neural activity between the correct classification of old and new items (Donaldson & Rugg, 1999; Paller & Kutas, 1992; Wilding & Rugg, 1997a). This effect has a left parietal maximum around 500-800 ms post-stimulus and it has been traditionally linked to conscious recollection (Rugg & Curran, 2007; Vilberg et al., 2006). On the other hand, the pupil old/new effect refers to the greater pupil size in response to old compared to new items (Montefinese et al., 2013; Vo et al., 2008). Although the interpretation of this effect remains unclear, it has been suggested that it is related to recollective processes, analogous to the left parietal old/new effect (Brocher & Graf, 2016; Otero et al., 2011; Vo et al., 2008).

Our results showed that retrieval of consolidated and reconsolidated memories elicits different patterns of brain activity and pupillary response. We believe that the current study is an important step towards a better understanding of the brain mechanisms underlying retrieval of reconsolidated declarative memories.

2. Materials and Methods

2.1 Participants

A sample of 92 volunteers (24 males, 68 females, mean age = 21.4 years [S.D=2.3]) participated in the experiment. All participants were recruited from the student population of the Pontificia Universidad Católica de Chile.

All volunteers were native spanish speakers, had normal or corrected-to-normal vision and no reported history of psychiatric or neurological disorders. Additionally, participants were instructed to refrain from caffeine, alcohol and taking naps during the experimental days.

EEG data from 22 participants and pupillary data from 24 participants were excluded from further analysis due to technical reasons (i.e., poor signal quality or excessive artifacts in EEG or pupillary recordings) or because they did not reach the minimum score at the training session (70% of performance, see Memory Task). For the EEG dataset, the final sample comprised 70 participants (16 males, 54 females, mean age = 21.3 years [S.D=2.4]), with 17 participants in the reactivation group (4 males, 13 females, mean age = 22.3, [S.D=1.6]), 18 participants in the reactivation/interference group (4 males, 14 females, mean age = 20.8, [S.D=2.0]), 18 participants in the interference group (4 males, 14 females, mean age = 21.3, [S.D=2.5]) and 17 participants in the no-reactivation/no-interference group (4 males, 13 females, mean age = 20.8, [S.D=3.0]). For the pupil dataset, the sample was composed of 68 subjects (16 males, 52 females, mean age = 20.9 years [S.D=2.0]), with 16 participants in the reactivation group (5 males, 11 females, mean age = 21.9, [S.D=1.4]), 19 participants in the reactivation/interference group (3 males, 16 females, mean age = 20.2, [S.D=1.4]), 16 participants in the inference group (5 males, 11 females, mean age = 21.4, [S.D=2.6]) and 17 participants in the no-reactivation/no-interference group (3 males, 14 females, mean age = 20.3, [S.D=2.0]).

Because the final selection of participants (and acceptable trials) differed for EEG and pupil recordings after the preprocessing stage, independent datasets of participants were used for EEG and pupil size analyses. However, as shown in the behavioral results, both populations are comparable (see Table S1).

This study was approved by the Ethics Committee of the Pontificia Universidad Católica de Chile and all participants gave informed, written consent before taking part in this investigation.

2.2 Stimuli

Stimuli were 120 pictures of everyday objects and situations (48 for old and 24 for new conditions; and 48 for interference by new learning). From this pool of pictures, 72 were used in a previous study of memory reconsolidation (Forcato et al., 2016) and 48 were added to guarantee a sufficient number of trials for reliable ERP and pupil analyses.

Each picture of the old condition or the interference list (see Figure 1) was paired with a 3-syllable spanish word, not directly related. All the words were nouns and were composed by six letters. The stimuli were presented using Psychopy (Peirce, 2007) on a Tobii Pro TX300 monitor (Tobii Technology, Inc., Sweden). All the pictures were equated in contrast and luminance and were presented in the center of the screen, in grayscale with a gray background, within a rectangular area of approximately 9 cms (h) x 13 (w) cms and at a viewing distance of 65 cms, providing a visual angle of approximately 7° (h) x 11° (w). All the words and syllables were presented in uppercase and in black over a white rectangle superimposed on its associated picture. Additionally, a white central fixation cross was displayed during intertrial intervals to avoid unnecessary eye movements that could affect electroencephalographic and pupillary recordings.

2.3 Experimental Design

We used a modified version of the paradigm described by Forcato et al., (2016). In this context, three sessions took place within a week, being separated by 48 hours (monday, wednesday and friday) (see Figure 1). Each subject participated in 2 or 3 sessions, depending on the experimental group assigned (see Table 1).

Session 1: Training

At the beginning of session 1, participants were instructed to learn 48 picture-word pairs (for instance, a picture of clouds and the word "PALOMA", pigeon in spanish). Each trial started with the presentation of a fixation cross for 3 seconds. Then, each picture was shown for another 3 seconds. Immediately after, the associated word was superimposed onto the upper part of the picture for 1 second. This procedure was repeated until the 48 pairs were presented.

Five minutes later, learning was evaluated. Test trials began with the presentation of a fixation cross for 3 seconds. Then, each picture was presented alone for 3 seconds followed by the presentation of the picture and only the first syllable of the associated word, for 1 second (for instance, the picture of the clouds and the syllable "PA"). Afterwards, and every time an image of a microphone appeared, participants had 2 seconds to say aloud the associated word of the respective picture. Then, the picture and the associated word were presented for 1 second. This procedure was repeated for the total of picture-word pairs.

Session 2: Memory Reactivation and/or Interference

In the second experimental session, forty-eight hours later, memory was reactivated in order to trigger the labilization-reconsolidation process. As was described earlier, a proper reminder structure is crucial to destabilize a consolidated memory and

trigger the reconsolidation process. Specifically, previous findings have demonstrated that the detection of a prediction error (a discrepancy between what is expected and what actually happens) is necessary for the destabilization of an existing memory (Fernandez et al., 2016; Forcato et al., 2016; Pedreira et al., 2004). Accordingly, only unpredictable cues were used, and the prediction error was incorporated in the reminder structure as an interruption of the task that occurred before the participant could answer in each trial. This session was composed of two blocks: the memory reactivation and the subsequent interfering task.

During the reactivation procedure, participants were instructed to say the associated word aloud when a microphone image appeared on the screen. Each trial began with a fixation cross in the center of the screen for 3 seconds. Then, a studied picture was presented for 3 seconds followed by the simultaneous presentation of the picture and the first syllable of the associated word, for 1 second. Afterward, the experiment was interrupted with the text "INTERRUPCIÓN" ("interruption") for 2 seconds, followed by the text "RETOMA" ("trial continues") for another 3 seconds, indicating that the task continues in the next trial. This procedure was repeated for all the 48 picture-word pairs. Because no microphone images were presented during this block, participants could not provide an answer during the trials. To maintain the expectation of the appearance of the microphone and to avoid distraction and habituation, subjects were instructed to pay attention to the computer screen until all images and texts were presented and were told that the microphone image could appear at any time during the task.

To target the reconsolidation process (Forcato et al., 2007; Hupbach et al., 2007), some participants performed an interfering task five minutes after memory reactivation (see Figure 1 and Table 1). In this task, using the same procedure as in the training session, participants were asked to learn a new list of 48 picture-word pairs. Five minutes later, learning was evaluated. This procedure allowed us to investigate the retrieval of declarative memories whose reconsolidation process was interfered, preventing its subsequent effects on memory.

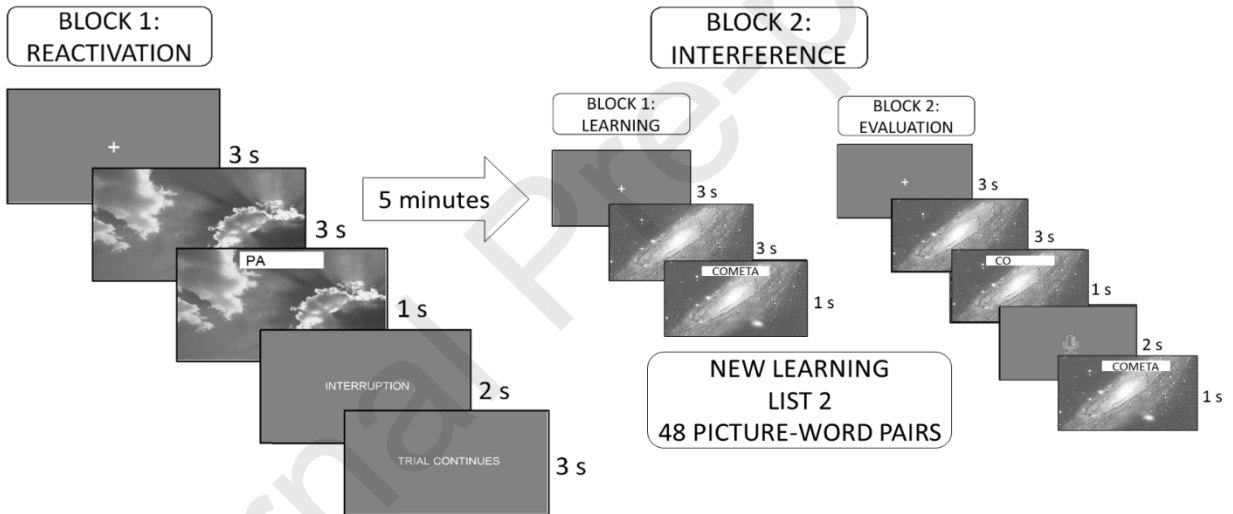**Session 3: Memory Testing**

Forty-eight hours after memory reactivation and/or interference, memory was evaluated in an associative recall old/new task (Donaldson & Rugg, 1999) while brain activity and pupillary responses were simultaneously recorded by means of EEG and eyetracker, respectively. At the beginning of each trial, a fixation cross appeared in the center of the screen for a randomly selected duration of 2, 3 or 4 seconds. Pictures studied on session 1 (list 1, old pictures) and an additional set of 24 non-studied pictures were randomly shown one at a time for 4 seconds each, followed by the microphone image for another 3 seconds. Participants were instructed to say aloud, during the presentation of the microphone image, "VISTA" ("seen" in spanish) when an item was

recognized as old or "NO VISTA" ("not seen") when an item was recognized as new. Additionally, for pictures recognized as old, the participant had to say the associated word aloud or "No sé" ("I don't know"), in case it was not possible to recall it. To obtain a sufficient number of trials in each condition for ERP and pupil old/new effect analyses, the task was repeated 3 times in 3 independent blocks, separated by five minutes.
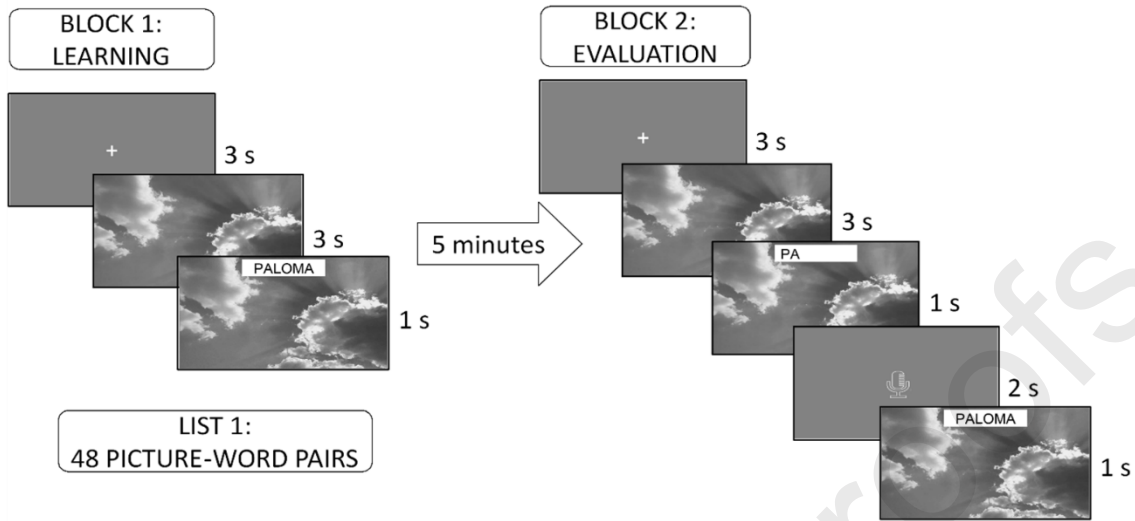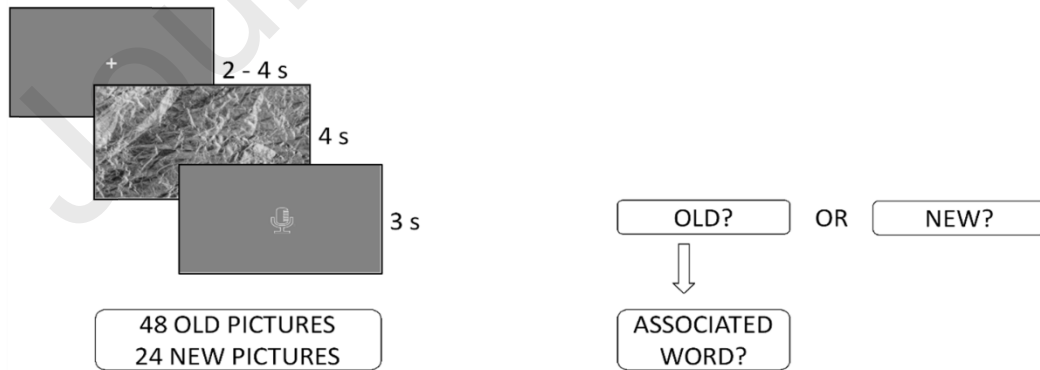
Figure 1. Experimental design (adapted from Forcato et al., 2016).

2.4 Experimental Groups

Participants were randomly assigned to one of the four following experimental groups: reactivation group, reactivation/interference group, interference group and no-reactivation/no-interference group. All groups were exposed to identical training and testing procedures in session 1 and session 3, respectively. During the second session, the reactivation group only underwent memory reactivation; the reactivation/interference group underwent memory reactivation followed by the interference task (new learning); the interference group was only exposed to the interference task; and the no-reactivation/no-interference group did not come back to the laboratory for the session 2 to prevent any kind of unintentional memory reactivation (See Table 1).

EXPERIMENTAL GROUPS	SESSION 1	SESSION 2	SESSION 3
REACT	TRAINING	REACTIVATION	TESTING
REACT/INT	TRAINING	REACTIVATION + INTERFERENCE	TESTING
INT	TRAINING	INTERFERENCE	TESTING
NO-REACT/NO-INT	TRAINING	-	TESTING

Table 1. Experimental Groups.

2.5 Behavioral Analysis

Learning in session 1 was measured by quantifying correct responses (correct recall of the associated word) and expressed as a percentage of the total number of picture-word pairs. Following Donaldson and Rugg (1999), to evaluate memory in session 3, we calculated the percentage of old responses with the correct recall of its associated word, the percentage of old responses with no word recall ('don't know' response) and the percentage of old responses with incorrect recall for those items that were correctly recognized as old in the initial old/new judgment. Additionally, we calculated the recognition accuracy, measured as the difference between hits (correctly recognized old pictures) and false alarms (incorrectly classified new pictures). In all cases, memory evaluation considered the average scores from the three independent test blocks.

2.6 EEG Recording and Analysis

A continuous EEG recording was acquired from 64 Ag-AgCl active electrodes - mounted in an elastic cap according to the extended International 10-20 System - using the Biosemi ActiveTwo system (Biosemi, Amsterdam, Netherlands). Additionally, left and right mastoids were recorded to be used as reference electrodes in offline analyses. During the recording, impedances were kept below 20kΩ. The continuous data was recorded at a sampling rate of 2048 hz and stored offline for later analysis.

For ERP analyses, based on Donaldson and Rugg (1999), two response categories were considered: correct old responses (correct classification of old pictures and recall of their associated words) and correct new responses (correct classification of new pictures).

The data was preprocessed and analyzed with MATLAB (MathWorks, Inc., Natick, MA) using EEGLAB toolbox (version 14.1.1) (Delorme & Makeig, 2004) and ERPLAB (version 6.1.3) (Lopez-Calderon & Luck, 2014). For each subject, the three independent test blocks were imported and concatenated. Then, the data was resampled to 1024 hz, bandpass filtered using a Butterworth filter (half amplitude cutoffs at 0.1 and 100 hz, 12 dB/octave roll-off) and re-referenced to the average of the left and right mastoids. Continuous data was then epoched into 3000 ms segments (1000 ms before and 2000 ms after the picture onset). Segments of EEG were visually inspected to identify and remove bad channels and muscular activity artifacts. Additionally, blinks and eye movements artifacts were identified and manually removed using independent component analysis (ICA) (Delorme & Makeig, 2004; Jung, Makeig, Humphries, et al., 2000; Jung, Makeig, Westerfield, et al., 2000). After ICA, removed channels were interpolated using spherical-spline interpolation, as implemented in EEGLAB.

To compute ERPs, the data was resampled to 512 hz and bandpass filtered using a Butterworth filter (half amplitude cutoffs at 0.1 and 30 hz, 12 dB/octave roll-off). Remaining artifacts were automatically rejected using the moving peak-to-peak algorithm (Lopez-Calderon & Luck, 2014) (with a voltage threshold of ±100 mV, moving windows full width of 200 ms and window step of 50 ms) in epochs from -200 to 2000 ms relative to the picture onset. The pre-stimulus window of 200 ms was used to correct for baseline activity in each trial. Then, the ERP old/new effect was calculated as the difference between ERPs elicited by the correct classification of old items (and the recall of their associated words) and new items. For tmax permutation analyses (Groppe et al., 2011a, 2011b) (see Statistical Analyses section), the data was downsampled to 128 hz to decrease the number of comparisons and increase statistical power (Luck, 2014). Following an examination of the permutation results, the difference score (old minus new) of the peak latencies, peak amplitudes (200-800 ms time window) and mean amplitudes (450-800 ms time window) relative to the 200 ms pre-stimulus baseline, were independently computed for two regions of interest (ROIs): left parietal (CP1, CP3, CP5, P1, P3, P5) and right parietal (CP2, CP4, CP6, P2, P4, P6).

For the tmax permutation procedure and traditional ERP analyses, time windows and electrode sites (parietal and midfrontal ROIs) were selected based on existing literature on the temporal course and topographical characteristics of the left parietal and mid-frontal old/new effects (Rugg & Curran, 2007).

2.7 Pupil Recording and Analysis

A Tobii TX300 eye tracker system was used during session 3 to acquire the pupil size, with a sampling rate of 300 hz. The Tobii Studio software was used to record the pupillometry data and export it for later analyses. The system was calibrated before each test block using the Tobii Studio's 9-point automated calibration routine. The exported data were processed using custom MATLAB scripts.

For pupil diameter analyses, pupil size of the right eye was chosen. Participants with excessive missing data due to head movements, pupil signal loss and blinks during the total recording time were excluded from analysis. Specifically, trials containing less than 50% of valid pupil recordings were excluded and, if 50% of all trials for each condition were missing after the former correction, the participant was no longer considered in further analyses. Then, missing data were linearly interpolated, signals were low-pass filtered at 3 hz and data was epoched from -1000 to 4000 ms relative to the image onset (time interval from -1000 to 0 ms was used as baseline). Only correct old (old response + recall of the associated word) and correct new responses were considered. Afterwards, maximal pupil dilation from baseline was independently calculated for each trial (Wainstein et al., 2017) and the difference between the post-stimulus and baseline values were computed. For each subject, the pupil old/new effect was calculated subtracting the mean maximal pupil diameter associated to correct old responses from the mean maximal pupil diameter associated to correct new responses. As a complementary measure, the mean pupil diameter was computed in the 1500-2500 ms time window for correct old and new responses and the pupil old/new effect was calculated as the difference. Finally, for each participant, the old/new effect was calculated, from which we extracted the peak latency (in a 500 – 3300 ms time window). To find the maximum in this case, we looked for the local maximum (i.e., the point within the curve in which the derivative is 0, being positive in the left side of the point and negative in the right side).

Selection of time windows was based on a visual inspection of the waveforms and on the literature (Montefinese et al., 2013; Vo et al., 2008)

2.8 Statistical Analyses

Behavioral data for the ERP and pupil datasets were analyzed with non-parametric Kruskal-Wallis test, followed by planned comparisons (corrected by post-hoc Dunn's comparisons). Four contrasts of interest were considered due to their theoretical relevance and importance for the hypotheses proposed. To test our hypothesis that the exposure to the reconsolidation process may strengthen memories and modulate the neurophysiological correlates of retrieval, the comparison between groups reactivation and no-reactivation/no-interference was made. To test our hypothesis that the interference by new learning may affect the reconsolidation process initiated by retrieval, preventing memory strengthening and neurophysiological changes, we compared reactivation and reactivation/interference groups. Complementarily, as control protocols,

we made the comparisons reactivation/int vs interference and interference vs no-reactivation/no-interference to investigate the specific effects of the behavioral interference on previously reactivated and non-reactivated memories.

All p-values reported were two-tailed and were considered significant if they were less than 0.05.

Differences between ERPs elicited by old and new categories were detected using a two-tailed permutation test based on the tmax statistic (Blair & Karniski, 1993). One critical advantage of the tmax procedure is that uses the characteristics of the actual data to evaluate statistical significance. Specifically, the tmax permutation test permutes the labels of the samples in order to estimate the null distribution of the tmax values (i.e., the largest t value computed across all electrodes and time points in each iteration). To assess statistical significance, the real t values from the recorded data are calculated for each electrode/time bin and compared to the extreme values of the estimated distribution of tmax values. This method was chosen because it allows to determine, with strong confidence and without previous assumptions, the temporal dynamic and topographical distribution of an effect (Luck, 2014).

We performed the tmax permutation procedure on mean difference wave amplitudes considering five time windows (300-390, 400-490, 500-590, 600-690 and 700-790 ms), including 25 representative electrodes from occipital, parietal and mid-frontal areas (O1, Oz, O2, PO7, POz, PO8, P7, P3, Pz, P4, P8, TP7, CP3, CPz, CP4, TP8, AF3, AFz, AF4, F3, Fz, F4, FC3, FCz and FC4), with a family-wise alpha level of 0.01 and 100.000 permutations, as implemented in the Mass Univariate ERP Toolbox for MATLAB (Groppe et al., 2011a, 2011b). The selection of time windows and the electrodes sites had the objective of maximizing statistical power (Luck, 2014).

To investigate differences between groups in the mean amplitude (450-800 ms time window), peak latency and peak amplitude (200-800 ms time window) of the old/new effect, an one-way ANOVA was performed on the difference scores between old and new items in planned comparisons (the same contrasts of interest considered in behavioral analyses, Bonferroni-corrected). All p-values were considered significant if they were less than 0.05.

To estimate reliable differences in pupil size between old and new responses in each group (pupil old/new effect), we performed a permutation test over consecutive time windows of 100 ms each (family-wise alpha level of 0.01 and 10.000 random permutations). Following the observation of the permutation results, two-tailed independent t-tests were computed for the maximal and mean pupil diameter elicited by correct old and new responses, for each group. Subsequently, an one-way ANOVA was used to compare the difference scores of the maximal and mean pupil diameter between groups and a two-way ANOVA (with reactivation and interference as the between-subject factors) to compare the peak latency of the pupil old/new effect between groups.

T-tests, Kruskal Wallis and ANOVA analyses were performed using Prism software (version 7, GraphPad Software, San Diego, CA) and MATLAB.

3. Results
3.1 Behavioral Data

In session 3 (testing session), both in EEG and pupil datasets, significant differences between groups were detected in the percentage of correct old responses with correct recall, showing the reactivation group a higher percentage of correct responses compared to the reactivation/interference group and in comparison to the no-reactivation/no-interference group, as shown in Figures 2b, S1b and Table S1 (Kruskal-Wallis test, $p<0.0001$ and $p<0.0001$, for EEG and pupil dataset, respectively; reactivation vs reactivation/inference, $p=0.0463$ in EEG and $p=0.0441$ in pupil dataset; reactivation vs no-reactivation/no-interference, $p<0.0001$ in EEG and $p<0.0001$ in pupil dataset, Dunn's post-hoc test).
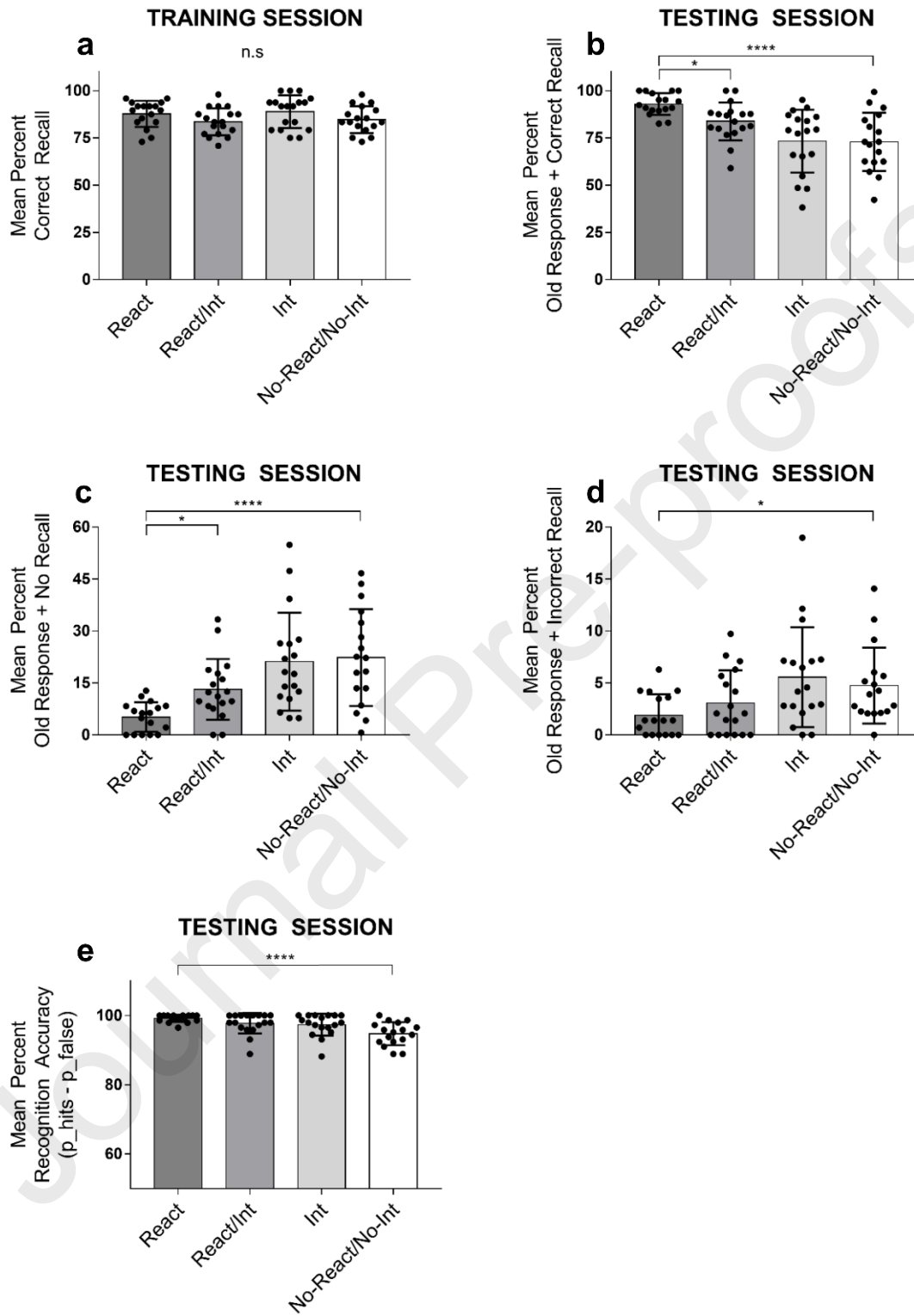
Differences between groups were also detected in the percentage of old responses with no word recall. Specifically, in the EEG dataset, the reactivation group had a lower percentage of old responses with no word recall compared to the reactivation/interference and the no-reactivation/no-interference groups, as shown in Figure 2c and Table S1 (Kruskal-Wallis test, $p<0.0001$; reactivation vs reactivation/interference, $p=0.0408$; reactivation vs no-reactivation/no-interference, $p<0.0001$, Dunn's post-hoc test) . In the pupil dataset, the reactivation group showed a lower percentage of old responses with no word recall compared to the no-reactivation/no-interference group but no differences were observed between reactivation and reactivation/interference groups, although there was a trend towards significance, as shown in Figure S1c and Table S1 (Kruskal-Wallist test, $p<0.0001$; reactivation vs reactivation/interference, $p=0.0731$, reactivation vs no-reactivation/no-interference, $p<0.0001$, Dunn's post-hoc test).

Additionally, there were significant differences between groups in the percentage of old responses with incorrect recall, in both EEG and pupil datasets, showing the reactivation group a lower percentage compared to the no-reactivation/no-interference group, as shown in Figures 2d, S1d and Table S1 (Kruskal-Wallis test, $p=0.0121$ and $p=0.0023$ for EEG and pupil datasets, respectively; reactivation vs no-reactivation/no-interference, $p=0.0458$ for EEG and $p=0.0066$ for pupil datasets, Dunn's post-hoc test).

Regarding hit rate, false alarm rate and recognition accuracy (hits – false alarms), differences between groups were detected in both EEG and pupil datasets. In the EEG dataset, the reactivation group had a higher percentage of hits and a lower percentage of false alarms compared to the no-reactivation/no-interference group (see Table S1) (Kruskal-Wallis test, $p=0.01$, reactivation vs no-reactivation/no-interference, $p=0.023$, for hits; Kruskall-Wallis test, $p=0.024$, reactivation vs no-reactivation/no-interference, $p=0.009$, Dunn's post-hoc test). In the pupil dataset, the reactivation/interference group showed a higher percentage of hits compared to the interference group (see Table S1) (Kruskal-Wallis test, $p=0.009$, reactivation/interference vs interference, $p=0.021$, Dunn's post-hoc test). Finally, in both EEG and pupil datasets, the results showed differences between reactivation and no-reactivation/no-interference groups in the percentage of recognition accuracy, as shown in Figures 2e, S1e and Table S1 (Kruskal-Wallis test,

p=0.0002 and p=0.007 for EEG and pupil datasets, respectively; reactivation vs no-reactivation/no-interference groups, p<0.0001 and p=0.0048, for EEG and pupil datasets, Dunn's post-hoc test).

In summary, the reactivation group (in which memory was successfully reconsolidated) showed an overall better performance compared to the reactivation/interference and no-reactivation/no-interference groups in the memory task in session 3. This result cannot be explained by differences in the initial learning in session 1 (see Figure 2a, S2a and Table S1).

TRAINING SESSION

a

n.s

Mean Percent Correct Recall

React React/Int Int No-React/No-Int

TESTING SESSION

b

*

****

Mean Percent Old Response + Correct Recall

React React/Int Int No-React/No-Int

TESTING SESSION

c

*

****

Mean Percent Old Response + No Recall

React React/Int Int No-React/No-Int

TESTING SESSION

d

*

Mean Percent Old Response + Incorrect Recall

React React/Int Int No-React/No-Int

TESTING SESSION

e

****

Mean Percent Recognition Accuracy (p_hits - p_false)

React React/Int Int No-React/No-Int

Figure 2. Behavioral results (EEG dataset). (a) Mean percent of correct responses achieved by each group in session 1 (training); (b) mean percent of old responses with correct recall, (c) no recall and (d) incorrect recall; and (e) mean percent of recognition accuracy in session 3 (testing), for each group. Error bars depict ± S.D. and asterisks indicate statistically significant differences for contrasts of interest (React vs React/Int, React vs No-React/No-Int, React/Int vs Int, No-React/No-Int vs Int; n.s: no significant; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001, Kruskal-Wallis test, followed by Dunn's post-hoc comparisons).

3.2 Electrophysiological Results

Representative plots of the left parietal old/new effect, quantified in measures of mean amplitudes, can be seen in Figure 3.

Tmax permutation tests showed differences between old and new categories in all groups. These results are illustrated in raster diagrams in Figure 4 and in topographical plots in Figure 5.

In the reactivation group, the permutation test revealed a positive difference between old and new categories over occipital, parieto-occipital, parietal and temporo-parietal regions in the 400-490 ms (PO7, O1, Oz, TP8, P8, PO8, O2) and in the 500-590 ms (P7, PO7, O1, POz, Oz, P8, PO8, O2) time windows. Additionally, a later reversed old/new effect was detected over mid-frontal electrodes (AF3, F3, FC3, AFz, Fz, FCz, AF4, F4, FC4, TP8) in the 600-690 ms time window.

In the reactivation/interference group, the tmax permutation test revealed a positive effect over occipital electrodes in the 400-490 ms window (O1, Oz) and in occipital, parietal and parieto-occipital sensors (P7, P3, PO7, O1, Pz, POz, Oz, P8, PO8, O2) in the 500-590 ms time window.

In the interference group, a positive difference between old and new categories was observed in occipital (O1, Oz, O2) and left parietal electrodes (P7, P3, PO7) in the 500-590 ms time window. An old/new significant difference was also detected in the P7 electrode in the 400-490 ms time window.

Finally, in the no-reactivation/no-interference group, a positive effect was detected in centro-parietal, parietal, parieto-occipital and occipital electrodes (CP3, P7, P3, PO7, O1, CPz, Pz, POz, Oz, P8, P4, PO8, O2) in the 500-590 ms time window. This effect extended to the 600-690 ms time window in the P7 and Oz electrodes.

In summary, the tmax permutation tests revealed a consistent parietal old/new effect in all groups. However, retrieval of reconsolidated memories showed a distinct temporal and topographical profile compared to retrieval of memories that were consolidated or interfered during reconsolidation.
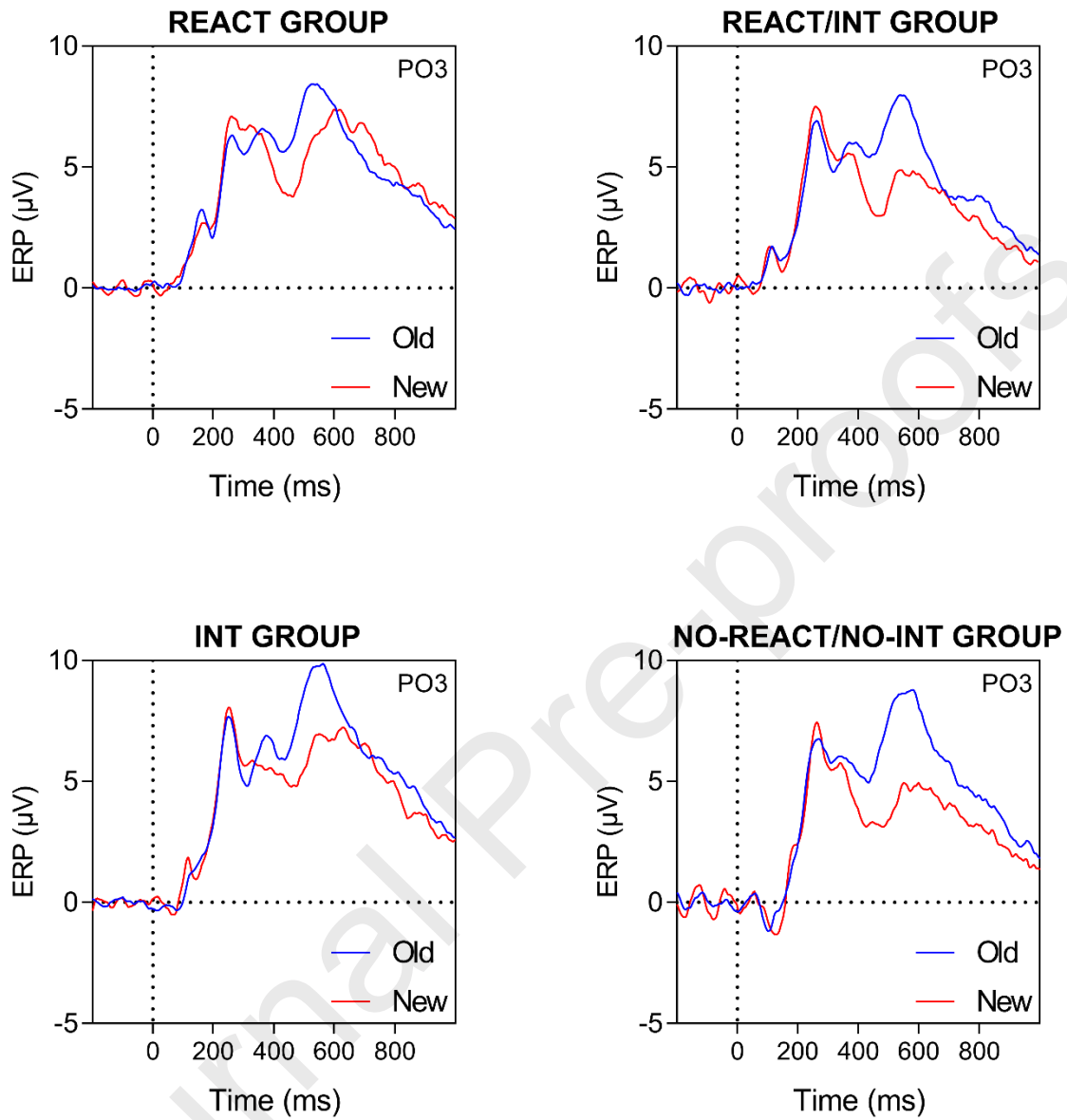
Figure 3. Grand-averaged ERP waveforms from a representative electrode (left parietal region, PO3) for correct responses. In blue, the potential elicited by the correct classification of an old image and the recall of the associated word. In red, the potential elicited by the correct classification of a new image.
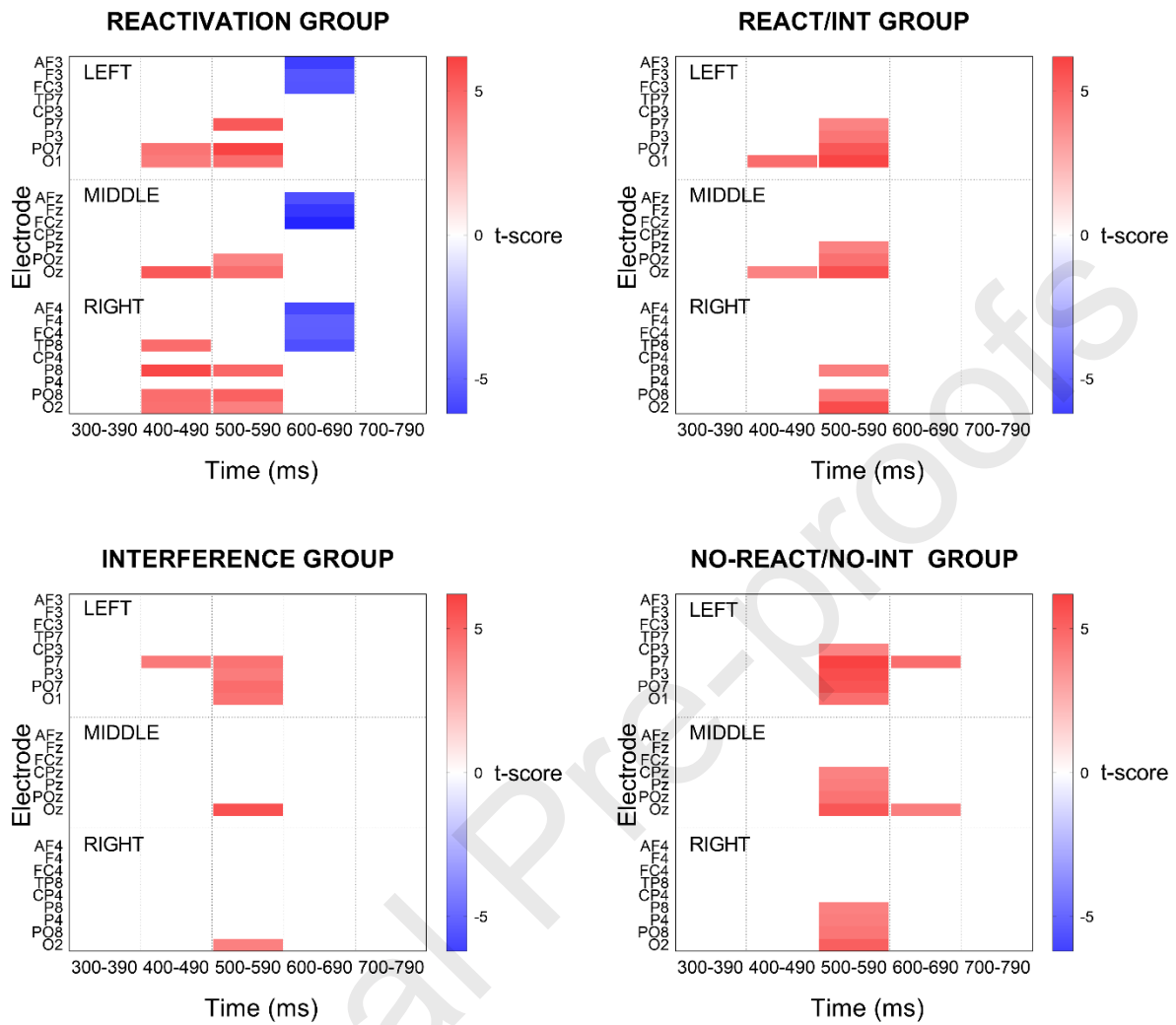
Figure 4. Raster diagrams illustrating the temporal-spatial distribution of the significant effects for each time-electrode bin, according to the tmax permutation tests (in red, positive t values indicating positive differences, old>new; in blue, negative t values indicating negative differences, new>old).
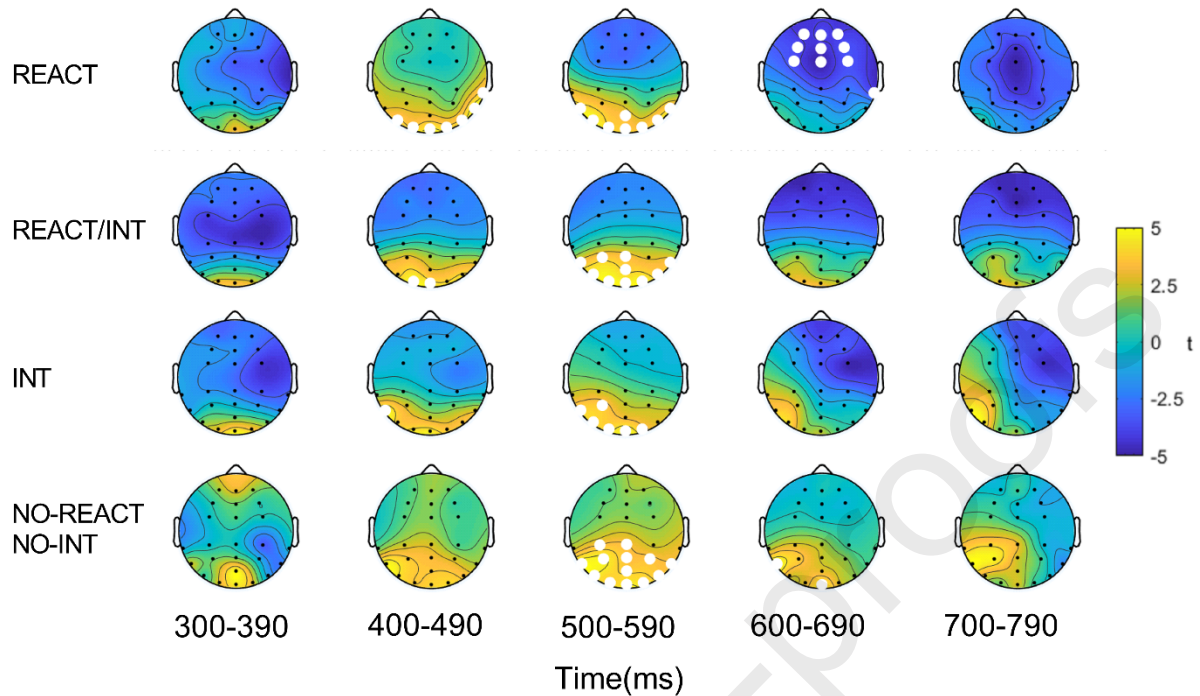
Figure 5. Topographical maps showing the scalp distributions of the significant differences between old (correct old + correct recall) and new responses as obtained by the tmax permutation test, in five temporal windows (300-390, 400-490, 500-590, 600-690 and 700-790 ms), for each experimental group. Note that, in the case of the reactivation group (first row), a reversed old/new effect (new>old) was detected in the 600-690 ms time window.

To evaluate group differences in the peak latency, peak amplitude and mean amplitude of the parietal old/new effect, differences scores (old minus new) were compared independently for left and right parietal ROIs.

In the left parietal ROI (CP5, CP3, CP1, P5, P3, P1), significant differences between groups were observed in the peak latency (200-800 ms time window), showing the reactivation group an earlier peak latency of the parietal old/new effect compared to the reactivation/interference and the no-reactivation/no-interference groups, as shown in Figure 6a (one-way ANOVA, $F_{(3, 66)} = 4.3$, p=0.0076; reactivation vs reactivation/interference, p=0.0238, reactivation vs no-reactivation/no-interference, p=0.015, Bonferroni-corrected).

Differences in the peak amplitude were also detected between groups in the left parietal ROI. Specifically, the reactivation group showed a smaller peak amplitude compared to the no-reactivation/no-interference group, as can be seen in Figure 6c (one-way ANOVA, $F_{(3, 66)} = 3.2$, p=0.0303; reactivation vs no-reactivation/no-interference, p=0.0451, Bonferroni-corrected).

Additionally, significant differences were observed between groups in the mean amplitude (450-800 ms time window), showing the reactivation group a smaller mean amplitude of the parietal old/new effect compared to the no-reactivation/no-interference group in the left parietal ROI (see Figure 6e) (one-way ANOVA, $F_{(3, 66)} = 2.951$, $p=0.0390$; reactivation vs no-reactivation/no-interference, $p=0.0324$, Bonferroni-corrected).

In the right parietal ROI (CP2, CP4, CP6, P2, P4, P6), no significant differences between groups were observed in the peak latency, peak amplitude and mean amplitude, as shown in Figure 6b, 6d and 6f.
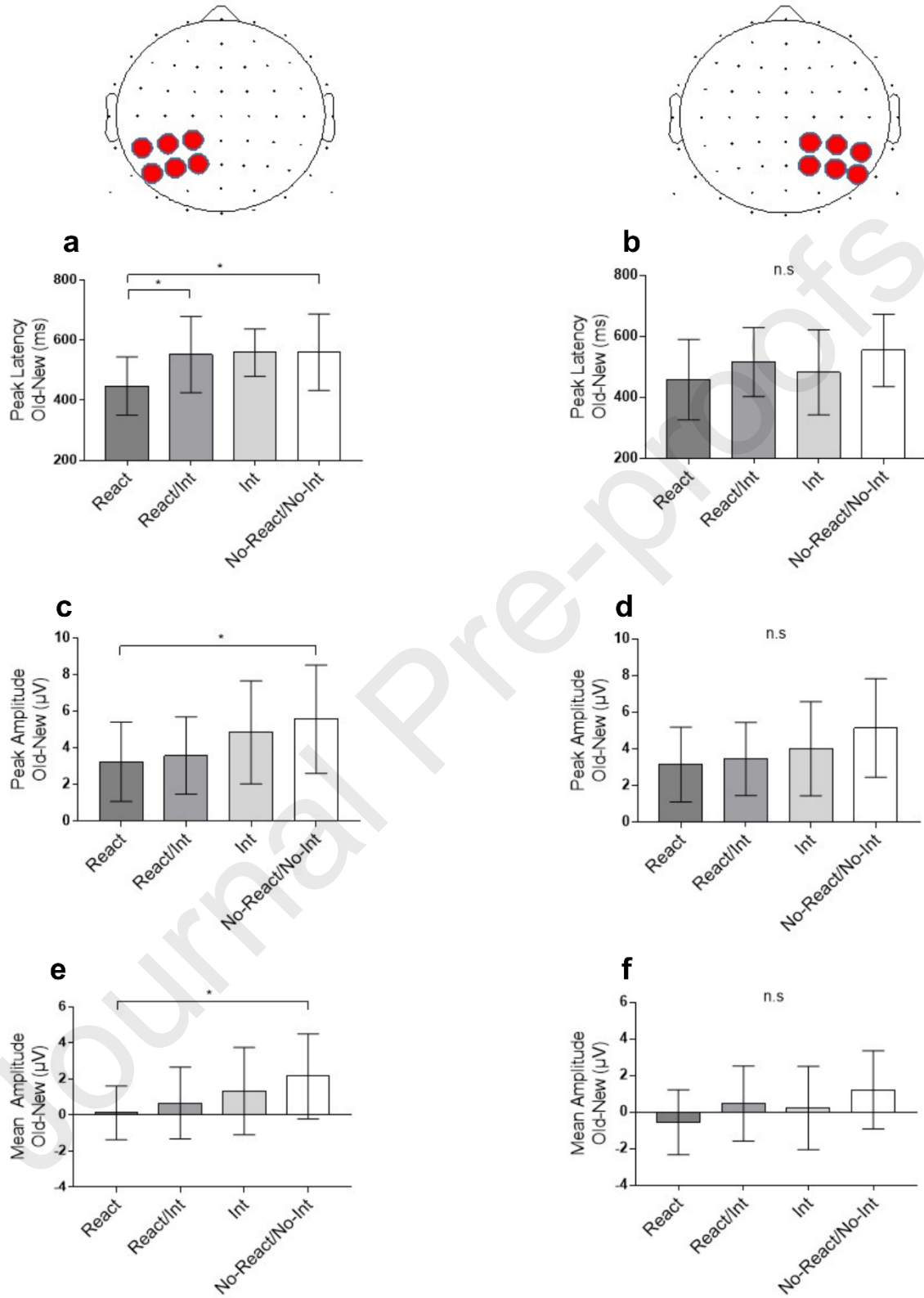
Figure 6. (a), (b) Peak latency; (c), (d), peak amplitude; and (e), (f) mean amplitude of the parietal old/new effect (difference scores) for left and right ROIs (left ROI: CP5, CP3, CP1, P5, P3, P1; right ROI: CP6, CP4, CP2, P6, P4, P2), for each group. Error bars depict ± S.D. and asterisks indicate statistically significant differences for contrasts of interest (React vs React/Int, React vs No-React/No-Int, React/Int vs Int, No-React/No-Int vs Int; n.s: no significant; *: p<0.05; One-way ANOVA, Bonferroni-corrected).

3.3 Pupillary Results

Two-tailed independent t-tests showed that the maximal pupil dilation was greater for old than for new responses in all groups, as can be seen in Figure S2a (reactivation group: t=7.195, df=14, p<0.0001; reactivation/interference group: t=10.35, df=18, p<0.0001; interference group: t=7.608, df=16, p<0.0001; no-reactivation/no-interference group: t=8.759, df=16, p<0.0001).

Complementarily, two-tailed independent t-tests showed that the mean pupil diameter between 1500 and 2500 ms was greater for old than for new responses in all groups, as shown in Figure S2c (reactivation group: t=8.268, df=14, p<0.0001; reactivation/interference group: t=12.73, df=18, p<0.0001; interference group: t=8.646, df=16, p<0.0001; no-reactivation/no-interference group: t=9.226, df=16, p<0.0001).

Finally, an independent permutation test for each group showed that the difference between old and new categories exceeded the t-value that corresponds to the 99th percentile (old>new) in the 1000-1100 ms time window in the reactivation, interference and no-reactivation/no-interference groups, and in the 700-800 ms time window in the reactivation/interference group (in all cases, the pupil old/new effect continued until the end of the 4000 ms epoch), as presented in Figure 7 (observed t-value, reactivation group: 1.8901; reactivation/interference group: 0.7571; interference: 1.5094; no-reactivation/no-interference: 1.7626, p<0.01). No differences in the magnitude of the pupil old/new effect were detected between groups (measured as differences between old and new responses for the maximal and mean pupil dilations), as can be seen in Figure S2b and S2d.

Finally, a two-way ANOVA with the factors "reactivation" and "interference" revealed a significant main effect of reactivation on the mean peak latency, as shown in Figure 8 (F (1, 64) = 10.63, p=0.0018).
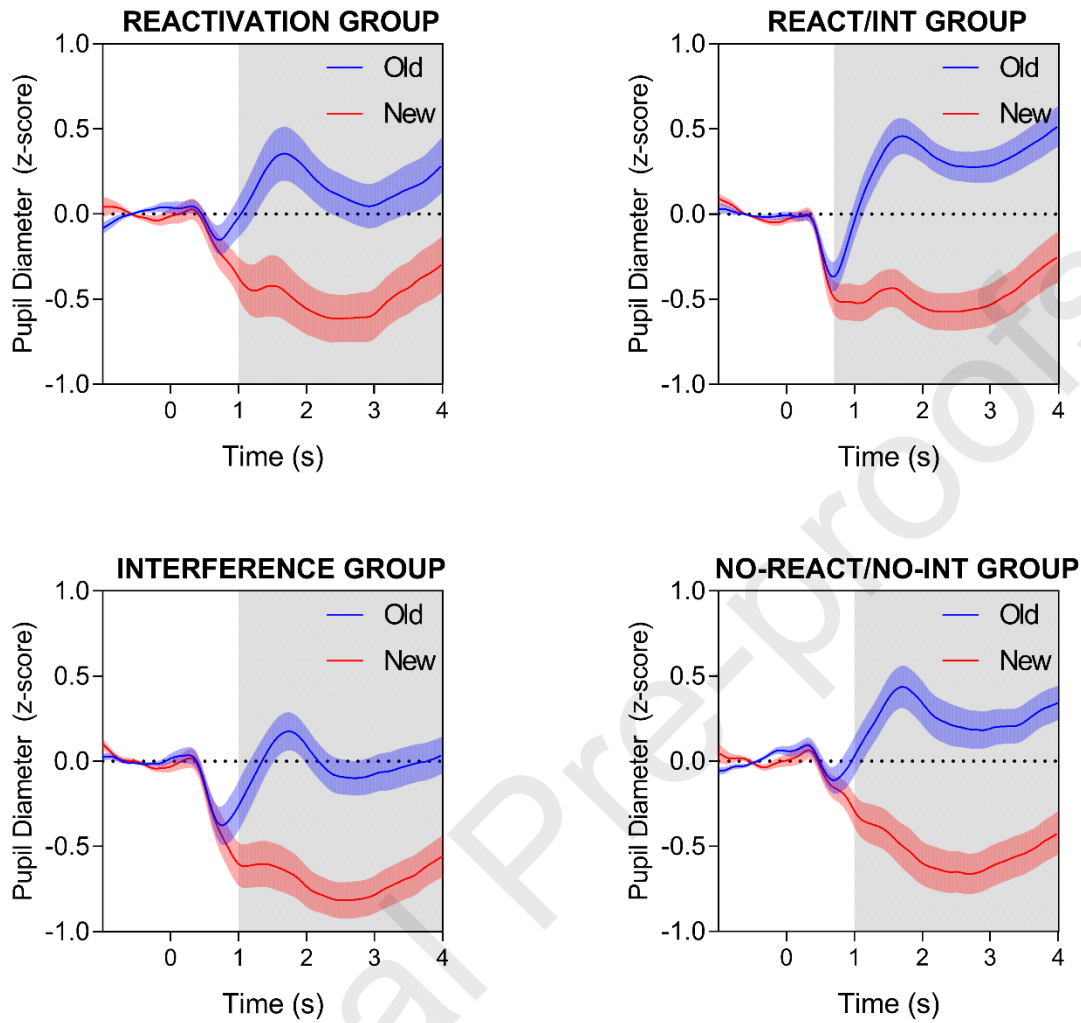
Figure 7. Pupil diameter elicited by correct answers (expressed in z-scores). In blue, the pupil diameter elicited by the correct classification of an old picture and the recall of the associated word. In red, the pupil diameter elicited by the correct classification of a new picture. Color shaded areas represents the standard error of the mean. Gray shaded areas define the temporal windows in which an independent permutation test detected positive significant differences between old and new categories in each group (p<0.01).
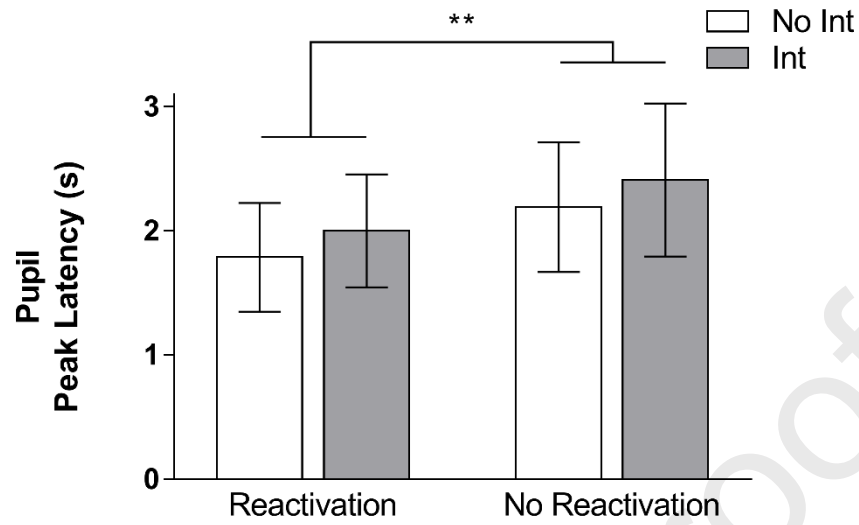
Figure 8. Latency of the pupil old/new effect, for each group. On the left, groups that were exposed to memory reactivation during session 2 (reactivation and reactivation/interference groups). On the right, groups that were not exposed to memory reactivation (no-reactivation/no-interference and interference groups). A two-way ANOVA revealed a main effect of reactivation on the peak latency of the pupil old/new effect. Error bars depict ± S.D. and asterisks indicate statistically significant differences (**: p<0.01).

4. Discussion

Reconsolidation refers to the process whereby a previously consolidated memory requires to be re-stabilized after its reactivation (Nader, Schafe, & LeDoux, 2000). However, despite the behavioral and pharmacological evidence of reconsolidation in humans (Agren et al., 2012; Forcato et al., 2007; Hupbach et al., 2007; Schwabe et al., 2012; Walker et al., 2003), little is known about the neurophysiological correlates of retrieval of reconsolidated memories.

The present study sought to identify the neural mechanisms underlying the retrieval of declarative memories modulated by reconsolidation. In order to accomplish this goal, ERPs and pupillary responses associated to retrieval of memories that were consolidated, reconsolidated or whose reconsolidation process was interfered by a new learning, were measured in an associative recall old/new task. Our main hypothesis was that retrieval of successfully reconsolidated memories is associated with distinct neurophysiological correlates. These modulations of neurophysiological markers of memory retrieval would be prevented in those memories whose reconsolidation process was interfered.

Based on the existing literature (Forcato et al., 2016), we predicted that the exposure to the reconsolidation process would improve memory performance. Moreover, we expected that the exposure to a new learning would interfere the re-stabilization process of the reactivated memory, preventing the memory strengthening predicted for reconsolidated memories. The behavioral results confirmed our assumptions (see Table S1, Figure 2 and Figure S1). According to the old/new effect literature, associative memory can be assessed through associative recall, operationalized in memory studies as the ability to retrieve additional information of a studied item presented in isolation (Donaldson & Rugg, 1999). In this context, we evaluated associative recall by asking participants to retrieve a word associated with an image. Our results, in both EEG and pupil datasets of participants, showed that the reactivation group performed better in the memory evaluation in session 3 compared to the reactivation/interference and the no-reactivation/no-interference groups, as was primarily expressed in a higher percentage of correct responses (correct old responses + recall of associated information) achieved by the reactivation group. Considered from the perspective of the previous definition, these results suggest that the exposure to the reconsolidation process may improve future associative recall. Complementarily, our experimental protocol allowed us to evaluate recognition memory, defined as the subjective judgment that a specific stimulus has been previously experienced (Curran, 2000; Friedman & Johnson, 2000; Rugg & Curran, 2007; Wilding, 2000). In our case, this was expressed as the ability to correctly discriminate between studied and non-studied pictures (old vs new). In this context, the reactivation group showed a higher percentage of recognition accuracy (hits – false alarms) in comparison to the no-reactivation/no-interference group (although EEG and pupil datasets showed specific differences between groups in hit rate and false alarm rate between groups, see Table S1). In general, these results suggest that reconsolidated

memories were associated not only to a better recall of associated information but also to a better recognition of pictures as old or new, as defined before. Interestingly, no statistical differences between the reactivation and the reactivation/interference groups were detected in recognition accuracy, indicating that the difference between these two groups was related only to associative recall and not to recognition. Taken together, these results confirm previous evidence showing that an unpredictable reminder can trigger the labilization-reconsolidation process, which in turn can improve existing declarative memories (Forcato et al., 2016; Forcato et al., 2014). In this context, these findings support the idea that memory strengthening is probably one of the main roles of the reconsolidation process (Forcato et al., 2014; J. L. Lee, 2008).

Our behavioral results also show that the post-reactivation behavioral interference partially prevented the memory improvement observed in the reactivation group, as was indicated by a lower percentage of correct responses in the reactivation/interference group compared to the reactivation group (see Figure 2b and Figure S1b). This result is in line with previous findings showing that a behavioral intervention, in the form of a new learning, may alter the reconsolidation of different types of memories in humans (e.g., motor, declarative and emotional memories), supporting the notion that reconsolidation is a dynamic mechanism by which memories are strengthened or updated (Forcato et al., 2007; Hupbach et al., 2007; Schiller et al., 2010). In this context, one possible explanation for our results is that, if new information is encoded after reactivation of an existing memory, the consolidation of the new material will temporally coincide with the reconsolidation of the previously reactivated memory, competing for those resources (i.e., neural circuitry and molecular mechanisms) needed for memory re-storage. This interpretation is in agreement with the idea that a behavioral interference (such as playing a videogame following the reactivation of an emotional memory or the exposure to a new learning) may engage cognitive processes that require neural resources in common with those required by memory re-stabilization, affecting subsequent retrievals (James et al., 2015; J. L. C. Lee et al., 2017). Future investigations are needed to clarify the mechanisms that underlie this interference during the reconsolidation process.

At an electrophysiological level, a positive parietal ERP old/new effect that peaked approximately 500 ms post-stimulus was present in all the experimental groups (see Figure 3, 4 and 5). This effect, traditionally known as "left parietal old/new effect", is assumed to reflect recollective processes (Donaldson & Rugg, 1999; MacKenzie & Donaldson, 2009; Opitz & Cornell, 2006; Rugg & Curran, 2007; Rugg et al., 1996; Wilding & Evans, 2012). In this context, it can be suggested that retrieval of memories that were consolidated, reconsolidated or interfered during reconsolidation share some neurophysiological mechanisms. In other words, we can assume that the left parietal old/new effect indexes processes related to memory retrieval regardless of whether the memory was previously modulated or not by reconsolidation. Our prediction was that, considering that the exposure to the reconsolidation process can restructure and strengthen declarative memories, retrieval of reconsolidated memories would present modulations of the left parietal old/new effect. Consistent with our assumption, the left

parietal old/new effect was detected from the 400-500 ms time window in response to retrieval of memories that were reconsolidated, while memories that were just consolidated or interfered during reconsolidation showed a left parietal old/new effect primarily starting from the 500-600 ms time window, as was evidenced by within-group permutation tests (see Figure 4 and 5). Concordant with this result, we found an earlier peak latency of the left parietal old/new effect (at 450 ms approximately) in reconsolidated memories in comparison to memories that were consolidated or whose reconsolidation process was interfered by a new learning (at 550 ms approximately) (see Figure 6a). One possible explanation is that reconsolidation, allowing reactivated memories to be enhanced, facilitates memory accessibility in future recalls. Because, as it was mentioned previously, the left parietal old/new effect is considered an index of recollection (Donaldson & Rugg, 1999; Wilding, 2000), it is plausible that an earlier onset and peak latency associated to retrieval of reconsolidated memories reflects a more effective recollection process as a result of a stronger memory trace. In consonance with this idea, previous studies have linked an earlier parietal old/new effect to an enhanced recollection. For instance, De Chastelaine et al., (2009), using a recognition memory task, demonstrated that improved memories as consequence of multiple study-test repetitions show an earlier parietally distributed old/new effect, suggesting an earlier recollection (De Chastelaine et al., 2009). Additionally, an earlier onset of the parietal old/new effect has been found when more details of items are retrieved or when the retrieved information is highly precise (Murray et al., 2015; Vilberg et al., 2006). In line with this idea, in our study it is possible that an earlier onset and peak of the left parietal old/new effect could reflect not only earlier but also enhanced retrieval processes that support the memory improvement observed in the behavioral results described above. More research is needed to validate this idea.

A second noteworthy finding was that the reactivation group, whose memories were successfully reconsolidated, was the only group that showed a significant reversed mid-frontal old/new effect following the parietal effect, in the 600-690 ms time window (see Figure 4 and 5). This result is in line with previous reports from humans (Sandrini et al., 2013) and animal models (Ye et al., 2017) indicating a role of frontal brain regions in the reconsolidation of different types of memories. Considering the topographical distribution and temporal course, we speculate that the observed effect could reflect control operations related to memory retrieval, supported by the prefrontal cortex (Velanova et al., 2003). In this context, this effect could be related to post-retrieval monitoring processes and evaluative operations over the retrieved information (Donaldson & Rugg, 1999; Rugg & Wilding, 2000; Wilding & Rugg, 1997b). In other words, it is plausible that the exposure to the reconsolidation affects not only to recall but also to post-retrieval processes, supporting in part the observed memory improvement in reconsolidated memories.

A third ERP-result of interest concerns the magnitude of the parietal old/new effect observed in the reactivation group compared to the no-reactivation/no-interference group. Previous studies have demonstrated that the magnitude of the left parietal old/new effect

is modulated by several factors. Importantly, in associative recall tasks, this aspect is modulated by recall of associated information during retrieval (Donaldson & Rugg, 1999; Rugg et al., 1996). Although our results confirm the previous evidence (in all groups, the category "old" showed a greater amplitude than the category "new"), also showed a smaller left parietal old/new effect associated to retrieval of strengthened memories by reconsolidation in comparison to retrieval of consolidated memories (and no differences between retrieval of memories that were reconsolidated and memories that were interfered during reconsolidation). One possible explanation is that memory strengthening minimizes cognitive demands by facilitating the selective retrieval of the necessary information to successfully complete the task. In this context, a smaller parietal old/new effect may partially reflect a decreased cognitive effort to recognize old items and recall associated information, while a greater effect could imply a more effortful search to retrieve the required information. The idea that retrieval of memories strengthened by reconsolidation would be less cognitively demanding is also in line with the earlier peak latency and onset of the old/new effect found in the reactivation group. Future research will be needed to confirm or reject this interpretation.

In summary, our electrophysiological results showed that reconsolidated memories elicit distinct patterns of brain activation during retrieval as compared to consolidated memories and memories whose reconsolidation process was interfered by a new learning.

Complementarily, a larger maximal pupil dilation and mean pupil diameter associated to old as opposed to new responses were found in all the experimental groups (see Figure 7, S2a and S2c). Our results not only confirm the pupil old/new effect previously described in recognition tasks (Brocher & Graf, 2017; Kafkas & Montaldi, 2015; Vo et al., 2008), but also expand the previous findings in two critical aspects. First, the pupil old/new effect, in our case, was found in an associative recall task that required participants not only to recognize items as old or new but also to consciously recall associated information. Second, to our knowledge, this is the first study that describes the pupil old/new effect in memories that were reconsolidated. Thus, these findings add new knowledge to the existing literature.

Although no differences between groups were detected in the maximal pupil dilation and mean pupil diameter (see Figure S2b and S2d), our analyses showed an earlier peak latency of the pupil old/new effect in those groups that were exposed to memory reactivation in comparison to non-reactivated groups (see Figure 8). Because the pupil old/new effect has been previously associated to recollection (Vo et al., 2008), we suggest that an earlier peak latency of this effect could reflect earlier and presumably facilitated recollective processes in those groups that were exposed to memory reactivation and, subsequently, to the reconsolidation process. This explanation is closely in line with our previous suggestion that the observed modulation of the parietal old/new effect in the reactivation group reflects enhanced recollective processes. Interestingly, despite the observed trend, no statistical differences in the peak latency elicited by

retrieval of memories that were successfully reconsolidated and memories that were interfered during reconsolidation were found. One possible explanation is that the behavioral interference (new learning) during reconsolidation did not fully prevent the memory improvement observed in the reactivation group and, therefore, an earlier peak latency of the pupil old/new effect is partially reflecting improved memories in both reactivated groups in comparison to non-reactivated groups. In this context, more research is needed to investigate the pupil old/new effect in the retrieval of consolidated and reconsolidated memories.

5. Conclusions

In summary, our study demonstrates that the reconsolidation process triggered by unpredictable reminders can modulate subsequent retrieval processing of declarative memories, as was evidenced by specific changes in ERP and pupil old/new effects. We argued that these changes may be associated with more effective, efficient and earlier recollective processes. Therefore, we can conclude that reconsolidation could have a significant impact in how memories are accessed in the future. A better understanding of how we retrieve memories that have changed over time as consequence of the reconsolidation process could have important implications for the treatment of patients with memory disorders and for the improvement of the learning process in educational contexts.

Conflict of Interest

The authors declare no competing financial interests.

Acknowledgement

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