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Memory timeline: Brain ERP C250 (not P300) is an early biomarker of short-term storage

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ABSTRACT

Brain event-related potentials (ERPs) offer a quantitative link between neurophysiological activity and cognitive performance. ERPs were measured while young adults performed a task that required storing a relevant stimulus in short-term memory. Using principal components analysis, ERP component C250 (maximum at 250 ms post-stimulus) was extracted from a set of ERPs that were separately averaged for various task conditions, including stimulus relevancy and stimulus sequence within a trial. C250 was more positive in response to task-specific stimuli that were successfully stored in short-term memory. This relationship between C250 and short-term memory storage of a stimulus was confirmed by a memory probe recall test where the behavioral recall of a stimulus was highly correlated with its C250 amplitude. ERP component P300 (and its subcomponents of P3a and P3b, which are commonly thought to represent memory operations) did not show a pattern of activation reflective of storing task-relevant stimuli. C250 precedes the P300, indicating that initial short-term memory storage may occur earlier than previously believed. Additionally, because C250 is so strongly predictive of a stimulus being stored in short-term memory, C250 may provide a strong index of early memory operations.

1. Introduction

There are many aspects of memory, including short-term (on the order of seconds) and long-term memory, and each is vital to everyday function. The ability to remember information for at least a short time is critical, but in some cases it may be equally important for the brain to discard irrelevant information. In performing many tasks, efficiency may be tied to identifying incoming information as task-relevant or task-irrelevant and storing only the task-relevant information that will be needed later.

Often emphasis is placed on where various functions occur in the brain. However, *when* functions occur is just as important in understanding brain processes. A timeline of memory storage and processing, from early post-stimulus coding of stimuli to short-term storage to using items saved in

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memory to complete tasks, must be developed. Brain event-related potentials (ERPs) are effective measures of underlying electrophysiological processes that can be linked to cognitive and behavioral functions. When manipulated by a cognitive task with separable task conditions, ERPs and their underlying components can provide direct, quantitative indices of abstract cognitive processes. ERP components can reflect brain activity both in time (high resolution on the order of milliseconds) and in space (electrode location), and their behavior under task conditions has been correlated with memory (Begleiter et al., 1993; Chapman et al., 1978a, 1981; Friedman et al., 1978; Polich, 2007; Ruchkin et al., 1990; Rugg and Curran, 2007), recognition and familiarity (Morgan et al., 2008; Pfütze et al., 2002; Trenner et al., 2004), semantic meaning (Chapman et al., 1978b), stimulus expectancy (Arbel et al., 2010; Walter et al., 1964), executive functioning (Begleiter and Porjesz, 1975), and stimulus relevance (Chapman and Bragdon, 1964; Chapman, 1965; Chapman et al., 2013), among others. While anatomical imaging methods, such as PET and MRI, may indicate where activity occurs during memory storage and processing, their poor temporal resolution (on the order of seconds) makes it difficult to separate the early post-stimulus sequence of events (Missonnier et al., 2004), including sensory processing, memory storage, and later executive functions such as solving the task. Defining and validating an ERP component directly linked to short-term memory storage would be of great importance, as memory processes are complex and it is difficult to parse and clearly define measures of short-term storage, task processing demands, and working memory operations.

There is a vast wealth of research on ERPs and memory mechanisms (Ruchkin et al., 2003). Much work has been reported on maintenance, rehearsal, and retrieval, but little is known about the timing or exact sequence of events that occur when items are first stored in working memory (Baddeley, 2003; Missonnier et al., 2004). The task-relevant ERP component P300 (Hillyard and Picton, 1987) was first reported roughly 50 years ago (Chapman and Bragdon, 1964; Chapman, 1965) and has been extensively studied in the context of working memory (for a review, see Kutas, 1988; Polich, 2007, 2012). The P300 component and its subcomponents of P3a and P3b are commonly linked to a host of memory operations. These include attentional processing of task-relevant stimuli and distractors, context updating, working memory storage, and task related memory operations. However, how and when items are stored immediately after initial sensory processing is not well-defined. While retrieval is an important aspect, the processes by which stimuli are first transferred from fleeting sensory registers (Begleiter et al., 1993; Chapman et al., 1981; Dick, 1974; Sperling, 1960) into a less transient short-term memory store is equally important and often over-looked. This step, where pertinent information is identified and preserved at least for a short time, is essential in successful problem-solving. Diseases and disorders that impact memory may disrupt these key, early processes. In addition, because so much interest has been placed on working memory retrieval, there has been necessary emphasis on longer-latency ERP components (Dunn et al., 1998; Friedman, 2000; Missonnier et al., 2004). This has perhaps caused earlier components that could be linked to storage to be overlooked.

Researchers have struggled with interpreting mixed results concerning early post-stimulus memory storage, and we suggest that measuring short-term memory storage may prove difficult for numerous reasons. The confusion surrounding how to disentangle and measure the early, short-latency underlying components of ERPs has contributed to the difficulties in linking an ERP measure directly with short-term memory storage. Often ERP components overlap in time, particularly in this early post-stimulus period when a large amount of processing occurs including sensory and perceptual functions. A formal multivariate method such as principal components analysis (PCA) allows for extraction of individual components in a parsimonious fashion that requires neither making strong assumptions about the nature of those components nor identifying particular time regions of interest (Chapman and McCrary, 1995). Differentiating ERP components by measuring peaks or broad time regions in averaged ERPs is not conducive to detecting underlying ERP components and may produce muddied measures of these components if they occur in rapid succession. In addition, longer latency components, such as contingent negative variation (CNV) (Chapman et al., 1981), may interfere with measuring and interpreting other components that occur during its latency window.

Another reason for the difficulty in defining an ERP component that directly indexes short-term storage lies with the cognitive task. Whether or not the stimulus is relevant to solving the cognitive task must be separable from whether or not it requires storage. A task design like this separates short-term storage from other working memory operations, including attentional and executive processes concerning task relevancy. Our Number–Letter task (Fig. 1) provides the aforementioned advantage in studying short-term memory storage. During the task, subjects see four simple number and letter stimuli flashed in a random sequence and are asked to compare either the two numbers or two letters. The type of stimuli used in the comparison is considered “relevant”. The interaction between stimulus relevancy and stimulus position within the sequence of the trial (called intratrial part) offers two key conditions: (1) stimuli for which memory storage is required to complete the task and (2) stimuli for which storage is optional. Storage of the first relevant stimulus, which randomly appears in part 1 or 2, is required in order to later perform the comparison with the second relevant stimulus, which randomly occurs in part 3 or 4. The second relevant stimulus can be immediately used to perform the comparison with the first relevant stimulus in short-term memory; therefore, storage of the second relevant stimulus is optional. Additionally, memory storage of irrelevant stimuli in any of the four intratrial parts is never required to complete the task successfully. By this reasoning, a sensitive ERP measure for short-term memory storage should have a pronounced effect elicited by the first relevant stimulus in intratrial parts 1 or 2 that is diminished or non-existent for the second relevant stimulus in parts 3 or 4 and for all irrelevant stimuli.

In this article, we examine a short-latency ERP component C250 (maximum at 250 ms post-stimulus), named the “memory storage component” (Chapman et al., 1978a), as an electrophysiological index of short-term memory storage. Earlier work with this component has shown it to be a candidate for an electrophysiological marker for memory storage, and in this paper we aim to further explore C250 by comparing it to other later ERP components P3a (C325) and P3b (C415) obtained in the same trials. We place C250 on a timeline of memory events, where it measures memory storage of stimuli which are later operated upon by P300 and its subcomponents. Also new in this study is our assessment of C250 activity in additional brain regions of interest. Our work validates C250 as a potential biomarker of short-term memory storage, which is a process that precedes and is distinct from the working memory and executive processes associated with P300.

2. Results

2.1. C250 distribution

Topographical maps of C250 brain activity (Fig. 2) showed a striking, positive response to relevant stimuli in intratrial parts 1 and 2 that encompassed most of the electrodes. This response was particularly large in the central and frontal regions. This effect was not observed in conditions where memory storage was optional, such as for the second relevant stimulus occurring in intratrial parts 3 and 4 and for all irrelevant stimuli. In general, we found no laterality differences in the patterns of C250 activation.

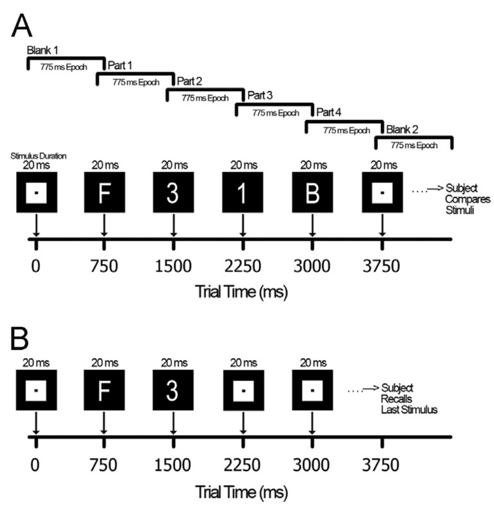

Fig. 1 – (A) A sample trial of the Number–Letter paradigm. Each trial consists of four intratrial parts (the first and last "blank" stimuli were not considered in this ERP analysis). Each part contains one stimulus, which can be a number or a letter and either relevant or irrelevant depending on the task condition. After Blank 2, the subject is asked to give an answer concerning the numeric (or alphabetic) order of the two relevant stimuli (which are numbers on one block of 102 trials and letters on another). The stimulus briefly appears at 0 ms (25 ms of pre-stimulus EEG is recorded as baseline). The stimulus lasts 20 ms and is followed by a 730 ms interval before the next stimulus presentation. (B) A sample random memory probe test. This infrequent behavioral test was conducted during the Number–Letter task. Subjects were randomly questioned after one of the four intratrial stimuli to name the last stimulus they saw. Two blank flashes were delivered at 750 ms and 1500 ms after the stimulus to be probed. There was one probe for each of the eight conditions (Relevant or Irrelevant by Intratrial Parts 1–4) for each block (Number-Relevant or Letter-Relevant) of 102 trials.

2.2. Number–Letter task effects

To determine how the varying Number–Letter task conditions affected C250 memory storage, we examined the interaction between the two main Number–Letter task effects: Task Relevancy (Relevant and Irrelevant) and Part (Intratrial 1–4) (Table 1, Fig. 3). Because the interaction between Relevancy and Part was not affected by Stimulus Type (Table 1), the two Stimulus Types are combined in Fig. 3. A strong, statistically significant Relevancy by Part interaction appeared in occipital, parietal, central, and frontal brain locations (Figs. 2 and 3). Young adults demonstrated a significantly larger and more positive C250 to relevant stimuli when memory storage was required (parts 1 and 2), suggesting they successfully recognized whether the stimulus was relevant and whether storage of that stimulus was necessary to complete the task (Figs. 2 and 3). These effects are brought into context by the mean component amplitudes, and C250 showed the largest response to the first relevant stimulus located in intratrial parts 1 or 2.

We also investigated how the same task effects recorded in the same trials manifested themselves for C325, which we have labeled as the P3a, and for C415, which is commonly known as P3b (Fig. 3, Table 1). While the C325 component also showed strong Relevancy by Part interactions, its highest mean component scores were in response to irrelevant stimuli, particularly in parts 2 and 4. These effects were more prominent in anterior areas of the electrode distribution. Similarly, C415 showed strong Relevancy by Part interactions such that a relevant stimulus in parts 3 or 4, which was the second relevant stimulus in the trial, produced the largest responses. This is nearly the opposite effect of what was observed with C250.

2.3. Behavioral recall

C250 amplitudes varied with task effects in a way that suggests it represents short-term memory storage of a stimulus. However, to confirm that interpretation, C250 amplitudes elicited by a stimulus should predict behavioral recall of that stimulus. The occasional memory probe test conducted during the Number–Letter paradigm (Fig. 1B) showed that mean recall of the stimulus in each of the sixteen task conditions was highly correlated with C250 amplitude. We tested correlations between the behavioral probe data and mean C250 component scores paired by each of the sixteen task conditions (Fig. 4). We found a large, positive correlation between successful storage (as indicated by the mean probe recall score) and C250 amplitude at central midline electrode CZ ($r=0.75$, $p<0.001$) and at parietal electrode PZ ($r=0.69$, $p<0.01$). This effect was reduced at frontal site F3 ($r=0.48$, $p=0.06$), though not significantly. Interestingly, C325 showed a significant negative correlation between its amplitude and memory probe recall, particularly at F3 ($r=-0.58$, $p<0.05$).

C325 correlation magnitude was smaller, however, than C250. C415 had practically zero correlation with memory probe recall at CZ ($r=0.00$, $p=0.99$), PZ ($r=0.17$, $p=0.53$), and F3 ($r=-0.08$, $p=0.99$). Thus P300 subcomponents (P3a and P3b) are not well correlated with memory storage as measured by the behavioral probe.

3. Discussion

Operation of the working memory system allows temporary storage of information so that it may be manipulated or acted upon (e.g. (Perez and Vogel, 2012)). Many cognitive processes that require information to be held in an online state, such as the Number–Letter task, are thought to utilize this working memory system as a form of "mental workspace" to perform the requisite operations. A fundamental characteristic of working memory is a severe limitation in its capacity to maintain information. We believe that short-term storage in this working memory system can be indexed by the C250 component, which is separable and distinct from both C325 (P3a) and C415 (P3b). Though these later components influence task performance, their impact is at a later, post-short term storage level. The evidence for C250's involvement in memory storage is two-fold: first, C250 amplitudes in response to task conditions match what is required by the task in terms of memory storage, and second, C250 predicts recall of stimuli in short-term memory with high accuracy.

3.1. *Number–Letter task effects*

The Number–Letter task affords the opportunity to separate stimuli (and thus potentials evoked in response to them) into those that are relevant and require storage, those that are relevant and do not require storage, and those that are irrelevant. C250 shows its largest, most positive amplitudes to stimuli relevant to the task objectives when those stimuli were located in intratrial positions where memory storage was required (relevant stimuli in parts 1 or 2). This was a widespread effect that encompassed much of the scalp (Figs. 2 and 3). This is not an effect solely due to Relevancy or to Intratrial Part, though both produced significant main effects, particularly in the central region (Table 1). Rather, it was the interaction between Relevancy and Part such that storage only occurred (as marked by larger C250 amplitudes) in response to stimuli whose relevance and position within the sequence of the trial dictated their remembrance was required, at least until the second relevant stimulus occurred.

These findings regarding C250 are in contrast to both C325 and C415. Although both of these components produced significant Relevancy and Part main effects as well as a significant interaction, the interpretation of these effects is strikingly different. Our evidence suggests that C325 may be the common ERP component P3a (Polich, 2007), which is thought to involve executive processes such as attention. C325 reflected processing of the requirements of the Number–Letter task (Fig. 3) as indicated by its large Relevancy by Part interaction, but its largest positive component scores occurred to irrelevant stimuli, particularly in the latter parts of the trial. Most interestingly, its negative significant correlation with the memory probe data suggests it may be involved with inhibition of processing irrelevant stimuli (Fig. 4). Other research has indicated that P3a may influence working memory via the P3b, which occurs slightly later and in more posterior brain locations (central and temporal-parietal areas) (Polich, 2007).

By spatial location of its effects and its post-stimulus timing, we relate C415 to P3b. Again, the large Relevancy by Part interaction for C415 showed an entirely different pattern of Number–Letter task processing than did C250 or C325

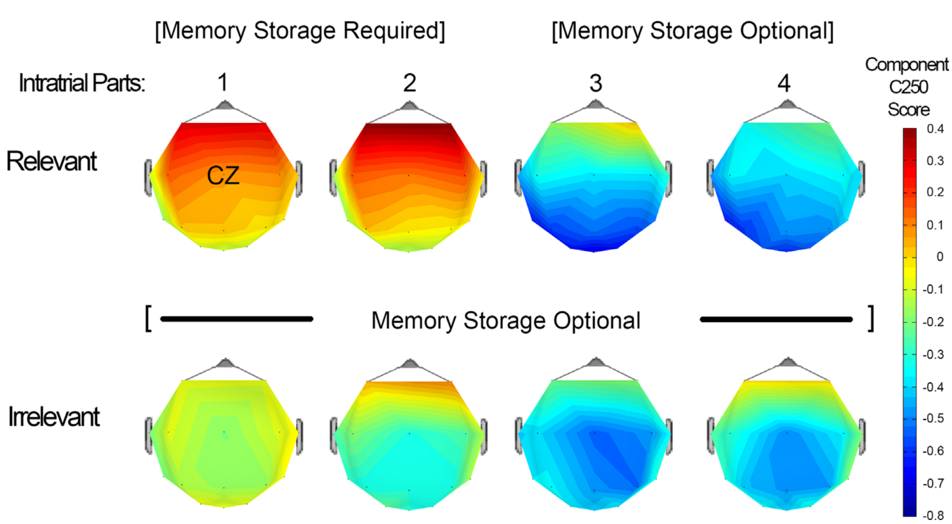

Fig. 2 – Topographical patterns for C250 component scores for young adults. Component scores (amplitudes) were averaged over Stimulus Type (Number, Letter). The first relevant stimulus appears in either part 1 or 2, and the second relevant stimulus appears in part 3 or 4. Memory storage of the second relevant stimulus is not required to complete the task because the comparison between the two relevant stimuli could be performed immediately after the second relevant stimulus appears. In addition, memory storage of irrelevant stimuli is never required. The term "Memory Storage Optional" refers to assumptions from the experimental design and should not imply no C250 activity occurred.

Table 1 – Number–Letter task effects for ERP components C250, C325, and C415 at occipital (posterior), parietal, central, and frontal (anterior) brain locations. In particular, a significant Relevancy by Part interaction is essential in both differentiating relevant from irrelevant stimuli and determining by stimulus order which stimuli require memory storage. The mean component scores for the task conditions must be considered to determine how the particular component represents task processing (Fig. 3). Significant effects (alpha < 0.05) are marked in bold.

	df	*Occipital*	*Parietal*	*Central*	*Frontal*
C250—memory storage					
Relevancy[a]	1.35	3.58	1.86	**9.18**[**]	1.70
Intratrial part[b]	3.15	**16.75**[***]	**24.21**[***]	**18.68**[***]	**7.73**[***]
Stimulus type[c]	1.35	**6.96**[*]	0.77	**12.07**[**]	**10.84**[**]
*Rel*Part*	3.105	**5.78**[**]	**5.70**[**]	**3.85**[*]	**5.21**[**]
*Rel*Stim*	1.35	1.97	0.94	0.11	0.45
*Stim*Part*	3.105	2.01	1.63	1.63	1.39
*Rel*Part*Stim*	3.105	2.05	1.94	2.30	2.29
C325 (P3a)					
Rel	1.35	**4.65**[*]	0.64	0.02	1.77
Part	3.35	1.99	**6.45**[**]	**12.2**[***]	**6.45**[***]
Stim	1.35	**63.22**[***]	**52.28**[***]	**25.02**[***]	**9.80**[**]
*Rel*Part*	3.105	**5.13**[***]	**7.48**[**]	**6.61**[***]	**5.72**[***]
*Rel*Stim*	1.35	**6.50**[*]	**5.11**[*]	0.28	0.22
*Stim*Part*	3.105	0.79	1.13	0.42	0.58
*Rel*Part*Stim*	3.105	0.11	0.23	0.23	0.66
C415 (P3b)					
Rel	1.35	**55.76**[***]	**56.73**[***]	**34.01**[***]	**10.69**[**]
Part	3.35	2.60	**6.61**[**]	**12.74**[***]	**7.42**[***]
Stim	1.35	2.13	0.02	0.03	0.76
*Rel*Part*	3.105	**36.85**[***]	**33.23**[***]	**28.76**[***]	**10.35**[***]
*Rel*Stim*	1.35	1.44	0.75	0.03	0.05
*Stim*Part*	3.105	1.33	0.54	1.56	1.01
*Rel*Part*Stim*	3.105	0.27	1.16	1.30	1.31

[a] Relevancy (Relevant, Irrelevant).
[b] Part (intratrial 1–4).
[c] Stimulus type (numbers, letters).
[*] $p < 0.5$, respectively.
[**] $p < 0.01$, respectively.
[***] $p < 0.001$, respectively.

(Fig. 3). P3b is sometimes considered a hallmark of working memory operations and may be produced in response to task demands as processed by P3a (Polich, 2007). C415 displayed its largest positive amplitudes to the second relevant stimulus in the trial (during parts 3 or 4). At this point, short-term memory storage has already occurred for the first relevant stimulus and the subject is observing the second relevant stimulus with which the stored first relevant stimulus must be compared for the order task. Therefore, P3b to the second relevant stimulus appears related to the comparison process. This is further supported by the memory probe data, as C415 (P3b) has essentially no correlation with immediate stimulus recall. Short-term memory storage is therefore independent of P3b activity.

3.2. *Behavioral probe data: C250 predicts recall*

The relationship between C250 and the memory probe data is the strongest evidence for C250's influence on short-term memory storage. The nature of the Number–Letter task with its varying levels of "importance" of stimuli (whether or not the stimulus was relevant and if it required storage) allowed us to confirm some aspects of how and when short-term memory storage occurs. A memory probe was randomly and rarely performed shortly (1.5 s) after a stimulus was observed; therefore, if the stimulus was correctly recalled, that particular number or letter had been stored. The mean memory probe recall scores (Fig. 4) were the lowest for irrelevant and relevant stimuli that did not require storage. Thus a direct behavioral index confirmed that relevant stimuli that required storage to complete the task were in fact stored.

Of any of the components studied, C250 had the highest correlation to these memory probe scores. C325 demonstrated a negative correlation with probe recall data, meaning that larger C325 responses to the stimuli requiring storage were related to poorer recall of those stimuli. This indicates it may be involved in some sort of inhibitory process. C415 showed no significant correlation with the probe recall data. Whether or not the subject was capable of recalling the stimulus he had just seen was independent of C415 activity, again furthering the conclusion that C415 has more involvement with solving the Number–Letter task than with storing the relevant stimuli. C250 component scores were the most positive of these components to those stimuli that required storage and the least positive to those that did not. C250 amplitudes during storage therefore predict later short-term memory recall, which is remarkable given the multitude of other factors that can influence retrieval.

3.3. Comparing C250 to other ERP components

In this article, we compared C250 to P300 and its subcomponents of P3a and P3b and found C250 was the best index of short-term memory storage. Other work has compared C250 to CNV (Chapman et al., 1981) and shown that CNV does not well correlate with short-term storage measured through a behavioral probe. Other investigators have also reported short-latency ERP components related to short-term memory operations. In a continuous recognition study, Dunn et al. (1998) measured ERPs to each visual stimulus in a long list of words of three conditions (randomly ordered): new words, immediate repetition, and delayed repetition. One of their ERP components was P200 (a positive-going component with a peak latency in the vicinity of 250 ms) which they reported was observed across all three stimulus-presentation conditions with similar amplitudes. They were unable to describe P200 definitely but suggested it could reflect earlier stages of word recognition, such as automated word categorization and storage. It is possible that P250 was not easily attributable to storage in Dunn et al.'s work because the task did not provide ample opportunity to study conditions in which storage was not required. The subject performing a continuous word recognition task may attempt to remember every

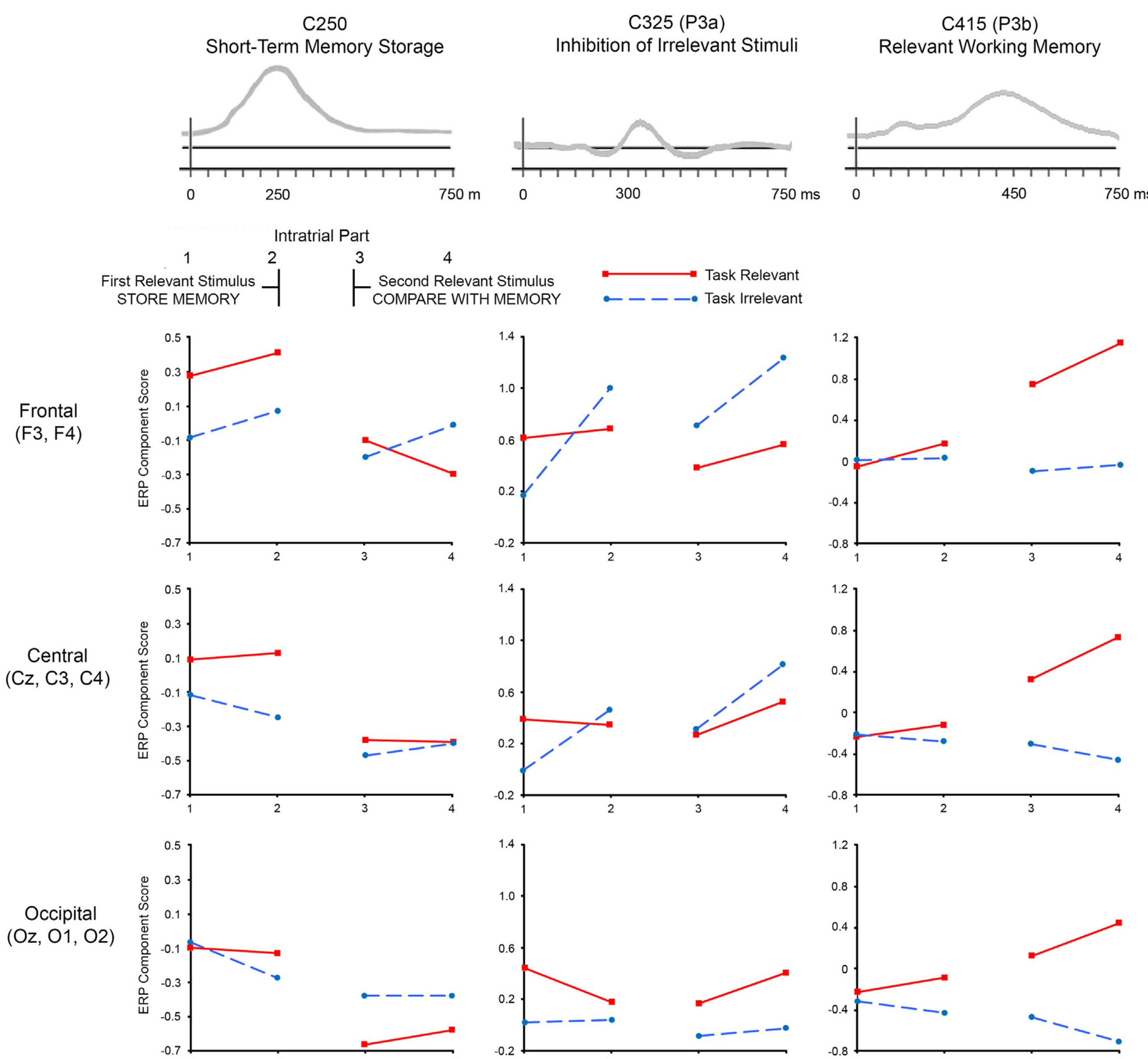

Fig. 3 – Number–Letter task effects for C250 ERP component and the P300 subcomponents (C325 (P3a) and C415 (P3b)) in frontal, central, and occipital brain locations. Component scores (amplitudes) were averaged over Stimulus Type (Number, Letter). C250 shows its largest amplitudes in response to first relevant stimuli in intratrial parts 1 and 2 (short-term memory storage required) in frontal and central regions. P3a shows its largest response to irrelevant stimuli, particularly in part 4 (inhibition of irrelevant stimuli). Finally, P3b has its largest response to the second relevant stimulus in parts 3 or 4 (task processing and comparison with the first relevant stimulus in working memory). For details of statistical testing, see Table 1.

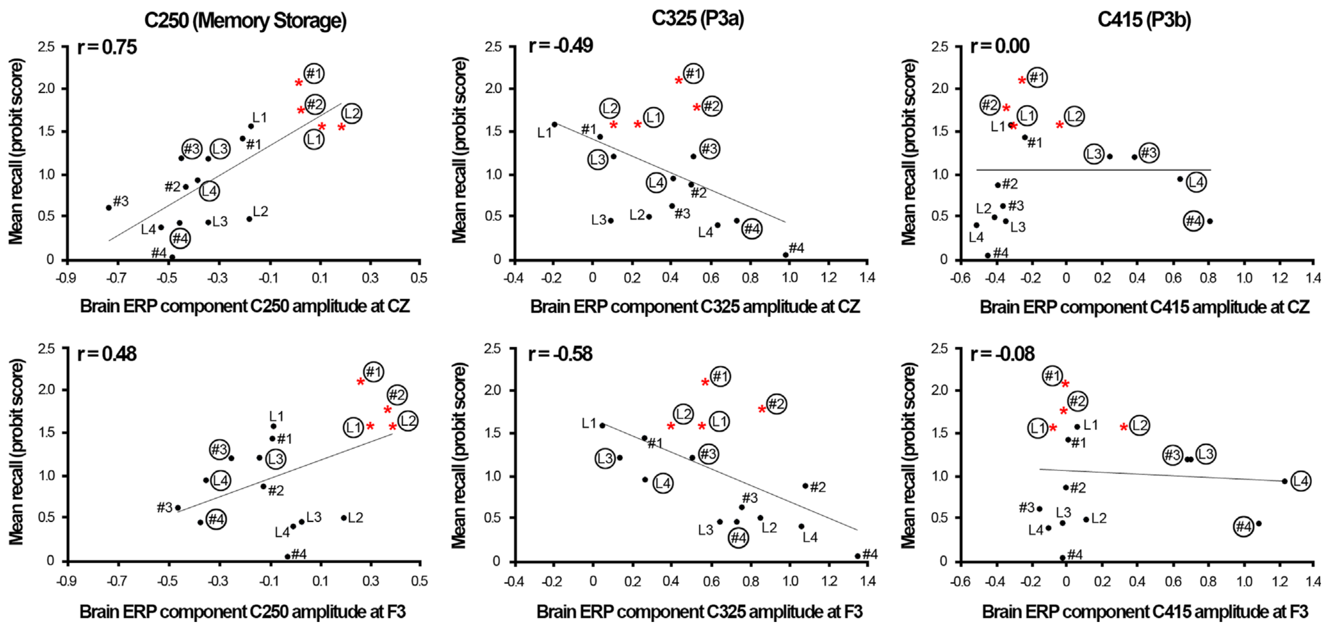

Fig. 4 – Correlations between behavioral probe recall data and brain ERP component scores (CZ in top row, F3 in bottom row) for the sixteen Number–Letter conditions. Each condition is denoted by a symbol composed of a Stimulus Type, an Intratrial Part, and its Relevancy. L=Letters. #=Numbers. 1–4=Intratrial parts. Circled symbols are task relevant. Stars indicate relevant numbers and letters in parts 1 or 2 (memory storage required). C250 showed a positive, strong correlation at the central midline (CZ) electrode with successful stimulus recall. C325 showed a negative correlation, particularly strongly in frontal regions (F3). C415 showed no relationship between its pattern of component scores and probe recall.

word, not knowing when or if it will be repeated. In a similar study featuring an n-back recognition task, Missonnier et al. (2003; 2004) describe a component they termed PNwm, which they consider to be an index of working memory load. Importantly, their study featured a well-defined control condition that required no memory storage but rather simple detection of stimulus types. ERPs for this control condition were subtracted from ERPs elicited by their n-back task. With this difference, Missonnier et al. detected PNwm, which occurred within a window of 140–280 ms post-stimulus. The amplitude of PNwm increased significantly with working memory demands. They postulate that short-term storage is separable from exogenous sensory processes and should occur during this early post-stimulus time period.

Begleiter et al. (1993), Zhang et al. (1997) discovered an ERP component that they called visual memory potential (VMP), which indexes visual short-term memory in humans and is maximal at 240 ms. VMP as described may be shorter latency than C250 because of overlap with an earlier component, whose influence was removed by the PCA in our analyses. Comparisons of VMP with C250 indicate a striking number of similarities. Begleiter et al. interpret VMP as reflecting retrieval from memory because VMP shows reductions in amplitude during the second stimulus of a matched pair as compared to the first stimulus of the pair in a short-term memory task. The first stimulus of this pair required memory storage in order to perform the delayed matching to sample task, not unlike the Number–Letter task necessitating the storage of the first relevant stimulus in order to compare it with the second relevant stimulus for order. The reduction of VMP in response to the second stimulus of a matched pair in Begleiter et al.'s work would be analogous to the reduction seen in C250 in response to irrelevant stimuli or stimuli that do not require storage in our experiment. Therefore, we think VMP may not reflect retrieval as Begleiter et al. suggest, but rather storage in that it had higher amplitudes to the first stimulus of the matched pair whose memory storage was required.

4. Conclusion

We have shown C250 produces a larger, positive response particularly in central regions with regard to stimuli whose short-term storage in memory is required by the Number–Letter task. In addition, correlation with memory probe data shows that C250 amplitude evoked by a stimulus is predictive of that stimulus' recall. The new group of 36 young adults used in the present work yielded a correlation of $r=0.75$ with the behavioral data, which confirms the correlation of $r=0.77$ obtained with earlier data (Chapman et al., 1978a). These results are seen in neither of the P300 subcomponents, C325 (P3a) and C415 (P3b). Considering C250 as an index of short-term storage produces an interesting timeline of task and memory processing. First, C250 directly indexes short-term memory storage with a larger amplitude to items that are successfully stored. This is a task-dependent event; only those stimuli whose storage is required by the task constraints (relevance and timing) elicit a strong, positive C250 detected over most of the scalp. After C250, C325 may inhibit incorrect processing of irrelevant stimuli or those relevant stimuli that do not require memory storage in this task. This could be a helpful process of filtering irrelevant or otherwise "distracting" information. Finally, C415 produces the post-storage comparison that is essential to completing the comparison task correctly. It is driven by executive processing of the second relevant stimulus, and working memory operations produce the comparison between the two relevant stimuli and the subsequent answer to the task. Clearly, brain ERP C250 adds an important, quantitatively verified biomarker of short-term memory storage.

5. Experimental procedures

5.1. Study subjects

We studied 36 young adults who were volunteers from the University of Rochester campus and surrounding areas. The group contained 22 women and 14 men with a mean age of 21.6 (SD=1.7) and a mean level of education of 15.3 (SD=1.1) years. Our study received IRB approval from the University of Rochester Research Subjects Review Board, and informed consent was obtained from each subject.

5.2. The Number–Letter paradigm

The Number–Letter task (Chapman, 1965; Chapman et al., 1979, 2007) manipulates working memory, stimulus relevancies and expectancies, and demands on executive functions. The variety of different task and stimulus conditions provides the opportunity to measure ERPs such that the corresponding underlying ERP components can be empirically manipulated and differentiated. Previous research with this task has shown it to manipulate many common and useful ERP components, including P300 (Chapman and Bragdon, 1964; Chapman, 1965; Chapman et al., 2007), contingent negative variation (CNV) (Chapman et al., 1979, 2007), C145 (Chapman et al., 2013), and other short- and long-latency ERP components.

An example trial of the Number–Letter paradigm appears in Fig. 1A. Four stimuli were flashed individually at intervals of 750 ms. The stimulus sequence contained two single-digit numbers (selected from 1 to 6) and two letters (selected from A to F). In addition, a white filled square containing a small fixation point of comparable size to the stimuli was used to initiate and terminate each trial. All visual stimuli were large (height of 5.3° visual angle), white (55 cd/m^2), and presented briefly (~20 ms) on a dark background in the same central location on a computer monitor in a darkened room. On a number-relevant block of trials, the participant compared the two numbers in each trial for numerical order, the letters being irrelevant to the task. On another block of trials, the numbers were irrelevant and the task involved comparing the two letters for alphabetic order. The same sequence of numbers and letters was used in both blocks of trials so that differences between relevant and irrelevant stimuli were not due to their visual properties or their order, which were identical. The subjects were given practice trials before the first experimental block to ensure they understood the task.

At the end of each trial, the participant said "Forward", "Backward", or "Same" to indicate the order of the two relevant stimuli. There was a delay of roughly 2–3 s between trials. The numbers and letters were randomly chosen with replacement, and the sequences of numbers and letters in the four temporal intratrial parts were randomized (constraint of two numbers and two letters per trial). The first relevant stimulus could occasionally occur in intratrial part 3. This was included so subjects would not anticipate that if the first relevant stimulus was not presented in part 1, it would be in part 2, and thereby perhaps process that first relevant stimulus differently. However, if the first relevant stimulus did not occur in part 1 or part 2, it would occur in part 3. To avoid the complexities of analyzing data when the first relevant stimulus did not occur until part 3, those data were not included in the behavioral or ERP analyses. In short, allowing the first relevant stimulus to sometimes occur in part 3 was done to reduce the role of expectancies in the subject's processing.

Every participant was shown a randomized sequence of trials. One block of 102 number-relevant and one block of 102 letter-relevant trials were completed in random order. The Number–Letter task permits examination of ERPs in response to 16 varying task conditions: two Task Relevancies (relevant, irrelevant), two Stimulus Types (letters, numbers), and four intratrial stimulus times (called Intratrial Parts).

5.3. Subject performance on the Number–Letter task

On average, the young adults correctly answered 98.6% (SD=1.5%) of the trials. There was no appreciable difference between how the women and men performed ($F(1, 35)=2.38$, $p=0.13$).

5.4. Short-term memory probe

A separate set of behavioral memory-recall data was obtained from a group of 52 young subjects (Chapman et al., 1978a). In addition to the primary task on each trial of comparing the two relevant stimuli, a memory probe test (Fig. 1B) was occasionally conducted during which subjects were randomly questioned after one of the four intratrial stimuli as to what the last stimulus they saw was. Two blank flashes (delivered at 750 ms and 1500 ms after the stimulus) were shown after the subject saw the stimulus to be probed. These flashes masked the probed stimulus, but, more importantly, delayed the recall report in order to reduce the effects of very short-term sensory (iconic) registers. This delay also allowed short-term storage to occur. An experimenter asked what stimulus was last presented and recorded the verbal response. This was performed without warning and rarely (7.8% of trials). There was one probe for each of the eight conditions (Relevant or Irrelevant by Intratrial Parts 1–4) for each block (Number- or Letter-Relevant) of 102 trials. Percentage of correct responses were converted to probit scores (z-score units); 50% and 98% correct equal 0.00 and 2.05 probit scores.

5.5. EEG recording

Scalp electrodes (a subset of the 10/20 electrodes including O1, O2, OZ, T3, T4, T5, T6, P3, P4, PZ, C3, C4, CZ, F3, F4, and EOG with reference to linked earlobes) recorded electrical brain activity while the participant performed the Number–Letter task. Frequency bandpass of the Grass amplifiers was 0.1 to 100 Hz.

Beginning 30 ms before each stimulus presentation, 155 digital samples were obtained at 5 ms intervals. Subsequently, the digital data were digitally filtered to pass frequencies below 60 Hz, and artifact criteria were based on the CZ and EOG channels to exclude those 775 ms epochs whose voltage range exceeded 200 μV or whose baseline exceeded ±250 μV from DC level (baseline was mean of 30 ms pre-stimulus). If the exclusion criteria were met, the 775 ms epoch for that stimulus was discarded on all channels. The ERPs were based on correct trials and data not rejected for artifacts. Mean artifact rejection rate was 4.9% (SD=9.9%).

5.6. Event-related potential components: Principal components analysis

We derived ERPs for each subject from his/her EEG vectors (155 time points per electrode for each stimulus (Fig. 1A)) by averaging each vector separately for each of the 16 task conditions in this experimental design (see above). ERPs were adjusted such that the mean prestimulus baseline for the first intratrial stimulus (Blank 1) was subtracted from the rest of the trial (which includes all stimuli). This permitted all the measures within a trial to have a common metric and preserved any effects that might carry over from one stimulus to another.

Because the ERP itself is a multivariate observation (due to its many post-stimulus time samples), we applied a multivariate measurement method, Varimaxed Principal Components Analysis (PCA) (Chapman and McCrary, 1995; Dien, 1998; Kayser and Tenke, 2005; Picton et al., 2000), to identify and measure the latent components of the ERPs. Volume conduction in the brain suggests an additive ERP model, which underlies the PCA process in extracting the component structure (Chapman and McCrary, 1995). PCA derives a solution that respects the possibility of components that overlap in time, which is of particular concern when measuring C250 which often appears as a shoulder to an earlier exogenous sensory component. Our approach to PCA uses ERP time points as the variables and subjects and task conditions as cases. This allows the computation of component scores (amplitudes of the components) for each of these cases.

The ERP component scores for this paper were measured using a previously derived set of ERP component loadings (used to create component waveforms—for waveforms of C250, P3a, and P3b, see the top of Fig. 3). This set of waveforms was created using a correlation matrix of the 155 time points on a group of 48 individuals: 12 with clinically diagnosed Alzheimer's disease (AD), 12 individuals with mild cognitive impairment (MCI), 12 elderly Controls, and 12 young subjects (totaling 1728 ERPs in the PCA—this number also includes the two blank conditions, as well as dividing the trials by odd and even trial number (Chapman et al., 2007). The subjects of the combined group all completed the same Number–Letter task under the same experimental and recording conditions as the current experiment. This set of varied subjects was used to create components that would be more generalizable to a wider array of individuals (Carroll, 1993) with varying cognitive and memory capabilities. This previous analysis (Chapman et al., 2007) produced eight ERP components that were retained by Kaiser's Eigenvalue>1 rule (accounting for 95% of the variance). These included well-known components, such as C415, which is sometimes called P300 (or P3b) (Chapman and Bragdon, 1964; Chapman, 1965; Chapman et al., 1978a; Polich, 2004, 2007), C325 (P3a) (Polich, 2007), CNV (Walter et al., 1964), C145 (Chapman et al., 2013), and other short- and long-latency components. Because of volume conduction, the central midline location (CZ) includes a composite of the brain activity from surrounding areas. Therefore, it is reasonable to apply the component structure derived at CZ to other electrode sites. Beginning with ERPs for each electrode, the PCA scoring procedure mathematically measured ERP component scores for each of the components for each electrode. The SAS 9.1.3 and 9.3 procedures FACTOR and SCORE were used to generate the component solution and calculate the ERP measures (Khattree and Naik, 2000).

The component scores for the C250, C325, and C415 components were retained here for further analysis. C325 and C415 were interpreted as P3a and P3b respectively due to their temporal and spatial properties. There were 16 ERP component scores, one for each Number–Letter task condition, and each subject had 16 electrodes. This equated to 256 ERP component scores for each subject. We visualized average component scores under various Number–Letter task conditions as topographical maps using the Bioelectromagnestism Toolbox (Weber, 2009) in MATLAB R2007b (MathWorks, 2011). There were no striking laterality effects based on the inspection of the maps for any of the components studied in this work; therefore, the occipital region was an average of electrodes OZ, O1, and O2, the parietal region was an average of electrodes PZ, P3, and P4, the central region was an average of CZ, C3, and C4, and the frontal region was an average of F3 and F4. For correlations with memory probe data, we selected midline electrodes CZ and PZ because there were no laterality effects. We selected F3 as representative of the frontal region (FZ was not available). F4 showed no major differences compared with F3 for both correlation results and Number–Letter task effects.

5.7. Statistical comparisons

To compare task effects, we used ANOVA with repeated measures. Using PCA to yield component scores that are based on a weighted combination of time points avoids multiple testing of measures at different time points. We performed all ANOVA procedures in SAS (SAS Institute Inc., 2014) using PROC GLM or PROC MIXED. Regression and correlation analyses were also performed in SAS using PROC REG and PROC CORR.

Conflicts of interest

There were no conflicts of interest regarding this research.

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