March 2021

Neurocognitive Interactions Between Anticipatory Anxiety and Memory Encoding

Felicia M. Chaisson
Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Cognition and Perception Commons, Cognitive Psychology Commons, and the Experimental Analysis of Behavior Commons

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_theses/5263

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
NEUROCOGNITIVE INTERACTIONS BETWEEN ANTICIPATORY ANXIETY AND MEMORY ENCODING

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Arts in The Department of Psychology

by

Felicia Marie Chaisson
B.S. Louisiana State University, 2017
B.A. Louisiana State University, 2017
May 2021
# Table of Contents

Abstract .................................................................................................................. iii

Introduction ......................................................................................................... 1

Methods ........................................................................................................... 11
  Participants .................................................................................................... 11
  Materials ........................................................................................................ 11
  Design ............................................................................................................. 13
  Procedure ....................................................................................................... 13

Analysis Strategy ............................................................................................. 16
  Arousal ............................................................................................................ 16
  Behavioral Analyses ....................................................................................... 16
  Event Related Potentials ................................................................................ 17

Results ............................................................................................................... 19
  Arousal ............................................................................................................ 19
  Behavioral Results ........................................................................................ 20
  Event related potentials ................................................................................ 20

Discussion ......................................................................................................... 26

Appendix A. Post-Test Questionnaire ............................................................... 31

Appendix B. Post-Test Feedback ..................................................................... 33

References ....................................................................................................... 34

Vita ..................................................................................................................... 40
Abstract

Although acute anxiety has been shown to improve encoding of threat-relevant information, its effects on threat-neutral information are less understood. Recent research suggests that anxiety can impair subsequent recall for neutral words, particularly following practice with the recall task. Here we use event-related potentials (ERPs) to test the notion that anxiety specifically disrupts the implementation of encoding strategies—such as elaborative encoding—that tend to develop with practice. ERPs were recorded as participants studied two sets of neutral words, one of which was presented in a stressful context using the threat-of-shock paradigm (threat block), and the other in a non-threatening context (safe block). A free recall test followed each block. As predicted, analyses of ERPs during study revealed that, relative to safe blocks, words studied during threat blocks evoked: 1) larger N400 amplitudes, consistent with impeded access to meaning, and 2) smaller amplitudes of a slow frontal positivity linked to elaborative encoding. The latter of these effects was selective to participants who received the threat block after the safe block, consistent with a dependence on task practice. In contrast to our previous work, we did not find differences in recall between conditions. However, exploratory analyses revealed that observed ERP differences were largest in those participants who recalled fewer words during threat than safe blocks. Overall, these data are consistent with models of acute anxiety that posit impairments to goal-directed internal attention, and further demonstrate their applicability to episodic memory encoding.
Introduction

Anxiety, defined as intense feelings of uncertainty directed at an impending outcome or problem, is a pervasive mental health concern in the United States (Gagnon & Wagner, 2016). Over 31.3% of adults in the United States experience some form of anxiety disorder throughout their lives (Kessler et al., 2005). Anxiety is also prevalent in formal learning settings. Of the students surveyed during the National College Health Assessment for the 2017 academic year, acute anxiety was one of the most frequently reported negative outside influences on grades. It is therefore essential to understand how anxiety impacts core cognitive processes that are vital for successful learning. In this study, we examine the neurocognitive mechanisms by which stress impacts memory formation.

One well-validated method for inducing acute anxiety involves introducing the threat of unpredictable shock using the so-called threat-of-shock paradigm (Baas et al., 2006; Bolton & Robinson, 2017; Robinson et al., 2013). In typical threat of shock studies, a block design is used to compare performance on a task when participants are told that they might receive a shock at any time (“threat” blocks), to performance when the threat of shock is removed (“safe” blocks). The resulting autonomic nervous system (ANS) response to anticipatory threat can be measured by recording electrodermal activity (EDA) to examine changes in tonic skin conductance levels (SCLs; Prokasy, 2012) between blocks.

Previous studies using threat of shock have revealed a mixture of positive and negative effects on cognition (for review, see Robinson et al., 2013). One consistent pattern that has emerged is that acute anxiety serves to enhance early sensory-perceptual processes involved in detecting environmental stimuli. For example, MacNamara and colleagues (2018) demonstrated that blocks cueing threat of shock (unpredictable) were associated with increased amplitudes of
the P200 component, an ERP waveform associated with early selective attention and visual processing of stimuli, for individuals compared to predictable shock cues or no cue, suggesting participants had increased attention when under heightened anxiety specifically. Moreover, Susskind et al. (2008) found that, when participants made fearful facial expressions, the size of their visual field increased and their search time decreased during a target localization task. Overall, these results are indicative of faster visual detection and more efficient responding to stimuli when participants are in a state of heightened anxiety.

In contrast to its facilitative effects on early detection and sensory-perceptual processing, acute anxiety has been shown to impair performance on tasks that involve goal-directed attention, particularly internally-directed attention (Derakshan & Eysenck, 2009). One of the most immediate neurophysiological effects of ANS arousal involves the release of catecholamines, which rapidly activate the prefrontal dopamine system (Arnsten & Li, 2004). Supraoptimal catecholamines in the prefrontal cortex (PFC) have been implicated in stress-related deficits in goal-directed processing, such as reduced performance on working memory tasks (Qin et al., 2009). This raises the possibility that anxiety can impede new learning by interfering with PFC-dependent cognitive control processes that would otherwise aid memory formation (e.g., Addis & McAndrews, 2006; Gagnon & Wagner, 2016).

One of the primary ways in which the PFC has been shown to contribute to memory encoding is by facilitating the implementation of “deep” or elaborative encoding strategies (Ragland et al., 2005). In the context of memory encoding, deep processing involves focusing on meaning-based information, and is typically described in contrast to the “shallow” processing of perceptual characteristics, with the former being associated with higher levels of retention (e.g., Craik & Tulving, 1975; Klein & Saltz, 1976). The term “elaborative encoding” can also refer to
encoding that focuses on meaning, but with an emphasis on deliberately integrating attributes of the to-be-remembered stimuli with each other or with prior knowledge. For example, imagining a situation in which multiple items from a word list would interact with one another is a form of elaborative encoding (Gardiner et al., 1998). Importantly, PFC activation has been observed in studies that require processing of semantic information (e.g. Baker et al., 2001; Wagner et al., 1998). These control processes, and therefore memory, have also been demonstrated to be further enhanced by the strength of the relationship between task items (Blumenfeld et al., 2011). This evidence suggests that regions in the PFC contribute to the ability to select goal-relevant item information and contribute to the ability to organize multiple pieces of information in working memory in service of effective encoding.

Thus far, the limited previous research examining the influence of anxiety on memory performance has yielded conflicting results. Work by Guez and colleagues (2015, 2016) used a social stress manipulation to demonstrate that acute anxiety during encoding of word and picture pairs impaired subsequent memory for both single items and associated pairs. Likewise, a study conducted by Schwabe and Wolf (2010) found a significant impairment of both free recall and recognition of words for participants who initially studied the words under stress compared to those in a no stress control group. By contrast, other studies have found either no effect of anxiety on memory encoding (Bolton & Robinson, 2017; Weymar et al., 2013), or even a facilitative effect (Cahill et al., 2003; Henckens et al., 2009; Payne et al., 2007; Smeets et al., 2009).

One important source of variability in previous studies is the emotional valence of the material being studied. Studies that test memory for both neutral and emotional stimuli, particularly when the emotional stimuli are negative or threat-relevant, are more likely to find
anxiety-induced enhancements of memory for the emotional materials (Cahill et al., 2003; Henckens et al., 2009; Payne et al., 2007; Smeets et al., 2009), with either no effect or a negative effect for neutral stimuli (Cahill et al., 2003; Payne et al., 2007). This phenomenon has been linked to an attentional bias toward potentially threatening information when under stress (Basanovic & MacLeod, 2017), as well as enhanced communication of the amygdala with the hippocampus due to the rapid release of norepinephrine (Joëls et al., 2006), which may selectively strengthen the encoding and consolidation of negative memories. In support of this idea, research conducted by Mather and colleagues (2016) proposed the Glutamate Amplifies Noradrenergic Effects (GANE) Model of arousal, according to which the simultaneous expression of norepinephrine and glutamate leads to the prioritization of information with high bottom-up saliency and top-down relevancy to the arousal (in spite of initial task demands). It is important to note that the inclusion of emotional stimuli thereby complicates the ability to interpret any negative effects of stress on neutral stimuli. Such findings could either reflect an inherently harmful effect of stress on neutral memory formation, and/or a tendency for the emotional stimuli to “pull” processing resources from the neutral stimuli.

My previous work (Chaisson et al., in preparation) more closely investigated the effects of acute anxiety on encoding for neutral, threat-irrelevant stimuli using the threat of shock paradigm. In this study, participants viewed and then attempted to recall two sets of neutral words, one of which was encoded in a stressful context using the threat-of-shock paradigm (threat blocks), and one of which was encoded without threat (safe blocks). Block order was counterbalanced between participants, resulting in one group of participants who received the safe block first (safe-first group) and the other of whom received the threat block first (threat-first group).
Self-report and analysis of skin conductance data confirmed that participants experienced more anxiety during threat than safe blocks. Moreover, threat during encoding had a significant negative impact on subsequent recall, such that participants recalled fewer words during the threat condition. Unexpectedly, however, this effect was only found in the safe-first group. As shown in Figure 1, participants in the threat-first group showed significantly improved recall in the second block (safe), relative to the first block (threat), whereas participants who completed the safe block first showed no such improvement.

Figure 1. Initial results from Chaisson et al. (in preparation) across both order groups, displaying proportion of correctly recalled words broken down by block and block order. There was no significant difference in performance during block 1. However, there was a significant difference in accuracy for block 2, with participants recalling more words when the second block was a safe block.

We considered two possible explanations for this pattern. One possibility is that the presentation of threat during the first block produced delayed enhancing effects that were not operative until the second block. Indeed, in addition to the rapid engagement of the ANS, the presentation of a stressor also results in the slow release of corticosteroids, which have been
shown to downregulate salience networks and enhance executive control networks, leading to possible improvements in new learning once time has passed (Gagnon & Wagner, 2016; Hermans et al., 2014).

Alternatively, the observed interaction with block order may pertain to the amount of practice or exposure to the task that participants had amassed prior to the onset of the threat induction. It could be that part of the mechanism by which acute anxiety impairs new learning involves disrupting the use of encoding strategies that participants may develop over the course of their first study-test block, which otherwise would have manifest as improvement via “practice effects” (e.g., Wesnes & Pincock, 2002) on the second block.

To distinguish between these accounts, we collected data from a post-hoc control group in which neither block occurred under threat. If the Condition X Order interaction shown in our original sample was the result of a post-stress enhancement to learning in the threat-first participants, then we would expect the performance of the control group to most closely resemble the performance of the safe-first participants, for whom all learning occurred prior to or concurrently with threat. By contrast, if the observed interaction resulted from an experience-dependent impairment to learning, then the performance of the control group should resemble that of the threat-first participants. As shown in Figure 1, the results best support the latter explanation, as performance in the control group did not statistically differ from the threat-first group. Indeed, the only significant difference among the three groups was the significantly lower recall performance on the second block in the safe-first participants relative to both the threat-first and the control group.
Figure 2. Recall accuracy results across all groups. The last column indicates the post-hoc control condition in which no threat was introduced. There were no significant differences in performance during the first block across all groups. However, there was a significant difference in accuracy for the second block, with participants recalling significantly more words during safe blocks following either a threat block or another safe block.

In summary, the results of Chaisson et al. (in preparation) provide evidence that acute threat-evoked anxiety can disrupt memory encoding for neutral information, and that the extent of this disruption may depend on participants’ pre-existing experience with the task. However, these behavioral results alone cannot tell us what processing stage or stages were most disrupted by acute anxiety. Event-related potentials (ERPs) provide temporally precise measures of brain activity that can be used to identify the neural indices associated with encoding processes. The goal of the present study is to replicate and extend our previous findings by examining the effects of threat-evoked acute anxiety on encoding-related ERPs.

To date, a modest number of studies have recorded ERPs to visual stimuli in conjunction with the threat of shock paradigm. Shackman and colleagues (2011) used ERPs to examine the effects of threat of shock on a visual discrimination task. Although task performance was
unaffected by the threat manipulation, electrophysiological analyses revealed that threat of shock was associated with larger amplitudes of the N100 component, an early negativity over posterior electrodes associated with early stimulus detection and discrimination processes (e.g., Luck et al., 2000). In addition, stimuli viewed under threat of shock elicited smaller amplitudes of the P300, a later component linked to goal-directed attention (Polich, 2007), consistent with predictions of the GANE model, that acute anxiety serves to divert processing resources toward bottom-up sensory processing.

Furthermore, and of relevance to the present study, Weymar and colleagues (2013) recorded ERPs while participants attempted to commit negative, neutral, and positive words to memory. Unlike most threat of shock studies, this study did not use a block design. Rather, half of the words in each study block were presented in a font color that indicated threat, while the remaining half were presented in a color indicating an absence of threat. The results revealed increased P2 amplitudes, another index of early selective attention, in response to cues indicating upcoming threat. In addition, amplitudes of a late (700-900 ms) frontal positivity were reduced when participants were presented with threat cues, activity researchers suggested represented a successful manipulation of arousal. However, this could be indication of slow frontal positivity associated with deeper levels of processing, discussed later.

Another ERP component relevant to the processing of words and other meaningful stimuli is the N400, a component occurring around 300-500 ms. The N400 has been characterized as an index of the processing demands or difficulty associated with extracting semantic information from a stimulus (Kutas & Federmeier, 2000, 2010). For example, the N400

---

1 It is important to note that Weymar (2013) found no significant influence of threat of shock on memory performance in their study, which limits the ability to establish a functional relationship between observed ERP differences and any behavioral effects of threat on encoding found in other studies.
exhibits increased (more negative) amplitudes in response to words that are not easily integrated with the current semantic context. N400 amplitudes during encoding have also been found to be sensitive to participants’ strategy use. Dunn and colleagues (1998) conducted a study examining the relationship between encoding-related ERPs and subsequent memory for lists of words when participants performed either a rote memorization task or an elaborative encoding task. During the elaborative encoding task only, participants who subsequently recalled fewer words expressed significantly higher (less negative) amplitudes of the N400 component at left central and posterior sites. These results thus provide evidence of a functional relationship between N400 amplitudes and the effective use of semantic elaboration during memory encoding. As such, it is possible that disruptions to strategic encoding processes due to threat of shock will manifest as larger N400 potentials.

Also associated with elaborative encoding strategies is a slow frontal positivity (SFP), which occurs between ~400-800 ms post-stimulus (Schupp et al., 2006). Mangels and colleagues (2001) reported an increased SFP amplitude during incidental encoding for participants who subsequently remembered studied words, which tended to be those identified during recognition as having evoked strong associations, elaborative imagery, and/or episodic links upon their first presentation. Furthermore, incidental learning experiments conducted by Paller, Kutas, and Mayes (1987) found similar late positive potentials at encoding for items that were studied in the context of a semantic relative to a non-semantic task, further suggesting that these late potentials may correlate with depth of processing.

To summarize, in the context of a memory encoding task, the trade-off between stimulus-driven and goal-directed processing, as identified in previous studies of threat of shock, might manifest as an enhancement of earlier processing stages (N1 and P2), combined with a disruption
of later processing stages involved in semantic processing and elaborative encoding, as indexed by the N400 and SFP. Given that the latter stages of processing are important for subsequent memory, this trade-off should behaviorally manifest as a decrease in word recall during threat relative to safe blocks. Moreover, in our prior work, the behavioral impact of threat was magnified when the threat was introduced mid-task. This pattern may reflect the fact that participants develop more effective encoding strategies over time (e.g., Delaney & Knowles, 2005), and as such these strategies are more susceptible to disruption after a moderate amount of practice.

Thus, the goals of this study were twofold. The first goal was to replicate the behavioral findings of Chaisson et al. (in preparation). In line with these results, it was predicted that: 1) subsequent recall would be worse during the threat relative to the safe conditions, particularly when the threat condition is presented second, and 2) the threat condition would be associated with greater ANS arousal, as evidenced by higher skin conductance levels relative to the safe condition. The second goal was to harness the temporal resolution of ERPs to determine which stage(s) of memory formation were most influenced by acute anxiety, if any. In particular, this study sought to examine how anxiety impacts: 1) early brain potentials implicated in the detection and early perceptual analysis of incoming stimuli, and 2) later brain potentials linked to the use of elaborative encoding strategies. I hypothesized that, similar to the results seen from Shackman (2011) and Weymar (2013), there would be increased amplitudes of the N1 and P2 components for words presented in the threat versus safe condition, reflecting facilitated sensory processing. Moreover, I expected to find decreased (more negative) N400 amplitudes and reduced SFP amplitudes for words during threat blocks, demonstrating an interruption in meaning-based or elaborative encoding processes.
Methods

Participants

Employing a power analysis on an estimated effect size of Cohen’s $d = 0.50$ from our previous study (Chaisson et al., in preparation), a sample size of 44 participants was required to achieve 90% power to detect a main effect of condition on recall scores given an alpha level of .05. However, data collection was terminated early due to the COVID-19 pandemic. At that time, 36 participants were recruited. All participants were recruited from Louisiana State University for course credit using the online SONA Research Participation System. Additionally, all participants were native English speakers, right-handed, and between the ages of 18 and 35 (age range 18-23, mean age $= 19.84$, $SD = 1.80$, 23 female). Of these, four participants were excluded from all analyses, with one participant removed due to performance that was more than three standard deviations away from the mean for recall, one due to lack of attentiveness during the task, one due to experimenter error, and one due to a history of brain surgery. Thus, analyses are reported for 32 participants, resulting in a power of 78%. This experiment was authorized under the Institutional Review Board at Louisiana State University.

Materials

Stimuli. The same stimuli was employed as in Chaisson et al. (in preparation) which consisted of 144 concrete neutral English nouns derived from the Medical Research Counsel Psycholinguistic Database (Coltheart, 1981). Normed values ranged from 500-650, ($M = 576.22$, $SD = 31.94$) for concreteness, 370-630 ($M = 513.83$, $SD = 55.06$) for familiarity, and 460-670 ($M = 567.67$, $SD = 40.02$) for imageability. An additional eighteen words were selected for use, with four per block (eight total) used as primacy and recency buffers and ten words used during practice.
**Threat of Shock.** Shocks were administered via a MP-160 BIOPAC shock stimulator and a STMISOC Current Stimulus Isolation Adapter (BIOPAC Systems, Inc., Goleta, CA) to electrodes placed at the base of the ring and pinky finger. Each participant underwent a threshold test before beginning the experiment to determine the appropriate voltage level at which shocks were experienced as uncomfortable, but not painful (mean = 9.19 mA, range = 1-50 mA). Skin conductance levels (SCLs) were recorded throughout the experiment via the BrainVision GSR-MR Module (Brain Products GmbH, Munich, Germany) at a sampling rate of 5 kHz. This was done via two additional electrodes placed at the base of the opposite ring and pinky finger. Electrode type (shock versus SCL) hand placement was counterbalanced across participants to control for possible effects of shock delivery on button presses.

All SCL recordings were visually inspected in order to detect artifacts, noise, or non-responders. Based on this inspection, 5 participants were excluded from further SCL analysis due to a lack of galvanic skin response (GSR) in either condition. SCL recordings were further processed to remove portions of the signal that reflected activity directly related to the onset of a shock. This was done by removing 10 second intervals after the onset of each shock (Cacioppo et al., 2007). Following all preprocessing, mean SCL activity was calculated for each participant during each block.

**Electroencephalography.** Continuous EEG was recorded using a BrainVision acquisition system. Recordings were collected through a 32-channel Ag-AgCl electrode cap. Electrode placement adhered to the international 10–20 system. Two additional electrodes were placed below and adjacent to each eye to record ocular movements, such as saccades and blinks. EEG data were referenced online to the left mastoid electrode and re-referenced offline to averaged

---

2 Approximately 10% of the population have been found to display no SCL response (Braithwaite et al., 2015)
left and right mastoids. Data were recorded with an online bandpass filter of 0.01-100 Hz at a sampling rate of 1000 Hz. An additional bandpass filter of 0.1-30 Hz was applied offline during preprocessing, prior to statistical analysis, and a 10 Hz low-pass filter was used for display purposes only. Impedance for all electrodes was kept below 5 kΩ. Epochs were time-locked to stimulus onset, with a time window of -200 – 1000 ms. The mean amplitude of the 200 ms prior to stimulus onset was used for baseline correction. Epochs containing artifacts such as blinks or lateral saccades were excluded from ERP analysis (mean = 10% of trials, range = 1-23%). If a participant’s data exceeded a 25% rejection rate, Independent Components Analysis (ICA) was performed to correct for eyeblinks only. Nineteen participants required ICA, with an average of two components removed (range = 1-4).

**Design**

As in Chaisson et al. (in preparation), this study employed a 2 x 2 mixed factorial design, with Condition (Safe/Threat) as a within-subjects factor, and Order Group (Safe-First/Threat-First) as a between-subjects factor.

**Procedure**

Participants were asked to study and then attempt to recall a total of 144 nouns, divided into two study-test blocks of 72. During study, words were presented in white 35-point font overlaid on a black screen. Each study trial began with a 2000 ms presentation of the study word followed by a jittered interstimulus interval (ISI) of 3500-4500 ms. The assignment of words to both conditions and block orders was counterbalanced across participants. Within each block, word order was determined randomly for each participant. Threat and safe blocks were denoted by a colored border of either red or green, respectively (see Figure 3).
Participants were instructed to attempt to memorize as many words as possible. To ensure attention to the task, participants were also asked to make trial-by-trial judgements of learning (JOLs) indicating their belief about the likelihood that they would be able to recall each word on the subsequent free recall test. JOLs were made on a 1-6 scale, with 1 and 6 indicating the least and most likely to remember, respectively. Participants were instructed to make their ratings before the onset of the next trial. JOLs were not analyzed for the purposes of this study.

Halfway through each block, participants received a 10-second break. Primacy and recency buffers were placed at the beginning and end of each block and immediately before and after each break period. After each study block, participants were asked to count backwards from 1000 by 2s for 60 seconds as a distractor task. After this distraction period, participants completed a written free recall test for the most recent study block. Recall responses were briefly reviewed by an experimenter after each test block strictly for the purpose of clarifying illegible handwriting.

Shock electrodes were only connected during the threat blocks. During threat blocks, shocks took place on eight of the 72 study trials, as well as on both primacy buffers presented in
the block, totaling to ten shocks throughout the block. The shock timing was random for each participant with the constraints that: 1) an equal number of shocks took place during the first and second half of each block, and 2) shocks were never presented on two consecutive trials.

Prior to beginning the experiment, all participants underwent a short practice and study-test block consisting of ten stimuli. At the end of the study, participants completed a post-test questionnaire measuring subjective estimates of anxiety and task performance during the threat and safe blocks. A total of eight questions were presented, each asking about either the threat block (red border) or the safe block (green border). The questions were as follows (see also Appendix A): How much did you worry about or fear receiving the shock when the border was (red/green)?; How confident did you feel about your ability to memorize the words when the border was (red/green)?; How confident did you feel about your ability to make accurate decisions about individual words when the border was (red/green)?; To what extent did the possibility of receiving a shock distract you from the task when the border was (red/green)? Additionally, four open-ended questions were provided, focused on strategies employed and overall understanding of the task. The post-test forms are included in Appendix A and B.
Analysis Strategy

Arousal

_Sympathetic Activation._ Tonic skin conductance levels (SCL) for the threat and safe blocks were compared using a 2 (Condition: threat, safe) x 2 (Order Group: threat first, safe first) mixed factors ANOVA. Trials on which shocks occurred were removed prior to analyses along with the proceeding 10 seconds to account for the autonomic nervous system refractory period.

_Subjective Ratings._ Analyses of the post-test questionnaire was limited to the questions about how much the participants worried about or feared receiving a shock (Questions 1 and 2; see Appendix A). This analysis took the form of a 2 (Condition: safe, threat) x 2 (Order Group: safe first, threat first) mixed-factors ANOVA on participants’ mean rating, with Condition as a within-subjects factor and Order Group as a between-subjects factor.

Behavioral Analyses

_Recall._ Behavioral analyses focused on the effects of threat of shock during study on the proportion of words recalled. Participants’ hand-written recall responses were entered twice by two different researchers for each participant, and any inconsistencies in data entry were resolved by the first author prior to analysis. All words identified as misspelled were corrected prior to statistical analyses. During analysis, buffers, intrusions, and falsely recalled words were identified and excluded from the proportion of accurately recalled items. Words that were simultaneously presented with a shock during study were excluded from analyses. Thus, recall scores for each block were calculated as a proportion of all non-shock trials, leaving a maximum of 64 trials for threat blocks (and all 72 trials for safe blocks). Analysis was conducted via a 2 (Condition: threat, safe) x 2 (Order Group: threat first, safe first) mixed factors ANOVA on the proportion of words recalled.
Event Related Potentials

We took two approaches to analyzing the ERPs. First, we used a standard spatiotemporal analysis approach to compare the amplitudes of the N1, P2, N400 and slow frontal positivity (SFP) between conditions and order groups. We selected electrodes to analyze for each component using a “collapsed localizer” approach (Luck & Gaspelin, 2017, Psychophysiology), which involves averaging the waveforms across all conditions and groups and using the collapsed averages to identify the electrode at which each component of interest was most pronounced. We used a similar approach to identify the time windows that represent the temporal peaks of the N1 and P2 components. For the slower N400 and SPF components, we selected the a priori time windows of 300-500 and 500-1000 ms respectively based on prior research. Together, these methods led to the selection of the measurement window of 73-183 ms averaged over electrode Fp1 for the N100; 149-299 ms over electrode Cz for the P200; 300-500 at Cz, for the N400, and 500-1000 ms over Fz for the SFP. We then conducted separate 2 (Condition: threat, safe) x 2 (Order Group: threat first, safe first) mixed factors ANOVAs on the mean amplitudes of each component.

Our second analysis approach was to submit the difference between ERPs to threat and safe trials to a repeated-measures, two-tailed cluster-based permutation test using the Mass Univariate ERP Toolbox (Groppe et al., 2011). ERPs were down sampled to 100 Hz prior to analysis. This process involves conducting repeated measures t-tests on all time-points between 0 and 1000 ms post-stimulus onset across 32 scalp electrodes (i.e., 3200 total comparisons), in addition to 2500 random with-in participant permutations. All t-scores corresponding to uncorrected p-values of 0.05 or less were formed into clusters with any neighboring t-scores. Electrodes were considered spatial neighbors when they were within approximately 5.44 cm of
one another ($M = 3.6, SE = 1.3$ electrode neighbors) and adjacent time points were considered to be temporal neighbors. T-scores within each cluster were then summed to provide a cluster-level t-score (i.e., the “mass” of each cluster). The most extreme cluster mass was used to build a data-driven null hypothesis distribution and the percentile ranking of each cluster from the observed data was used to derive its p-value, keeping the family-wise alpha level at 0.05. This p-value was then assigned to each member of the cluster. T-scores that were not included in a cluster were given a p-value of 1.
Results

Arousal

*Sympathetic Activation.* Of the 27 participants included in final SCL analysis, 11 were in the safe-first group and 16 were in the threat-first group. Results of the 2 (Condition: threat, safe) x 2 (Order Group: threat first, safe first) mixed factors ANOVA revealed a main effect of Order Group \[F(1, 25) = 7.07, p = 0.01, \eta^2 = 0.22\], as well as a main effect of Condition \[F(1, 25) = 15.43, p < 0.001, \eta^2 = 0.38\]. These results indicate participants’ SCLs were 1) higher during threat blocks compared to safe blocks and 2) higher in safe-first relative threat-first participants (see Table 1 for *Means* and *SEs*). There was no significant Condition by Order Group interaction \[F(1, 25) = 3.91, p = 0.06, \eta^2 = 0.14\].

<table>
<thead>
<tr>
<th>Order Group</th>
<th>n</th>
<th>Mean</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threat First Group</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threat</td>
<td></td>
<td>2.57</td>
<td>0.20</td>
</tr>
<tr>
<td>Safe</td>
<td></td>
<td>2.38</td>
<td>0.22</td>
</tr>
<tr>
<td>Safe First Group</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safe</td>
<td></td>
<td>3.87</td>
<td>0.38</td>
</tr>
<tr>
<td>Threat</td>
<td></td>
<td>4.38</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*Subjective Ratings.* An ANOVA conducted on participants’ mean post-experiment fear ratings (see Appendix A, Questions 1 and 2) yielded a main effect of Condition \[F(30) = 131.97, p < .001, \eta^2 = 0.81\] but no main effect for Order Group \[F(30) = 0.88, p = 0.35, \eta^2 = 0.03\], nor significant interaction \[F(30) = 0.48, p = 0.49, \eta^2 = 0.02\]. These results, along with the results of the SCL analyses, indicate that our manipulation of anxiety was successful.
Behavioral Results

Recall. Results of the 2 (Condition: threat, safe) x 2 (Order Group: threat first, safe first) mixed factors ANOVA on the proportion of words recalled revealed no significant main effect of Order Group \[F(1, 30) = 0.88, p = 0.36, \eta^2_p = 0.03\] or Condition \[F(1, 30) = 0.04, p = 0.85, \eta^2_p = 0.001\], nor a significant Order Group x Condition interaction \[F(1, 30) = 3.16, p = 0.09, \eta^2_p = 0.10\]. Thus, in contrast to the data obtained in Chaisson et al (in preparation), recall did not differ across conditions. Results are depicted in Figure 4.

Figure 4. Overall proportion of recall for each block in the safe-first group and threat-first group. Note that within each group, the condition that came first is plotted to the left of the condition that came second. Recall did not differ between condition or presentation order group. Threat trials on which shocks occurred were excluded from analyses. Error bars show ±1 standard error.

Free Recall Performance by Block and Block Order

Event related potentials: Spatiotemporal average-based analyses

Analyses were conducted on the mean amplitude for each participant per condition block across the four previously mentioned time windows and electrode sites. Each analysis was performed using a 2 (Condition: threat, safe) x 2 (Order Group: threat first, safe first) mixed factors ANOVA. Grand average ERP waveforms for frontal, central and parietal electrode sites
alongside topographical plots for amplitude difference between threat and safe for each
examined time window are depicted in Figure 5.

**N100.** From 73-183 ms, neither the main effect of Condition \([F (1, 30) = 0.04, p = 0.84, \eta_p^2 = 0.001]\) or Order Group \([F (1, 30) = 12.19, p = 0.15, \eta_p^2 = 0.07]\) was significant, nor was the
Condition by Order Group interaction \([F (1, 30) = 0.22, p = 0.64, \eta_p^2 = 0.01]\).

**P200.** No significant main effects or interactions appeared over the time window for 149-
299 ms for Condition \([F (1, 30) = 0.41, p = 0.53, \eta_p^2 = 0.01]\), Order Group \([F (1, 30) = 2.72, p =
0.11, \eta_p^2 = 0.08]\), or the Condition by Order Group interaction \([F (1, 30) = 0.61, p = 0.44, \eta_p^2 =
0.02]\).

**N400.** Analysis of activity from 300-500 ms revealed a main effect of Condition \([F (1,
30) = 4.31, p = 0.05, \eta_p^2 = 0.13]\), indicating that N400 amplitudes were larger (more negative)
during threat blocks \((M = -2.65, SE = 0.86)\) relative to safe blocks \((M = -1.86, SE = 0.71)\). There
was no significant main effect of Order Group \([F (1, 30) = 1.68, p = 0.21, \eta_p^2 = 0.05]\) or
Condition by Order Group interaction \([F (1, 30) = 2.84, p = 0.10, \eta_p^2 = 0.09]\).

**Slow Frontal Positivity.** Analyses of the 500-1000 ms time window analysis exposed a
significant main effect of Condition \([F (1, 30) = 8.14, p = 0.01, \eta_p^2 = 0.21]\), demonstrating more
positive amplitudes during safe blocks \((M = 0.12, SE = 0.41)\) compared to threat blocks \((M = -
1.11, SE = 0.48)\). Additionally, analysis revealed a significant Condition by Order Group
interaction, \([F (1, 30) = 11.70, p = 0.002, \eta_p^2 = 0.28]\). Results of follow-up paired t-tests
established that this interaction was driven by a significant difference in frontal positivity
between threat and safe blocks specifically in the safe first group \((M = -0.03, SE = 0.66\) for safe
blocks versus \(M = -2.20, SE = 0.60\) for threat blocks, \(t(15) = 3.66, p = 0.002, Cohen’s d = 0.86\). 
By contrast, no corresponding effect was present in the threat-first group. \((M = -0.21, SE = 0.51\)
for safe blocks; \( M = -0.01, SE = 0.66 \) for threat blocks, \( t(15) = 0.55, p = 0.59, \) Cohen’s \( d = 0.08 \)

There was no significant main effect of Order Group \( [F (1, 30) = 1.61, p = 0.21, \eta_p^2 = 0.05] \). In other words, even though we did not replicate the Condition x Order Group interaction on recall that was demonstrated in Chaisson et (in preparation), we did find a parallel pattern on SFP amplitudes, which were reduced in the threat condition only when the threat block came second.

Figure 5. A) Grand average ERPs for Safe (black line) versus Threat (red line) trials at encoding. Waveforms are shown from frontal, central, and parietal electrode sites. B) Grand average topographic plots of ERP differences between threat and safe trials (threat minus safe) for each analyzed component and corresponding time window.
Event related potentials: Mass univariate analyses

Cluster-based mass univariate analysis conducted on condition based (safe and threat) ERP differences revealed a total of 14 clusters. However, none emerged significant, with [all ps > 0.12]. Thus, the main effect of Condition on the SFP did not survive the more conservative threshold for significance imposed by the mass univariate approach.

Post-hoc analyses: Individual differences in behavioral and ERP responses to threat.

To summarize, ERP results reveal clear differences in electrophysiological activity when encoding occurred under threat of shock versus when it did not. Threat of shock was associated with more negative amplitudes for late time windows associated with deeper levels of processing, specifically the N400 and SFP components. However, our ability to understand the functional significance of these ERP differences is limited by the lack of significant behavioral effects of the threat manipulation. Indeed, although our prior study (Chaisson et al., in preparation) demonstrated lower rates of recall for words studied during threat than safe blocks, we did not replicate this pattern in the present study. Thus, while the increased N400 amplitudes and reduced slow frontal positivity for threat blocks could reflect less efficient encoding (albeit not robustly enough to affect behavior), these differences could also reflect either: 1) compensatory processes, by which the participants were able to maintain their memory performance despite the presence of threat, or 2) aspects of threat processing that are entirely orthogonal to memory processing. To gain traction on this issue, we conducted exploratory analyses in which we examined the ERP differences between threat and safe conditions separately for participants who performed more poorly in the threat than the safe condition (safe > threat; n = 14; mean recall = 0.12 (se = 0.02) and 0.18 (se = 0.02) for the threat and safe conditions, respectively), and those who showed the opposite pattern (threat > safe; n = 18, mean
recall = 0.19 (se = 0.02) and 0.15 (se = 0.01) respectively). Insofar as the observed ERP effects reflect processes that interfered with successful encoding, we would expect them to be more pronounced in the safe > threat group.

The results are depicted in Figure 6. In the safe > threat group, the pattern of smaller (less positive) SFP amplitudes for the threat relative to the safe block was significant \([t(13) = 3.27, p = 0.01, \textit{Cohen’s }d = 0.67].\) By contrast, this effect was not significant in the threat > safe group \([t(17) = 1.14, p = 0.27, \textit{Cohen’s }d = 0.24].\) This suggests that disruption in late-stage processing associated with the SFP is driven by decrements in recall performance induced by threat, corroborating findings demonstrated in Chaisson et al. (in prep). Parallel comparisons were conducted on N400 amplitude activity; however, there were no significant differences between the safe > threat group \([t(13) = 1.73, p = 0.11, \textit{Cohen’s }d = 0.24]\) compared to threat > safe groups \([t(17) = 1.24, p = 0.23, \textit{Cohen’s }d = 0.14].\)
Figure 6. Grand average ERPs for Safe (black line) compared to Threat (red line) conditions when grouped by recall performance. A) Grand average ERPs for participants who had better recall during Safe trials relative to Threat. B) Grand average ERPs for participants who had better recall during Threat trials relative to Safe. Waveforms are shown from midline electrodes frontal, central, and parietal electrode sites, (Fz, Cz, and Pz respectively).
Discussion

The goals for this study were: 1) to replicate the threat-induced memory deficits demonstrated in Chaisson et al. (in preparation) and 2) to harness the temporal resolution of ERPs to identify the stage(s) of processing during memory encoding that are impacted. To achieve these goals, ERPs were recorded while participants encoded threat-irrelevant stimuli in both safe and threatening contexts, the latter of which was induced via the threat of shock method. In doing so, this study directly examined whether and how anxiety impacts: 1) early brain potentials implicated in the detection and early perceptual analysis of incoming stimuli, and 2) later brain potentials linked to semantic access and the use of elaborative encoding strategies. Specifically, it was predicted that threat-evoked anxiety would facilitate early sensory processes, indexed by increased N1 and P2 amplitudes, but reduce amplitudes for the N400 and late, slow frontal positivity amplitudes, indicating a trade-off between (enhanced) early and (disrupted) late-stage processing that should be detrimental to subsequent memory.

Behaviorally, we were unable to replicate the finding from Chaisson et al., (in preparation) of overall lower recall levels in threat relative to safe blocks. The reason for this discrepancy is unclear, particularly given the close alignment of the methodology used in both studies, with the largest difference being the introduction of the EEG cap and environment in the present study. In theory, this change may have affected both the participant make-up and experience in ways that impacted the results, although we can only speculate as to how. One might imagine that participants who volunteer for EEG studies may find experimental equipment less threatening, or perhaps the longer set-up time increased comfort in the research environment. However, the fact that both self-report data and electrodermal recordings were consistent with greater anxiety in threat than safe blocks in both experiments argues against this
type of explanation. Future research will be required to determine whether the results of Chaisson et al (in preparation) are simply not robust, or if some peculiarity of the EEG experiment is to blame for the non-replication.

In contrast to the behavioral effects, we did find significant ERP differences between the threat and safe conditions that were partially in line with our hypotheses. In particular, in our spatiotemporal averaging-based analyses, we found both larger (more negative) N400 amplitudes and smaller amplitudes of the slow frontal positivity during threat than safe blocks. Given extensive previous evidence (e.g., Kutas & Federmeier, 2011) that N400 amplitudes are inversely related to ease of semantic processing, this pattern suggests that threat-induced anxiety may have impeded access to meaning-based information during encoding. Moreover, as outlined previously, it has been demonstrated that SFP amplitudes are positively associated with elaborative encoding processes important in successful memory formation, (Karis et al., 1984; Mangels et al., 2001; Weyerts et al., 1997). As such, the finding of reduced amplitudes in the present study implies that acute anxiety can interfere with participants’ ability to engage in effective encoding strategies, such as making meaningful connections among the to-be-remembered words or between these concepts and prior knowledge.

Of course, the absence of robust behavioral effects of threat in this study limits the confidence with which we can functionally interpret these electrophysiological effects. That said, multiple aspects of the data at least tentatively suggest that the observed ERP differences—particularly with respect to the slow frontal potentials—do reflect some level of decreased encoding effectiveness when learning under threat. To begin with, exploratory analyses revealed a relationship between across-participant variability in the effects of the threat manipulation on recall and the corresponding effects on ERPs, with slow frontal potential differences being larger
in people who performed more poorly in the threat condition relative to the participants who did not.

In addition, the slow frontal positivity was reduced in amplitude to the greatest degree in participants who received the threat block second. This pattern mirrors the effect of threat on recall shown in Chaisson et al. (in preparation). In our prior work, we tentatively interpreted this interaction between condition and block order as reflecting a disruptive effect of threat on test-potentiated new learning, which occurs when the experience of taking a test prompts learners to adopt more effective encoding strategies on subsequent study blocks (Chan et al., 2018; Yang et al., 2018). Similarly, attenuation of the slow frontal potential in this study when words were studied under threat after, but not before having already completed a study-test block may be a neural signature of this disruption, particularly given previous links between these ERPs and use of elaborative encoding strategies. By this account, ERP correlates of encoding effectiveness may simply be more sensitive to the effects of threat than behavioral measures, such that effects that are too subtle to affect memory performance can nonetheless manifest in these components (see Weymar et al., 2013, for a similar finding).

In contrast to previous studies that have recorded ERPs during acute anxiety in other cognitive tasks (Eldar et al., 2010; MacNamara & Barley, 2018; Shackman et al., 2006; Tanovic et al., 2018; Weymar et al., 2013), we found no significant effects of threat on early sensory components, specifically the N100 and P200. Thus, although our data are consistent with the notion that acute anxiety disrupts the use of controlled, top-down processing strategies, this disruption did not seem to go hand-in-hand with an enhancement of bottom-up processes involved in stimulus detection.
There are at least two possibilities for the lack of this type of “trade-off”. The first explanation could relate to a lack of power to detect an effect resulting from a smaller sample collected than anticipated. Alternatively, the absence of threat effects on early components could be attributed to the nature of the task and stimuli in the present study compared to those used in previous research. For example, in Shackman et al. (2011), participants underwent a visual discrimination task in which they were required to identify target shapes when flanked by distractors. It could be reasoned that performance on this task relied more on early perceptual processes than the task employed here, where individuals studied neutral words, each of which was presented for multiple seconds in a consistent font size and color. Said differently, anxiety-based enhancement of early sensory components seen in other studies could reflect a greater task-relevance of bottom-up processing in comparison to the present study.

Finally, it is important to note that, because the design of this study specifically evaluated memory for neutral stimuli, the ERP results may not generalize to the effects of threat on emotionally salient information. Indeed, while the limited amount of prior behavioral work on the effects of threat on memory for neutral stimuli have typically yielded negative (Guez et al., 2015, 2016; Schwabe & Wolf, 2010; Chaisson et al., in preparation) or no effects (Bauch & Bunzeck, 2015; Bolton & Robinson, 2017), facilitative effects of acute anxiety on memory have been reported for studies that used emotionally valent or threat-relevant stimuli. Evidence in both the current study and Chaisson et al. (in preparation) indicate that memory processing for neutral stimuli can be disrupted by acute anxiety even absent the presence of emotional stimuli, although the inclusion of emotional stimuli may exacerbate the disruption by usurping cognitive resources.
In summary, analysis of ERPs associated with memory encoding revealed that acute anxiety can disrupt encoding-related processes reflected in specific ERP components, particularly those linked to semantic processing and the use of elaborative encoding strategies. Moreover, while participants in this study did not show robust memory deficits when shock was introduced, there were individual and group differences in how much threat impacted memory that seemed to co-occur with the magnitude of the ERP effects, and which paralleled similar findings in our previous work. In conclusion, this study provides insight into the neural mechanisms by which stress disrupts learning and provides a foundation for future experiments to examine the efficacy of specific interventions—by targeting acute anxiety directly and/or focusing on compensatory learning strategies—to combat these difficulties.
Appendix A. Post-Test Questionnaire

1. When the border on the screen was **RED**, how much did you worry about or fear receiving the shock?

   1  2  3  4  5  6
   Not at all  Very much so

2. When the border on the screen was **GREEN**, how much did you worry about or fear receiving the shock?

   1  2  3  4  5  6
   Not at all  Very much so

3. When the border on the screen was **RED**, how confident did you feel about your ability to memorize the words?

   1  2  3  4  5  6
   Not at all  Very much so

4. When the border on the screen was **GREEN**, how confident did you feel about your ability to memorize the words?

   1  2  3  4  5  6
   Not at all  Very much so

5. When the border on the screen was **RED**, how confident did you feel about your ability to make accurate decisions about individual words?

   1  2  3  4  5  6
   Not at all  Very much so

6. When the border on the screen was **GREEN**, how confident did you feel about your ability to make accurate decisions about individual words?
7. When the border on the screen was **RED**, to what extent did the possibility of receiving a shock distract you from the task?

```
1 2 3 4 5 6
Not at all Very much so
```

8. When the border on the screen was **GREEN**, to what extent did the possibility of receiving a shock distract you from the task?

```
1 2 3 4 5 6
Not at all Very much so
```
Appendix B. Post-Test Feedback

1. Describe any strategies you employed in order to later recall words.

2. What strategies did you use to assign high versus low confidence rating to words?

3. Did you find certain words easier to later recall? Why?

4. What did you think this study was about?
References


with the degree of arousal at encoding. Learning & Memory, 10(4), 270–274. https://doi.org/10.1101/lm.62403


selectively and lastingly promotes learning of context-related high arousing information. *Psychoneuroendocrinology, 34*(8), 1152–1161. https://doi.org/10.1016/j.psyneuen.2009.03.001


Vita

Felicia M. Chaisson was born in Houma, Louisiana in 1990. After graduating high school in 2008, she enlisted into the United States Marine Corps where she served as an Intelligence Analysis and Arabic Linguist until 2013. In May 2017 she graduated from Louisiana State University with a Bachelor of Science degree in Psychology and a Bachelor of Arts degree in Sociology. During her time as an undergraduate Felicia worked as a research assistant in Dr. Steven Greening’s Cognitive Neuroscience of Affect and Psychopathology Laboratory, where her interest in cognitive research began. She entered the Cognitive and Brain Sciences doctoral program at Louisiana State University in 2018 and began working with Dr. Heather Lucas in her Brain and Memory Laboratory, researching the influence of acute anxiety on memory functions. Felicia plans to receive her Master’s in May 2021, after which she will continue to work with Dr. Lucas to complete her doctorate.