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The Effects of Threat-Evoked Anxiety on Visuospatial Memory Encoding

by

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Abstract

Much remains to be understood about the impact of acute anxiety on memory for emotionally neutral information. In general, anxiety has been shown to disrupt performance on tasks that involve visuospatial working memory, perhaps by diverting attentional resources from the task at hand to ensure optimal threat detection. However, no study thus far has examined the effects of threat-induced anxiety on the ability to form new memories for visuospatial information. In this experiment, we tested the hypothesis that threat-induced anxiety will disrupt the ability to use eye movements efficiently in service of memory formation, as measured by performance on a spatial reconstruction task. Participants completed 80 trials of a spatial reconstruction task, half of which were conducted in a threatening context (Threat condition), and half of which were not (Safe condition), in counterbalanced order. Eye movements were recorded during the study phases of the task, and the amount of *entropy*, or disorganization in participants scanpaths, served as an inverse measure of effective visual study. Contrary to predictions, spatial reconstruction accuracy did not differ by condition, nor did overall scanpath entropy during study. However, a significant Order x Condition interaction revealed that participants who viewed the Safe condition first showed a marginally significant *decrease* in scanpath entropy in the Threat relative to the Safe conditions. While preliminary, these results may suggest mild facilitative effects of threat-induced anxiety on the ability to form new memories under certain situations, such as when overall arousal is low due to task fatigue.

Keywords: visuospatial memory, threat-induced anxiety

The Effects of Threat-Induced Anxiety on Visuospatial Memory Formation

Throughout history, anxiety has aided humans in growth and development. When faced with life-threatening situations, humans can use anxiety to engage core fight or flight mechanisms necessary for their survival. For example, Robinson et al. (2013) reviews evidence that anxiety can heighten our abilities to detect danger, which can be useful in situations like walking home alone at night. While anxiety can, in some cases, be adaptive and facilitate cognition, there is also evidence that anxiety can have detrimental effects on cognitive performance. For example, Cassady and Johnson (2002) performed an experiment on a population of university undergraduate students that measured test anxiety and its relationship to performance on a classroom test. The results indicated a strong correlation between performance and the scores on the test anxiety scale, with the students in the group with higher test anxiety performing significantly worse than those that did not suffer from any test anxiety.

Anxiety is a pervasive public health problem. Population-based surveys (reviewed in Bandelow & Michaelis, 2015) estimate that up to 33.7% of the population experience pathological anxiety levels at some point in their lives. Moreover, in the American College Health Association’s Fall 2017 National College Health Assessment Survey, 25.1% percent of students reported that anxiety had negatively affected their academic performance within the past 12 months. Understanding when and how anxiety negatively impacts cognitive abilities is a critical aspect of assessing the full societal burden of anxiety. This is particularly prevalent among students who engage in cognitively demanding tasks on a regular basis. In this study, we tested the specific hypothesis that anxiety negatively impacts learning by interfering with the ability to gather information effectively in service of forming new memories.

Thus far, existing evidence for negative effects of anxiety on cognitive performance is strong in the domain of working memory, with a focus on the disparity between visual and spatial working memory. Visuospatial working memory is believed to be particularly susceptible to disruption from anxiety. For example, Lavric et al. (2003) used an n-back test with two manipulations that required participants to remember either spatial arrangements (spatial working memory task) or letter identities (verbal working memory task). Within each task, anxiety was induced on a subset of trials using a paradigm termed the threat of shock paradigm. The threat of shock paradigm involves attaching shock electrodes to the participants, typically on their fingers, and informing them that an occasional shock would be administered throughout the task. Although no shocks were actually administered during Lavric et al. (2003), the mere threat of shock had the effect of inducing anxiety, and thus could be used to measure the effects of acute anxiety on cognitive performance. The results of this experiment showed that participants were significantly impaired when they were threatened with a shock in the spatial working memory task; however, the threat of shock did not influence any performance on the verbal working memory task.

Shackman et al. (2006) found similar results to further support that threat-induced anxiety disrupts spatial, but not verbal, working memory. In this experiment, similar to Lavric et al. (2003), an n-back test was used with spatial and verbal tasks. The key defining difference from the prior experiment was that in the threat condition, an occasional shock was actually delivered. Again, participants showed significant impairment in spatial working memory performance during the threat conditions, whereas verbal working memory performance was unaffected by the threat of shock manipulations. These findings further support the evidence for the selective disruption of spatial working memory performance from induced anxiety.

Additional research has been conducted to determine why anxiety might disrupt spatial working memory, but leave verbal working memory unimpaired. Lavric et al. (2003) proposed that threat-induced anxiety and spatial working memory rely on a common visuospatial attention mechanism. There is evidence that the active maintenance of visuospatial information in working memory is mediated by shifts of attention (either covert or overt) from one-to-be-remembered location to the next during the delay. In support of this idea, Awh et al. (1998) found that visual information is processed more efficiently when it appears at locations being held in working memory. In this study, participants were asked to hold a specific stimulus in working memory, and to indicate whether it did or did not match a second letter (a memory probe) that appeared 5000ms later at a random location on the screen. Results showed that participants responded to the probe's identity as matching or different faster and more accurately when the memory probe was closer in proximity to the memorized target's location. This effect of distance on accuracy signifies that the act of holding the target stimulus in working memory over a delay results in participants directing spatial attention to the location at which that stimulus had originally been presented.

These findings support the notion that the cognitive resources used to maintain information in spatial working memory are shared with those used in selectively attending to spatial information. Importantly, there is also evidence that anxiety has the effect of "co-opting" some of these resources, perhaps in order to facilitate the ability to detect and process threat-relevant information that may be present in the environment. Shackman et al. (2011) describes the process as shifting the balance of attention from a "task-directed" or top-down mode of processing (in which attention is allocated to stimuli in a way that is determined by participants' cognitive goals) to a "sensory-vigilance" or "bottom-up mode" (governed by attentiveness toward detecting potential threats). In accordance with this notion, Shackman and colleagues combined a threat of shock manipulation with recording event-related potentials (ERPs) while participants completed a visual discrimination task for neutral stimuli. Although task performance did not differ between Safety and Threat blocks, ERP analyses revealed that the threat of shock enhanced the amplitude of the N1 potential, which is implicated in early sensory processing. By contrast, the amplitude of the P3, a late potential linked to later, task-directed processing, was smaller during Threat blocks relative to Safe blocks. Therefore, attentional processing in threat-induced anxiety might limit visuospatial processing resources during a demanding WM task. In other words, anxiety might impair spatial but not verbal WM rehearsal because only the former requires the ability to exert top-down control of visuospatial attention.

Interestingly, in contrast to working memory performance, studies of long-term memory have found neutral or even facilitative effects of anxiety at encoding on subsequent performance (for review, see Robinson et al., 2013). These beneficial effects have been attributed to dense anatomical connections between the amygdala, which is crucially involved with the expression and acquisition of fear and the hippocampus, which is important for the encoding of new memories (Richardson, Strange, & Dolan, 2004). That said, there is reason to suspect that, at least under some circumstances, detrimental effects of anxiety on visuospatial working memory abilities should have downstream negative effects on long-term memory formation. Visuospatial working memory allows people to hold information in mind across short delays, which is an important part of gathering information in order to construct complex visuospatial memory representations. For example, specific patterns of eye movements during encoding have been found to be predictive of subsequent memory accuracy on a spatial reconstruction task (Lucas et al., 2019). Eye movement reinstatement during study-test delays has been shown to be beneficial for later memory (Olsen et al., 2014). This implies that the act of moving one’s eyes to the (now-empty) locations at which to-be-remembered stimuli had been presented during encoding can serve to strengthen memory representations of those stimuli, perhaps by engaging in a form of mental “rehearsal”. Together, these findings suggest that the same visuospatial rehearsal process that is: 1) important for working memory tasks, and 2) disrupted by anxiety, may contribute to the process of forming certain types of long-term visuospatial memories.

In light of the above considerations, we suggest that the discrepant findings between the generally negative effects of anxiety on visuospatial working memory performance and the neutral or positive effects of anxiety on long-term memory may be partially explained by differences in the type of stimuli that have been used in prior experiments. In some studies, the stimuli used for encoding were often inherently threatening, or negatively portrayed in some way. For example, Henckens et al. (2009) incorporated negative or neutral images into sections of short films that were displayed to participants. Anxiety was manipulated by altering whether or not the films contained distressing content, such as people acting violently or displaying anger, distress, or pain. When memory for the embedded images was tested the next day, the negative images were better remembered than the neutral images, particularly in the context of distressing films. There is also evidence that individuals who are higher in trait anxiety show enhanced explicit memory specifically for threat-related words (Mccabe, 1999). Therefore, the threatening connotation associated with stimuli may have an effect on the results of prior experiments.

A second difference between the stimuli used in most working memory versus long-term memory experiments pertains to the complexity of the to-be-remembered information. Studies of anxiety and long-term memory (reviewed in Robinson et al., 2013) have tended to use relatively simple stimuli, such as words or images, which tend to be high in pre-experimental familiarity. Committing these simple stimuli to memory may place relatively minimal demands on top-down control of visuospatial attention. For example, even neutral images in Henckens et al. (2009) showed a memory benefit of being initially encoded in a stressful context. However, participants were asked to provide written description of the images in this memory test, suggesting that verbal rather than visuospatial processing was emphasized. By contrast, the ability to acquire memories for complex, multi-item displays places a greater burden on visuospatial working memory, as the learner must make constant choices about where and how to direct visual attention during encoding. The effects of anxiety on long-term memory encoding may depend on the extent to which visuospatial processing must be used strategically at encoding to optimize memory. To test this idea, in the present study, we examine the effects of anxiety, provoked by the threat of shock, on the ability to optimize encoding behaviors on a memory test for complex, visuospatial displays with no inherently threat-relevant content.

Lucas et al. (2019) recently reported a pattern of eye movement behaviors during encoding that correlated with successful performance on long-term memory tests. Within these tests, the used stimuli were complex, multi-item visual displays that contained non-threatening abstract shapes. The researchers found that the lower the entropy of the participant’s eye movements, the higher the participant’s scores. Entropy can be defined as the general decline of predictability; when a participant’s eyes move in an orderly, directed fashion, they have little to no entropy. If a participant’s scanpaths, or path of eye movements, appear to be random and unpredictable, he/she would be considered to have high entropy. In Lucas et al. (2019), moving the eyes in an organized, low-entropy fashion led to more accurate memory representations, supported by the finding of better performance on a subsequent test in which participants were asked to reconstruct the spatial displays from memory. Moreover, a follow-up study (Lucas et al., 2019) showed that individuals who obtain higher scores on tests that involve cognitive control also tend to perform better on, and use lower-entropy scanpaths during, spatial reconstruction tests. This finding further implicates top-down control over visuospatial attention in the ability to memorize complex spatial displays.

In the current study, we test the hypothesis that anxiety will disrupt the ability to organize eye movements and thereby have detrimental effects on subsequent memory performance. To manipulate anxiety, we used a threat of shock paradigm similar to those used in previous studies that have provided evidence of negative effects of anxiety on visuospatial working memory (Lavric et al., 2003; Shackman et al., 2006). We hypothesized that, compared to the blocks conducted without threat of shock, participants' scanpaths during encoding would be more random and disorganized (higher in entropy) when threat of shock was used to induce anxiety. Moreover, consistent with previous findings (Lucas et al., 2019) this increase in scanpath entropy should be associated with worse subsequent memory performance. Therefore, we expected the results of the study to provide evidence that the detrimental effects of anxiety on spatial working memory can have downstream consequences for memory formation.

Method

Participants

Participants included 30 undergraduates at Louisiana State University with an age range of 18-23. One participant was excluded due to incompletion of the experiment, and three others were excluded due to poor eye tracking data quality, for a final sample of 26 participants. Of the remaining data, 9 of the participants were male, and 17 were female. All had normal or corrected-to-normal vision and were tested individually. Informed consent was obtained from each participant.

Materials and Procedure

The stimuli used was the same stimuli used in Lucas et al. (2019) which consisted of forty visual displays, each with six novel abstract line drawings (“objects”) presented in a different spatial configuration. Object locations for each display were assigned randomly with the constraint that distance between any two objects within a display had to be at least 280 pixels.

Eighty trials were given to each participant total, in eight blocks of ten with an eye tracking calibration after each block. The Safety and Threat conditions were counterbalanced, with participants either receiving four Safety blocks followed by four Threat blocks or four Threat blocks followed by four Safety blocks. The purpose of blocking the Safety and Threat blocks in this manner was to reduce any carry-over effects of anxiety that may persist. The Threat blocks induce anxiety; therefore, the more often a Safety block immediately follows a Threat block, the more likely that lingering anxiety will be present. A Safety block was determined first by the presence of a green background with large words labeling the block as a “Safety” block, a green border that surrounded the screen throughout the trials, and the removal of the shock electrode from the participant’s finger. A Threat block was determined by a red screen that labeled the block as a “Threat” block, a red border that surrounded the screen throughout the trials, and the addition of the shock electrode to the participant’s finger.

Procedure

On each trial, participants were given 16 seconds to study the display, followed by a 4 second delay in which a blank screen will be presented (see Figure 1). A self-paced reconstruction test followed in which the items from the previous display appeared at the top of the screen. Participants then used a mouse to drag each object back to its original location. Eye movements were recorded throughout the experiment at a rate of 1000Hz using an Eyelink 1000 eye tracking system (SR Research, Ontario, Canada).

During threat blocks, shocks were delivered through the pointer and the index finger, using a shock stimulator MP-150 BIOPAC system (BIOPAC systems, Goleta, CA). Prior to the experiment, we obtained the intensity of shock that each participant individually determines to be “uncomfortable but not painful” by attaching the shock stimulator to the participants’ pointer and index fingers and delivering increasingly intense shocks until the appropriate level is reached. This individually-determined level was then used when delivering shocks during the experiment.

The shock stimulator was attached to a participant’s fingers throughout the Threat conditions. To ensure that the Safety conditions have the absence of the induced anxiety, the shock electrode was removed during the entire portion of the Safety trials.

Once the participants completed their experiment, we administered a post-experiment questionnaire. The questionnaire consisted of six self-evaluating questions that asked the participants to rank how they were affected on a 7-point scale, with one representing “not at all” and seven representing “very much so.” The questions focused on how participants’ perceptions during the red/green border (e.g., shock/no shock) conditions were of: 1) the extent to which they feared or worried about receiving a shock; 2) their overall ability to perform the memory task; and 3) the extent to which the possibility of receiving a shock distracted them from the memory task (see Appendix for the list of questions used).

Eye Tracking Methods and Analysis Strategy. Only trials that met the following quality criteria were included in analyses: 1) total gaze time recorded accounted for at least 67%

of the total trial duration; and 2) of the total gaze time recorded, the participant spent at least 67% of that time looking at one of the six objects. Participants (n = 3) for whom more than 50% of trials during safe and/or threat blocks did not meet these criteria were excluded from analysis. On average, 38/40 trials from the safe condition were included for each participant (range = 20–40), and 27/28 were included from the threat condition (range = 28-19). Note that the smaller number of threat trials is due to the need to exclude the 12 trials on which shocks were administered.

Analyses of eye movement data focused on the entropy of participants' eye movements as they study the displays. The procedure for calculating scanpath entropy is illustrated in part in Figure 2 (Lucas et al., 2019; Althoff and Cohen, 1999). We first entered each participant's item-to-item transition patterns from each trial into a matrix, with the columns representing the current focus of gaze and the rows representing the previously-fixed object. The matrix contains the proportion of total transitions that occur from each item to each other item in the display. The amount of entropy contained within each matrix was calculated using the following formula:

$$H = \sum_{i=1}^{n} P(i) * \log_2 \left(\frac{1}{P(i)}\right)$$

The resulting measure of entropy ranges from zero to one, with zero being complete constraint (organization) and one being complete entropy (randomness).

Memory Analysis Strategy. Subsequent memory was assessed based on the number of times participants commit swap errors during spatial reconstruction. A swap error was counted anytime the signs of both the X and Y coordinates of one object relative to the other are reversed, such as when an object that was initially studied above and to the right of another is placed below it and to its left during the memory test (see Figure 1). Swap errors were of particular interest because they specifically reflect failures of the ability to remember object-location bindings within an array.

We also examined overall misplacement error, or the average distance in pixels from each item's actual and reconstructed location. Misplacement error can be taken as an overall measure of reconstruction accuracy.

Results

Subjective Reports of Threat-induced Anxiety. We used paired t-tests to examine whether participants' self-reported fear of the shock, ability to perform the task, and level of distraction due to the shock differed between the Threat and Safe Conditions. The effect of condition on self-reported fear was significant [$t(28) = 8.02$, $p < 0.001$]. Participants reported significantly more fear in the Threat versus the Safe condition (mean ratings = 2.96 and 1.04, respectively, based on a 7-point scale). There was also a significant difference between the two conditions in the extent to which participants indicated that they were distracted by the possibility of receiving the shock [$t(28) = 7.62$, $p < 0.001$], with mean ratings of 2.96 and 1.08 for the Threat and Safe conditions, respectively. However, participants' mean ratings of overall ability to perform the task did not differ significantly between the two conditions [$t(28) = 1.31$, $p = 0.20$], with mean ratings of 3.50 and 3.96 for the Threat and Safe conditions, respectively. Thus, while participants reported higher anxiety and distraction in the Threat relative to the Safe condition, they nonetheless perceived themselves as able to maintain a similar level of performance on the memory test.

Scanpath Entropy and Memory Performance. We examined the overall, across-participant relationship between scanpath entropy and memory accuracy, to see if we replicated the finding from Lucas et al. (2019) in which entropy during study correlated with the number of swap errors committed. The correlation was indeed significant [$r(24) = 0.55$, $p = .004$, see Figure 3A]. A similar correlation was present for total misplacement error [$r(24) = 0.52$, $p = .005$, see Figure 3B].

Threat of Shock and Memory Performance. Next, we examined the extent to which overall memory performance (e.g., total swaps and misplacement error) differed between the threat and safe blocks, excluding trials for which shocks were actually administered. Analyses consisted of two 2 x 2, Condition (Safe/Threat) x Order (Threat First/Safe First) repeated measures ANOVAs with swap errors and overall misplacement as independent variables. Order was included as a factor to account for variability related to practice with task and/or fatigue effects, both which may affect memory performance.

The results are shown in Figures 4 and 5. In the analyses of swap errors, the main effect of threat was non-significant [$F(1,24) = 0.03$, $p = 0.86$], as was the main effect of order [$F(1,24) = 0.80$, $p = 0.38$] and the Order x Condition Interaction [$F(1,24) = 0.02$, $p = 0.91$]. Analyses of misplacement error likewise revealed no significant main effects [$F(1,24) = 0.03$, $p = 0.86$ for Condition; $F(1,24) = 0.80$, $p = 0.38$ for Order] nor a significant interaction [$F(1,24) = 0.02$, $p = 0.91$]. Thus, contrary to predictions, spatial reconstruction accuracy was similar in the Threat and Safety conditions.

Threat of Shock and Scanpath Entropy. Our analyses of scanpath entropy mirrored those of reconstruction accuracy, except with study-phase scanpath entropy as the dependent variable (see Figure 6). A 2 x 2, Condition (Safe/Threat) x Order (Threat First/Safe First) repeated measures ANOVA revealed that neither the main effect of Condition nor the main effect of Order was significant [$F(1,24) = 0.52$, $p = 0.48$ for Condition; $F(1,24) = 0.08$, $p = .78$ for Order]. However, a significant Order x Condition interaction emerged [$F(1,24) = 5.25$, $p = .03$]. Thus, we conducted follow-paired t-tests comparing scanpath entropy between the Threat and Safe conditions separately for participants who completed the Threat blocks first and participants who completed the Safe blocks first. No difference in scanpath entropy between the Threat and Safe block was present for participants who completed the threat blocks first. [$t(10) = 1.1$, $p = 0.30$]. By contrast, people who completed the Safe blocks first showed a marginally significant effect of condition, with entropy levels slightly *lower* in the Threat block relative to the safe Block [$t(10) = 1.9$, $p = 0.08$]. In other words, an effect of Threat on scanpath entropy during study was present; however, this effect was: 1) limited to the people who completed the Threat block in the second half our experiment, and 2) in the *opposite* direction of what we predicted.

Supplementary Analyses – Scanpath Entropy, Memory Performance, and Proximity to Shock. Unexpectedly, the above analyses suggested that, for some participants, being under threat of shock marginally increased rather than reduced the tendency to use constrained, low-entropy scanpaths. This pattern suggests that any additional stress or arousal experienced as a result of threat may have been mildly beneficial to participants later in the experiment, perhaps by countering negative effects of fatigue or boredom. That said, our ability to interpret this pattern is limited, both because the effect was marginally significant and because there was no evidence that this lowered scanpath entropy was accompanied by improved memory performance.

To shed additional light on this unexpected finding, we conducted supplementary analyses comparing scanpath entropy and memory errors in the Threat blocks specifically for those trials that immediately followed the administration of a shock, relative to the trials that neither contained nor immediately followed the administration of a shock. The rationale for these analyses was that, if the threat of shock helped performance by increasing arousal and combating fatigue or boredom, these effects might be most apparent in close proximity to an actual shock, when the threat manipulation is most salient. These analyses were limited to trials that occurred during the Threat blocks, and took the form of 2 x 2 ANOVAs with Order (Threat First, Safe First) as a between-subjects variable, and Trial Type (Post-Shock, Not Post-Shock) as a between-subjects variable.

The analysis for scanpath entropy revealed a significant main effect of Trial Type [$F(1,24) = 5.63$, $p = 0.03$], reflecting lower average scanpath entropy on threat trials that followed a shock (mean = 0.39, se = 0.01) versus threat trials that did not (mean = 0.41, se = 0.01), see Figure 7A. The main effect of order was not significant [$F(1,24) = 0.90$, $p = 0.35$], nor was the interaction [$F(1,24) = <0.001$, $p = 0.99$].

In the analysis of misplacement error, neither main effect was significant (see Figure 7B). [$F(1,24) = 2.3$, $p = 0.14$ for Trial Type, $F(1,24) = 0.48$, $p = 0.50$ for Order]. However, the Trial Type X Order interaction was significant [$F(1,24) = 7.93$, $p = .009$]. Follow-up paired t-tests revealed that, for the participants who completed the Safe blocks first, misplacement error during the Threat blocks was significantly decreased on trials that followed a Shock (mean = 220, se = 23) relative to trials that did not (mean = 245, se = 26, $t(10) = 2.62$, $p = 0.03$. By contrast, no difference between these trial types was present in for the people who completed the Threat blocks first [mean = 211 and 215 for post-shock and non-post-shock trials, respectively, $t(14) = 0.82$, $p = 0.43$]

The analysis for swap errors yielded no significant main effects or interactions [*F*'s < 1.2, *p*'s > 0.18] (see Figure 7C).

Overall, these results lend further support to the notion that the threat of shock may have induced levels of stress that benefitted rather than harmed performance, particularly for participants who completed the threat trials later in the experiment. Below we discuss possible implications of these results for the relationship between acute stress and memory.

Discussion

The purpose of this study was to investigate the effects of acute anxiety (manipulated via threat-of-shock) on the ability to use eye movements effectively to gather information in service of forming new, complex visuospatial memories. Previous work (Lavric et al., 2003) has provided evidence that that anxiety specifically impairs performance on spatial working memory tests, suggesting threat-induced anxiety and spatial working memory rely on a common visuospatial attention mechanism (see also Shackman et al., 2011), and that putting more attentional resources toward threat detection may come at the expense of top-down control over visuospatial processing. We reasoned that anxiety might also disrupt the formation of new memories for complex visuospatial information. Based on the work of Lucas et al. (2019), we tested the hypothesis that spatial reconstruction performance would be negatively affected by threat-induced anxiety. In particular, we predicted that threat of shock would be associated with more random and disorganized (e.g., high in entropy) scanning behaviors during study, reflecting less effective information-gathering. We also predicted more swap errors and higher misplacement errors during the spatial reconstruction tasks in the Threat condition relative to the Safe condition.

We did not find the expected negative effects of threat of shock on memory accuracy in the spatial reconstruction task. Rather, both swap error rates and overall misplacement error was similar regardless of whether the participants were under threat. Moreover, while we did find marginally significant effect of threat of shock on scanpath entropy, this effect was: 1) only for participants who completed the Safe blocks prior to the Threat blocks, and 2) in the opposite direction of what we expected (lower entropy in the Threat Block). Thus, overall, these results do not match our predictions concerning how acute anxiety would affect performance.

One possible interpretation of this unexpected pattern of results comes from work in rodents demonstrating that the manner in which stress can affect learning may depend on the amount or intensity of the stress (Salehi et al., 2010). In this study, rodents were subjected to a radial arm water maze and placed in one of three different temperature conditions (25°C, 19°C, and 16°C, representing low, medium, and high levels of stress, respectively). The results showed an inverse “U-shaped” relationship between stress and performance: the rodents in the 19°C water made the least amount of errors, compared to the 25°C and the 16°C conditions. In other words, temperatures that were not stressful and temperatures that were too stressful both decreased performance, while the middle temperature obtained the optimal performance and showed an “ideal” stress level for performance. Additionally, Anderson (1994) studied the “U-shaped” relationship that exists between caffeine and task performance in impulsive and non-impulsive participants. In this study, results indicated that as caffeine intake increased, performance on an easy (letter cancellation) task first increased, but then peaked and began to decrease.

With respect to the present results, then, one possibility is that the shock did not create high enough levels of anxiety to impair visuospatial memory formation. Indeed, while participants did report feeling significantly more anxious and fearful in the Threat versus Safety condition, anxiety levels in the Threat condition were still only rated at a mean level of 2.96/7 (compared to 1.08/7 in the Safe condition). It is also crucial to consider the limitations of self-report measures in this context. Self-report measures have the potential to be contaminated by demand characteristics, a phenomenon studied by Allen and Smith (2012). This experiment focused on the effects of demand characteristics on participants' moods and alertness to a task while chewing gum. The participants, in a between-subjects design, were told that the effect of the gum on their task performance would either be positive, negative, or neutral. Results showed that participants alertness ratings significantly increased when they were told the gum's effect would be positive or neutral. This study confirms that demand characteristics are a legitimate factor involved in the significant results of a study, and may have affected our results as well. Therefore, it is possible that participants' self-reports may have over-exaggerated the extent in which their anxiety levels differed by condition. Future research could involve corroborating self-report measures with more objective measures such as galvanic skin responses or other physiological measures that are sensitive to stress and anxiety.

Another possibility for interpretation of the results includes the notion that there is a U-shaped relationship between anxiety and task performance, which may also account for the fact that it was only the participants who completed the Threat blocks *after* the Safe blocks who demonstrated lower scanpath entropy during the Threat blocks. This was a long experiment: each participant was asked to complete eight blocks of ten trials, with a total duration of approximately 90 minutes. Participants who engaged in the Safe blocks first may have gone into the Threat blocks with a lower level of "baseline" arousal. As a result, they may have already been further away than the Threat-First group from the "optimal" level of stress or arousal for this task, allowing the shock to move their performance closer to the "zenith" of the inverted U. Indeed, analyses of both scanpath entropy and misplacement error in trials that immediately followed shock trials substantiate this interpretation. Because of the small increase in performance in the trials after a shock was delivered, we can speculate that those participants were at a lower "baseline" and this small onset of stress helped them to move more towards their optimal performance. Of course, further research will also be necessary to substantiate this findings, particularly given that the decrease in scanpath entropy in the Threat conditions for the Safe-First group was not accompanied by an increase in spatial reconstruction accuracy. Additional future research could further examine results when using differing levels of threat to directly test for the inverse "U-shaped" relationship we believe to be present.

In conclusion, these results join others to illustrate that the relationship between stress and cognitive performance is complex, and can vary depending on a person's current stress level in relation to task demands. These results are not just limited to the room of the experiment, but can be applied to a person's everyday life where anxiety and tasks are often present. The unanticipated effects of threat-induced anxiety leave a surplus of opportunity for future experiments to focus on what amount of stress is the right amount of stress for optimal task-based performance.

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Figures

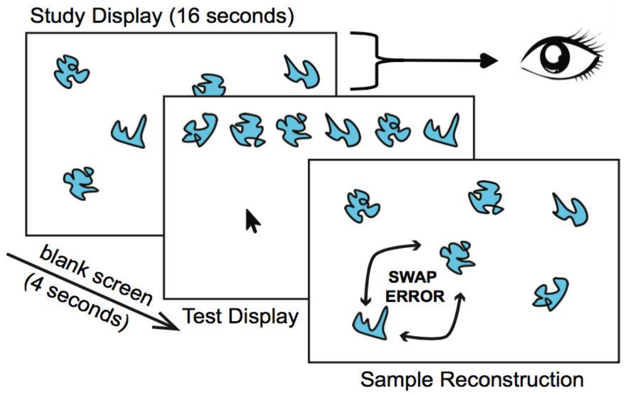
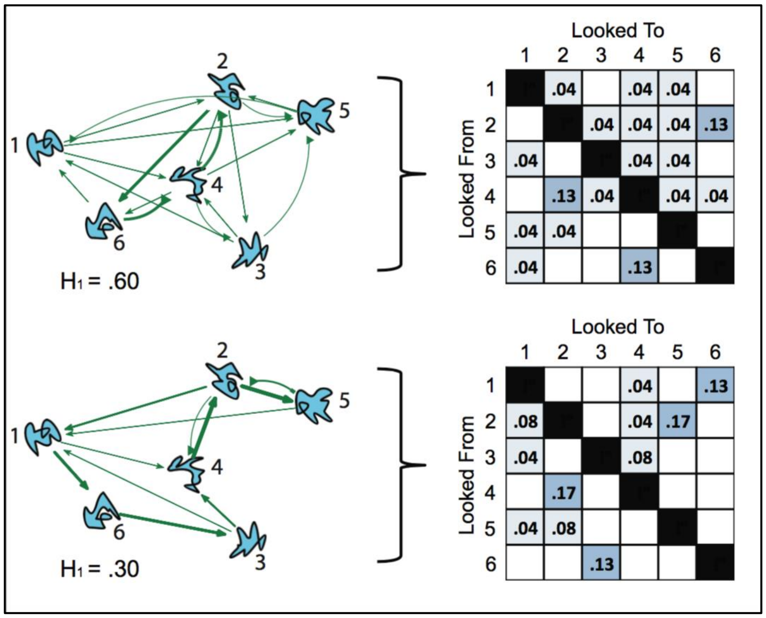


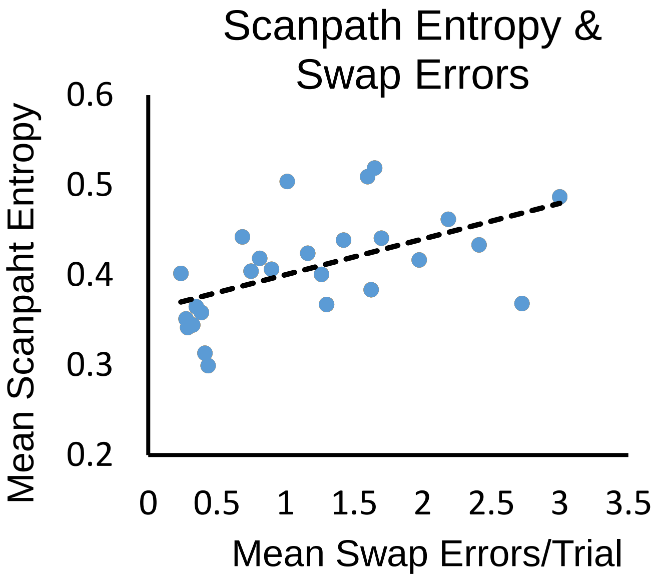
Figure 1. This figure depicts a visual representation of a sample trial from the experiment. Participants viewed the study display for 16 seconds while their eye movements were recorded. A blank screen appeared for 4 seconds followed by the test display where the target objects were rearranged in a line at the top of the screen, with instructions for participants to click and drag the target items back to their original positioning. The sample reconstruction shows an example of a swap error made: when the participant switches the placement of two target objects.



Looked From \ Looked To	1	2	3	4	5	6
1	■	.04		.04	.04	
2		■	.04	.04	.04	.13
3	.04		■	.04	.04	
4		.13	.04	■	.04	.04
5	.04	.04			■	
6	.04			.13		■

Looked From \ Looked To	1	2	3	4	5	6
1	■			.04		.13
2	.08	■		.04	.17	
3	.04		■	.08		
4		.17		■		
5	.04	.08			■	
6			.13			■

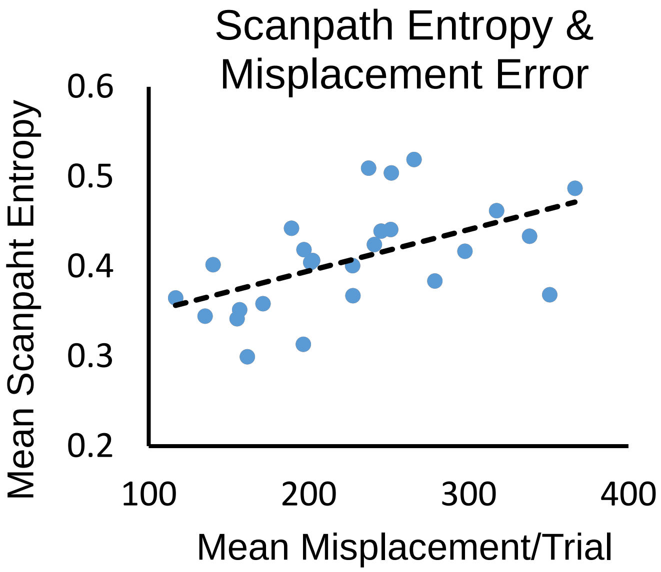
Figure 2. This figure depicts sample trials with high (top row) and low (bottom row) levels of entropy. The green lines in the figure outline the path that the participant’s eyes followed as they focused on each object. H1 is the measure of total entropy: the graph to the right depicts the probability of entropy that a participant will have looking from a specified number object to another. The top figure has a higher level of entropy because the participant shifted their gaze to and from the objects in a more disorganized and unpredictable manner, such that the transition probability graph more closely resembled a random distribution. Figure Adapted from Lucas et al. (2019).



***r*(24) = 0.55**

***p* = 0.004**

Figure 3A: Correlation of Mean Entropy and Mean Swap Errors



***r*(24) = 0.52**

***p* = 0.005**

Figure 3B: Correlation of Mean Scanpath Entropy and Misplacement Error

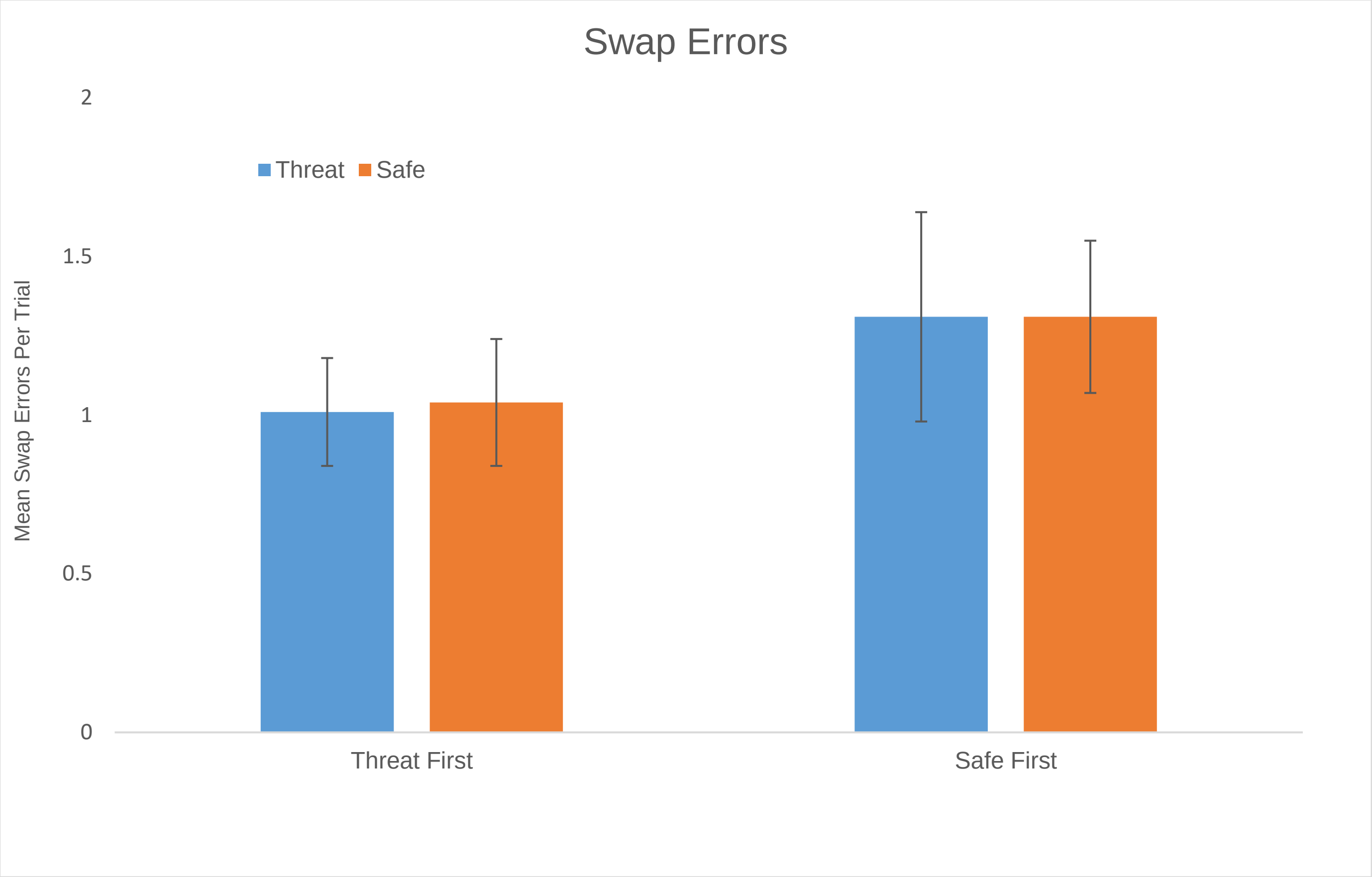


Figure 4. Total swap errors subdivided by Condition (Threat/Safe) and Order (Threat-first, Safe-first). Neither main effect was significant, nor was the interaction.

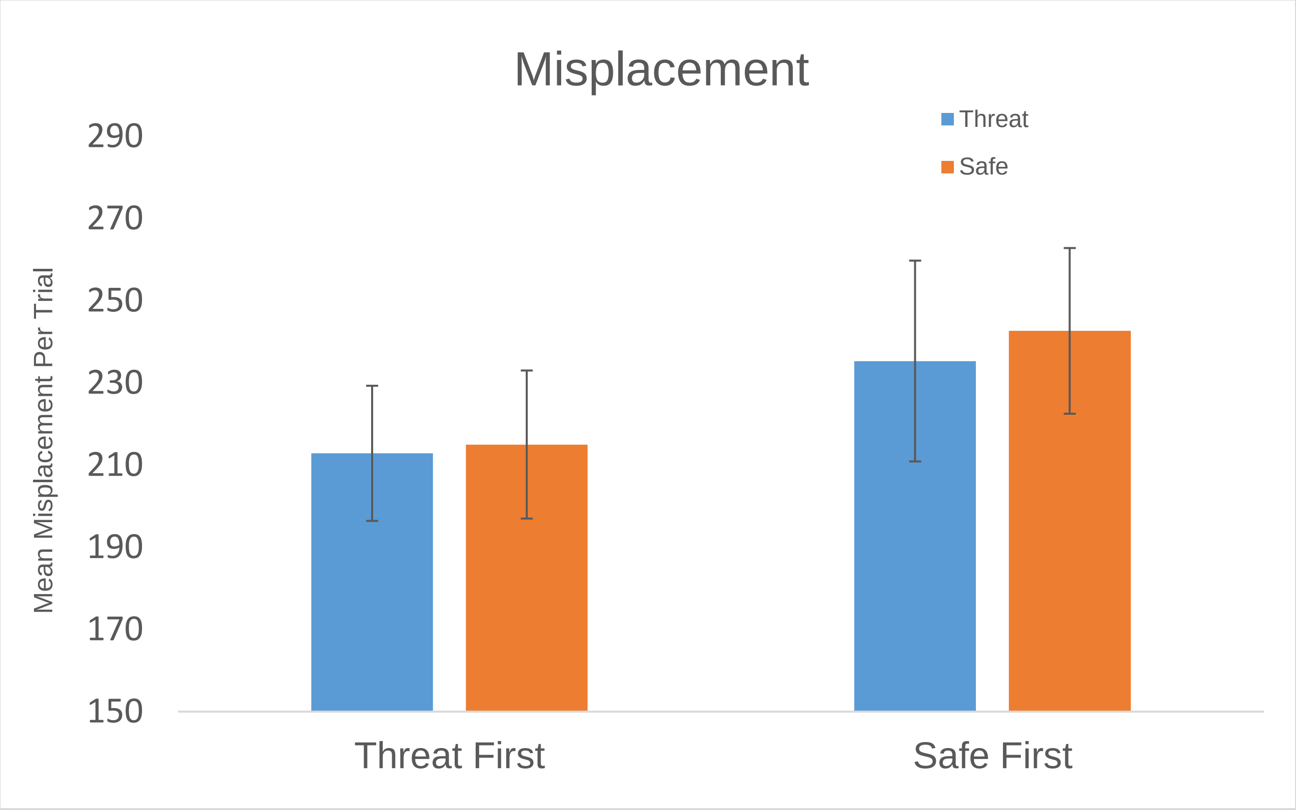


Figure 5. Total misplacement error subdivided by Condition (Threat/Safe) and Order (Threat-first, Safe-first). Neither main effect was significant, nor was the interaction.

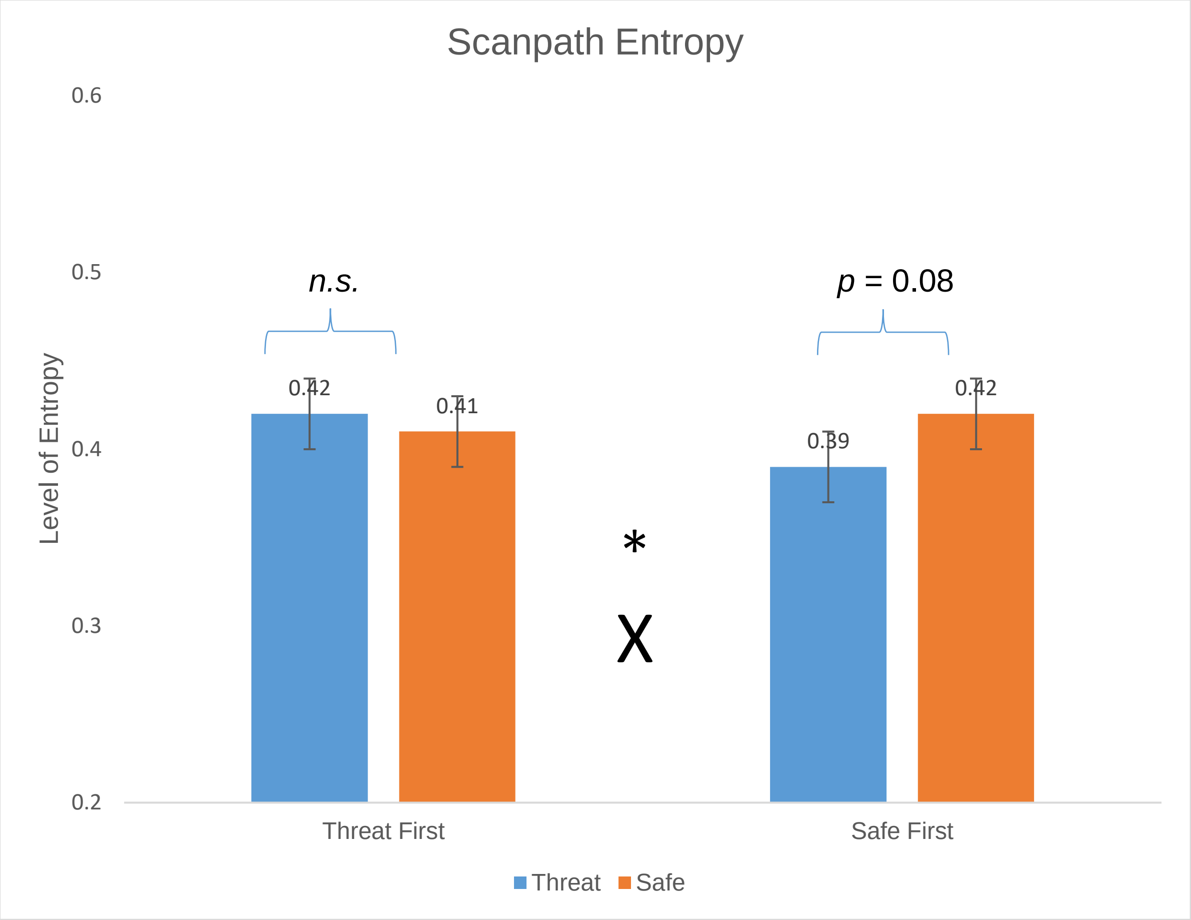


Figure 6. Results of participant scanpath entropy. There was no significant difference in entropy between the threat and safe conditions for the people who competed the Threat blocks first. However, follow-up tests to a significant interaction revealed a marginally significant effect whereby the participants who completed the Safe blocks first showed *less* entropy during Threat blocks than during Safe blocks.

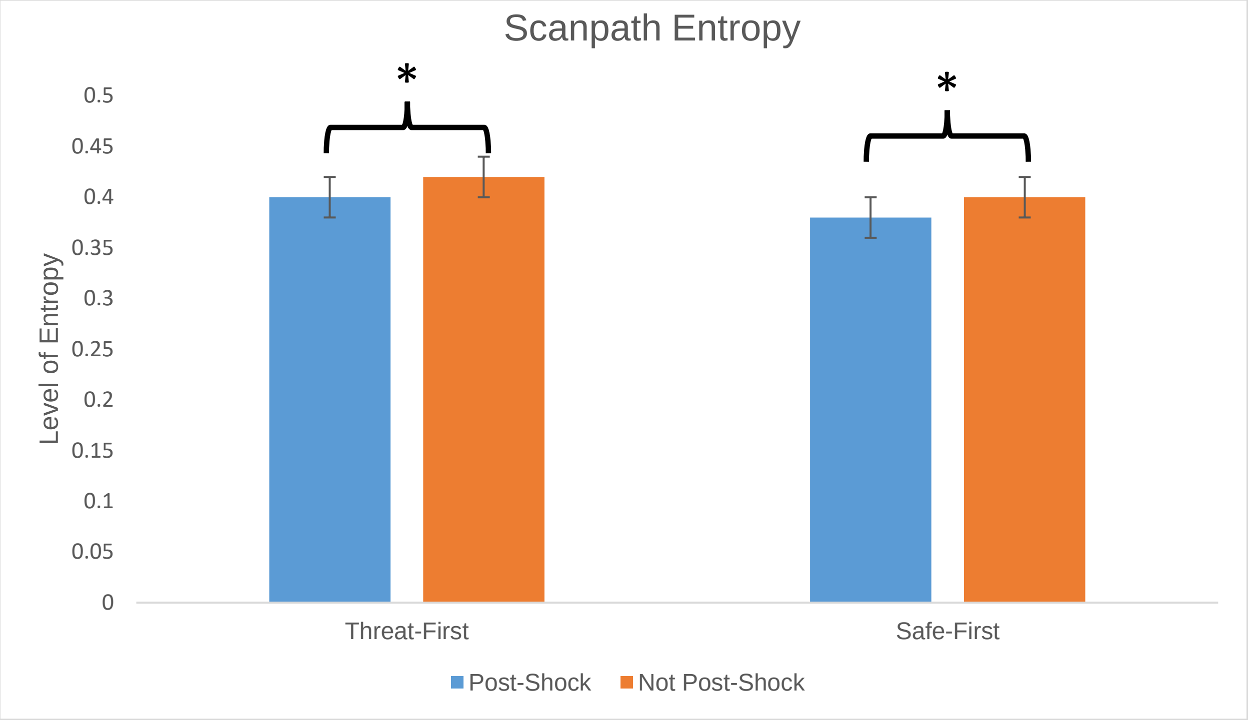


Figure 7A: Mean scanpath entropy during the Threat blocks on trials that did versus did not follow a trial on which a shock was administered. The significant main effect of Trial Type indicated that entropy levels were lower on trials that directly followed a shock trial. The main effect of order and the interaction were both not significant.

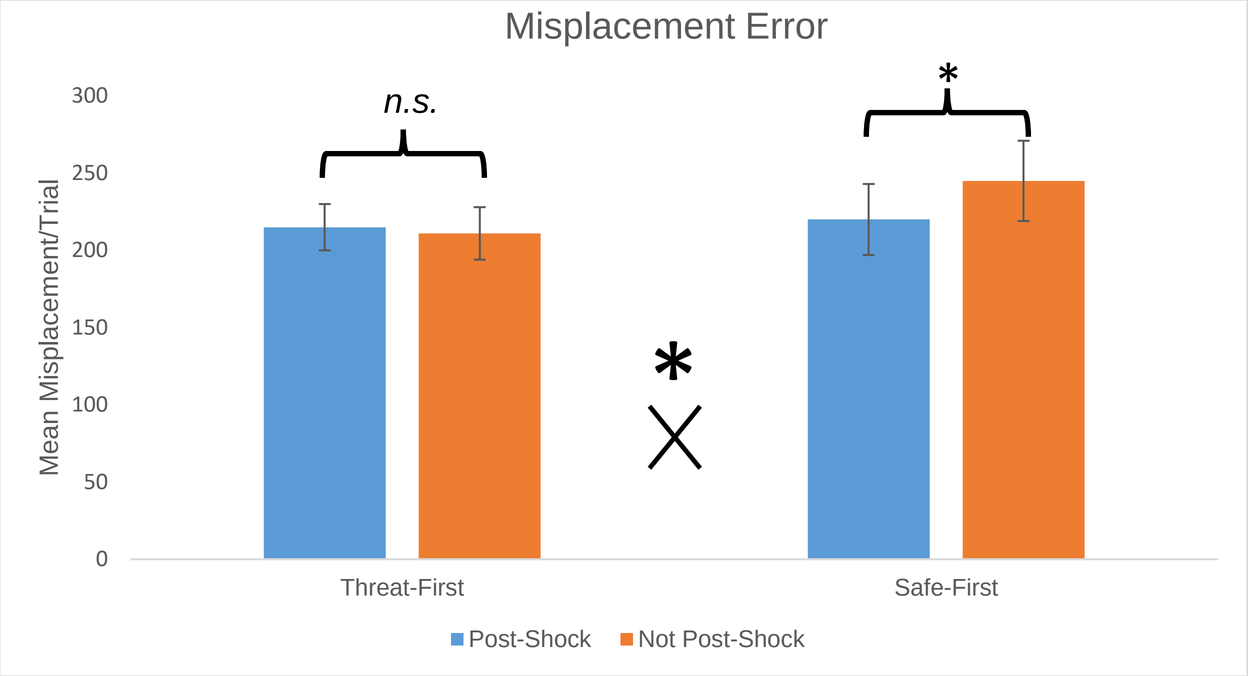


Figure 7B: Mean misplacement error during the Threat blocks on trials that did versus did not follow a trial on which a shock was administered. The significant Trial Type X Order interaction indicated that, for participants who completed the Threat blocks second, misplacement error was significantly lower on trials that directly followed a shock trial.

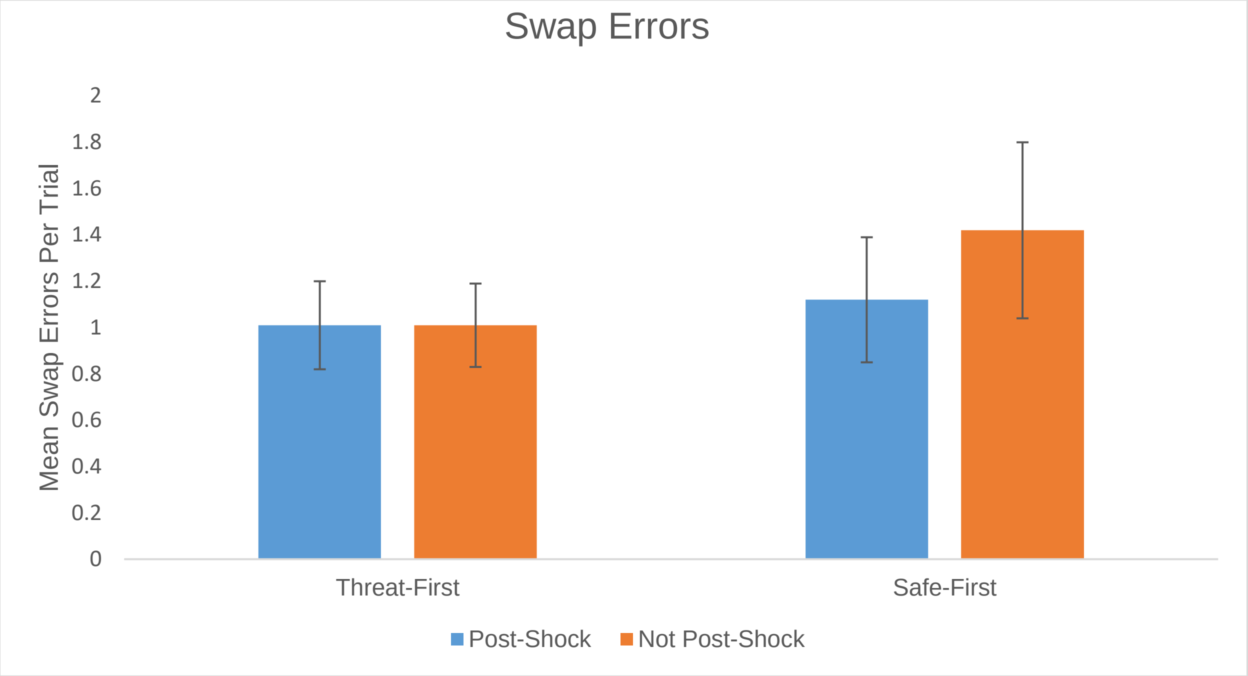


Figure 7C: Mean swap error rates during the Threat blocks on trials that did versus did not follow a trial on which a shock was administered. No significant main effects or interactions emerged.

Appendix

Study Name: ________________ Date: ____________ Participant ID: ________

Brain and Memory Lab

Post-Experiment Questionnaire

Instructions: Please circle a number 1-6 for the questions below. The information below will be kept confidential.

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 well did you feel that you were able to perform on the memory task?

1	2	3	4	5	6
Not well at all					Very well

4. When the border on the screen was GREEN, how well did you feel that you were able to perform on the memory task?

1	2	3	4	5	6
Not well at all					Very well

(continued on next page)

Study Name: ________________ Date: ____________ Participant ID: ________

5. When the border on the screen was RED, to what extent did the possibility of receiving a shock distract you from the memory task?

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

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

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