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Working memory maintenance is sufficient to reduce state anxiety

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Abstract

According to the attentional control theory (ACT) proposed by Eysenck and colleagues, anxiety interferes with cognitive processing by prioritizing bottom-up attentional processes over top-down attentional processes, leading to competition for access to limited resources in working memory, particularly the central executive (Eysenck, Derakshan, Santos, & Calvo, 2007). However, previous research using the n-back working memory task suggests that working memory load also reduces state anxiety. Assuming that similar mechanisms underlie the effect of anxiety on cognition, and the effect of cognition on anxiety, one possible implication of the ACT would suggest that the reduction of state anxiety with increasing working memory load is driven by activation of central executive attentional control processes. We tested this hypothesis using the Sternberg working memory paradigm, where maintenance processes can be isolated from central executive processes (Altamura et al., 2007; Sternberg, 1966). Consistent with the n-back results, subjects showed decreased state anxiety during the maintenance period of high load trials relative to low load trials, suggesting that maintenance processes alone are sufficient to achieve this state anxiety reduction. Given that the Sternberg task does not require central executive engagement, these results are not consistent with an implication of the ACT where the cognition/anxiety relationship and anxiety/cognition relationship are mediated by similar central executive mechanisms. Instead, we propose an extension of the ACT such that engaging working memory maintenance suppresses state anxiety in a load-dependent manner. Furthermore, we hypothesize that the efficacy of this effect may moderate the effect of trait anxiety on cognition.

The overall aim of this study is to examine whether increasing the number of items maintained in working memory (WM) is sufficient to reduce state anxiety. Anxiety is often considered to be a response to an uncertain or unpredictable aversive outcome (Grillon, 2008; Insel et al., 2010). Anxious states encompass both physiological (arousal) and psychological (worry) components (Robinson, Vytal, Cornwell, & Grillon, 2013), and the relationship between anxiety and cognition is complex. For instance, some of the major complaints of patients with anxiety disorders concern cognitive difficulties, such as excessive distractibility and poor concentration (Rinck, Becker, Kellermann, & Roth, 2003).

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However, cognition can also influence anxiety, a feature that is exploited for the treatment of anxiety disorders (Clark & Beck, 2010). A better understanding of the cognitive mechanisms contributing to downregulation of state anxiety may therefore have important clinical implications for psychological and pharmacological treatments and prevention.

According to the attentional control theory (ACT) put forth by Eysenck and colleagues, anxiety interferes with cognitive processing by prioritizing bottom-up attentional processes over top-down attentional processes, leading to competition for access to limited resources in WM (Eysenck, Derakshan, Santos, & Calvo, 2007). We have tested this possibility using n-back working memory (WM) tasks to study the effect of increases in state anxiety on WM performance (Patel et al., 2015; Vytal, Cornwell, Arkin, & Grillon, 2012; Vytal, Cornwell, Letkiewicz, Arkin, & Grillon, 2013). In these studies subjects performed verbal and spatial memory tasks of various loads (1-, 2-, 3-back) during periods of safety and shock threat, during which subjects were at risk of receiving unpredictable aversive electrical stimulation. State anxiety was measured on line with the startle reflex, a reliable measure of aversive state (Blumenthal et al., 2005; Grillon & Baas, 2003; Grillon, 2008). As expected, performance decreased as load increased and startle was potentiated in the threat compared to the safe condition (anxiety-potentiated startle; APS). The key findings were that 1) consistent with the ACT, threat of shock reduced performance on both the verbal and spatial n-back task, albeit when task demands were low but, 2) importantly, and not in line with ACT, increasing WM load decreased both APS and the deleterious effect of threat of shock on performance. The present study attempts to better understand the mechanism underlying this latter result.

According to Baddeley's revised tripartite model of working memory, WM is made up of four fluid components: the phonological loop, the visuospatial sketchpad, the episodic buffer, and the central executive (Baddeley, 1992, 2001). The first three components correspond to short term stores specialized for the maintenance of various types of information (verbal, spatial, and long term memories), while the final component represents the flexible manipulation of information within these short term stores. According to Eysenck's ACT, the central executive plays a key role in the interaction between anxiety and WM, such that worrisome thoughts interfere with allocation of attention toward task-relevant information in the short term stores (Eysenck et al., 2007). Although this theory is sufficient to explain the effect of anxiety on WM, it does not address the inhibitory effect of increasing WM load on anxiety.

The most parsimonious explanation is that the effect of WM on state anxiety is mediated by the same mechanisms responsible for the effect of anxiety on WM. Within the framework of the ACT, this should be the central executive. Therefore, assuming that this bi-directional relationship is mediated by a single mechanism, we would expect to see that the reduction of anxiety with increasing WM load is driven by activation of central executive attentional control processes. Alternatively, it is possible that the effect of WM load on state anxiety is mediated by mechanisms other than the central executive, such as competition for limited access to maintenance stores. Although both explanations can potentially be understood within the framework of the ACT (Eysenck et al., 2007), it is not possible to distinguish between these possibilities based on the n-back results alone, because the n-back is a

complex task highly dependent on executive function, requiring simultaneous encoding, maintenance, and retrieval. Furthermore, during the 2- and 3-back task, subjects must also shift their attention away from representations of the intermediate to-be-maintained items, further engaging the central executive.

One way to test this implication of the ACT is to examine the effect of WM load on state anxiety using a WM task where load can be increased without recruiting additional central executive resources. If we can replicate our effect with this task, then the central executive is not necessary to the effect of cognitive load on state anxiety. The Sternberg WM paradigm requires subjects to encode and then maintain a series of letters in WM for a brief interval fits this criterion, but does not involve manipulation of representations (i.e., central executive) (Altamura et al., 2007). Furthermore by separating the encoding, maintenance, and retrieval periods, it is possible to probe anxiety (with startle) specifically during the maintenance period, thereby minimizing the effects of encoding and retrieval.

Therefore, in the current study we used the Sternberg WM task to examine whether increasing WM maintenance alone is sufficient to reduce state anxiety. On the one hand, loading the storage capacity of WM could interfere with anxiety either by preempting the processing of threat information (i.e. crowding out worrisome thoughts) in the phonological loop (Elliman, Green, Rogers, & Finch, 1997; Rapee, 1991) or by engaging top-down emotion regulation strategies (Clarke & Johnstone, 2013). On the other hand, it is possible that the N-back task engages executive resources not recruited by the Sternberg task, and it is these executive demands that are necessary to affect the strong anxiety response evoked by shock anticipation (Konstantinou, Beal, King, & Lavie, 2014). The latter possibility is in line with the above proposed implication of the ACT, while the former possibility would suggest an alternate explanation. The differential impact of executive function and memory storage on processing task-irrelevant information has been demonstrated for pain. Indeed, pain is reduced during concurrent performance of an n-back task (Bingel, Rose, Gläscher, & Büchel, 2007; Buhle & Wager, 2010) but not during the Sternberg task (Houlihan et al., 2004). Therefore, the distinct WM processes engaged by the n-back and not the Sternberg task may be necessary for pain reduction. This study will examine whether a similar effect can be shown for state anxiety (i.e., APS).

In two experiments we asked subjects to perform a Sternberg WM task without manipulation (i.e. that do not engage the central executive) during periods of threat and safety, and probed their state anxiety during either the maintenance period or a blank intertrial interval (ITI). If central executive attentional control processes are necessary for anxiety reduction (as predicted by the above implication of the ACT), increasing the number of to-be-remembered items should not affect APS. Alternatively, if WM load alone is sufficient to reduce state anxiety, increasing the number of to-be-remembered items should reduce APS, but only if probed during the maintenance interval.

Method

Participants

Forty subjects were recruited from the community to participate in this study. Twenty subjects (14 female; age: $M = 28$, $SD = 6.8$) were included in Experiment I and twenty subjects (11 female; age: $M = 28$, $SD = 6.8$) were included in Experiment II. All subjects met the following inclusion criteria: 1) no current or past history of any Axis I psychiatric disorder as assessed by SCID-I/NP (First, Spitzer, Gibbon, & Williams, 2012), 2) no medical condition that interfered with the objectives of the study as established by a physician, and 3) no use of illicit drugs or psychoactive medications according to history and confirmed by a negative urine screen. One subject was excluded because their overall APS level (across all conditions) was two standard deviations below the collective mean of the other subjects. All participants gave written informed consent approved by the National Institute of Mental Health (NIMH) Combined Neuroscience Institutional Review Board and were compensated for participating.

Stimuli and apparatus

Stimuli were presented using the Presentation software package (version 14.6, Neurobehavioral Systems, Berkeley, CA) via a standard 19 inch LCD monitor, and responses were made using the arrow keys of a standard mechanical keyboard.

Shock—Threat of shock was used to induce anxiety (Robinson et al., 2013; Vytal et al., 2012, 2013) which was delivered via 6mm Ag/AgCl electrodes, using the SHK module of the Psychlab system (Contact Precision Instruments, London, UK). Prior to the experiment the subject was given a standard shock workup procedure to determine the intensity of the shock. Subjects rated presentations of a 100 ms shock, which gradually increased in intensity until the subject reached a level that they rated as “uncomfortable but not painful”. During blocks of threat, subjects received several unpredictable presentations of this shock.

Acoustic startle stimulus—The startle stimulus was a 40-ms bursts of a 103 dB white noise (near instantaneous rise/fall times) delivered by the TN-WN module of the Psychlab system and presented over headphones. Prior to the experiment, the subject received 9 presentations of the white noise to habituate the startle reflex.

Eyeblink reflex—The white noise elicited an acoustic startle reflex, which was measured via electromyographic (EMG) activity of the eyeblink reflex recorded from 6mm Ag/AgCl electrodes placed below the right eye over the *orbicularis oculi* muscle (Blumenthal et al., 2005). EMG was recorded at 1000 Hz and analyzed using the Psychlab version 7 software. The EMG signal was bandpass filtered (30–500 Hz), rectified, and smoothed with a 20-ms time constant. The peak startle/eyeblink magnitude was determined in the 20–100 ms after white noise onset. These scores were then transformed to *z*-scores and converted to *t*-scores for each subject in order to reduce large inter-individual differences in the overall magnitude of startle reflex (Blumenthal et al., 2005).

Affective rating scales—At the start of the experiment and after each block subjects were given affective rating scales, which were scored on a 1 to 10 scale: 1) How anxious are you (1 = *not anxious*, 9 = *extremely anxious*)? 2) How afraid are you (1 = *not afraid*, 9 = *extremely afraid*)? 3) How happy are you (1 = *not happy*, 9 = *extremely happy*)? 4) How would you rate the intensity of the electrical stimulation (1 = *not painful at all*, 9 = *uncomfortable but not painful*)?

Procedure

The setup was similar for both experiments. When subjects arrived to the laboratory, they signed the informed consent form and were given the Spielberger state anxiety questionnaire. Afterward, the shock and recording electrodes were attached. The subjects underwent the shock workup, followed by the startle habituation procedures. Next the subjects were given the instructions for the task, and asked to rate their emotional state on a set of affective rating scales.

On each WM trial, subjects saw a series of letters presented sequentially one at a time (encoding period) that was followed by a brief interval when subjects were required to maintain these letters (maintenance period). At the end of the maintenance period, subjects were prompted to make a response based on the task instructions (response period; Sternberg, 1966). At the beginning of each trial, subjects were presented with a 2000 ms cue that indicated how many letters would be presented. This was followed by a $\sim 2500 \pm 1000$ ms encoding period, a $\sim 9000 \pm 1000$ ms maintenance period, and a 2000 ms response prompt. Trials were separated by a variable duration ITI, which was dependent upon the timing of the within trial events such that each trial was 23 s long. The durations of the encoding, maintenance, and ITI periods were varied across trials by selecting a random duration (in milliseconds) between the ceiling and floor values for each period. For example, the encoding period could take any value from 1500 ms to 3500 ms on any given trial. This was done to maximize the unpredictability of the startle probe and to develop a paradigm that could be used for future functional neuroimaging studies. On low load trials, 5 (Experiment I) or 4 (Experiment II) letters were presented, while, on high load trials, 8 letters were presented (Figure 1A). The response prompt consisted of a letter and a number. The letter was chosen from the study series, and the number corresponded to a position in the series. The subjects had to indicate whether the position of the letter in the series matched the number. Half of the trials were matches, half were mismatches. On 50% of the trials a startle probe was presented during the maintenance period, while on the other 50% of the trials a startle probe was presented during the subsequent ITI. Startle probes during the maintenance periods were presented randomly within the maintenance interval, at least 3000 ms after the encoding period, and 1000 ms prior to the response period. ITI startle probes were presented randomly within the ITI, at least 2000 ms after the response period, and 4000 ms prior to the onset of the following trial.

Trials were grouped into alternating blocks of safety and threat (6 trials per block), where subjects could receive unpredictable shock during the threat blocks, but not during the safe blocks (Figure 1B). The shocks (1–2 per run) were either presented during the maintenance period or ITI, and trials with a shock were removed from the analysis. Blocks were grouped

into 4 runs with 4 blocks per run (2 per condition), and subjects completed the affective rating scales after each run. The order of the blocks was counterbalanced across runs, and the order of the runs was counterbalanced across subjects.

During Experiment I, high and low load trials were presented together during the blocks, and the order was randomly shuffled within a given block. However, we found a marginal effect of load on APS (See Results section). We hypothesized that because we used an event-related design with interspersed trials, the stronger results of the previous n-back studies might be due to the use of a blocked design with respect to load (Vytal et al., 2012, 2013). Therefore, we decided to conduct an additional study in a separate cohort using blocks of trials, rather than interspersed trials. Therefore, during Experiment II, high and low load trials were presented in separate blocks, and any given block contained either all high load trials, or all low load trials. Additionally, performance in the low load condition was reduced relative to that of the previous n-back studies (~85% correct here compared to ~95% in Vytal et al., 2013). Therefore, we also decided to reduce the number of items in the low load condition from 5 to 4. All other details remained the same across experiments.

Data analysis

Affective rating scales were averaged across blocks for safe and threat to create summary scores for the subjective levels of anxiety, fear, and happiness. Summary scores were calculated for startle, accuracy, and reaction time. Raw startle was converted to *t*-scores across trials for each subject, according to the guidelines in Blumenthal et al. (2005). These *t*-scores were then averaged across trials within each condition, and Threat-Safe contrasts were calculated independently for each level of Load (low vs. high) and Timing (maintenance vs. ITI). Accuracy was calculated as the percent of correct answers within a given condition independently for each level of Threat (threat vs. safe) and Load (low vs. high). Similarly, reaction time was averaged across trials within a given condition independently for each level of Threat (threat vs. safe) and Load (low vs. high). These summary statistics were used in the analyses described below.

Results

Affective rating scales

Experiment I—For the affective rating scales, we conducted paired sample *t*-tests (See Table 1 for a complete list of the results). As expected, subjects reported more anxiety ($t(18) = 8.85$; $p < 0.001$) and fear ($t(18) = 5.48$; $p < 0.001$) during the threat blocks than during the safe blocks. In contrast, they reported less happiness ($t(18) = -4.22$; $p < 0.001$) in the safe blocks than in the threat blocks.

Experiment II—As in Experiment I, we conducted paired sample *t*-tests (See Table 1 for a complete list of the results). Similar to Experiment I, subjects reported more anxiety ($t(19) = 6.99$; $p < 0.001$) and fear ($t(19) = 3.28$; $p < 0.001$) during the threat blocks than during the safe blocks. They also reported less happiness ($t(19) = -3.53$; $p < 0.001$) in the safe blocks than in the threat blocks.

Anxiety potentiated startle (APS)

Experiment I—For APS, we conducted a 2 (load: low vs. high) by 2 (timing: maintenance vs. ITI) repeated measures ANOVA. This analysis yielded a significant load * timing interaction ($F(1,19) = 5.93$; $p = 0.03$; See Figure 2A) but no significant main effects ($ps > 0.05$). To decompose the interaction, we conducted paired-sample t -tests contrasting low and high load for the maintenance and ITI periods. For the maintenance period, APS was marginally larger during low load trials, while for the ITI, APS was marginally larger during the high load trials, but none of these effects reached significance ($ps > 0.05$).

Experiment II—For APS, we conducted a 2 (load: low vs. high) by 2 (timing: maintenance vs. ITI) repeated measures ANOVA. This analysis yielded a significant load * timing interaction ($F(1, 18) = 16.63$; $p < 0.01$; See Figure 2B) but no significant main effects ($ps > 0.05$). To decompose the interaction, we conducted paired-sample t -tests contrasting low and high load for the maintenance and ITI periods. For the maintenance period, APS was significantly larger during low load trials than the high load trials ($t(18) = 3.92$; $p < 0.01$). However for the ITI, which should not be affected by load, there was no significant difference between APS for high and low load trials ($p > 0.05$).

Accuracy

Experiment I—For the accuracy and reaction time, we conducted a 2 (context: safe vs. threat) by 2 (load: low vs. high) repeated-measures ANOVA. As expected, participants were significantly more accurate on the low load trials than on the high load trials ($F(1,19) = 29.5$; $p < 0.01$; See Figure 3A). However, there were no other significant main effects or interactions ($ps > 0.05$).

Experiment II—As in Experiment I, participants were significantly more accurate on the low load trials than on the high load trials ($F(1,18) = 84.34$; $p < 0.01$; See Figure 3B). But there were no other significant main effects or interactions ($ps > 0.05$).

Reaction time

Experiment I—Consistent with the accuracy data, participants were also significantly faster on low load trials than on high load trials ($F(1,19) = 27.86$; $p < 0.01$; See Figure 4A). In addition to this load main effect, there was also a main effect for threat ($F(1,19) = 7.64$; $p = 0.01$), with faster reaction times during threat blocks than during safe blocks. However, there was no significant threat by load interaction ($p > 0.05$).

Experiment II—Again, consistent with Experiment I, participants were significantly faster on low load trials than on high load trials ($F(1,18) = 19.49$; $p < 0.01$; See Figure 4B). In addition to this load main effect, there was also a main effect for threat ($F(1,18) = 8.03$; $p = 0.01$), with faster reaction times during threat blocks than during safe blocks. However, there was no significant threat by load interaction ($p > 0.05$).

Discussion

The purpose of this study was to determine the effects of loading items in WM on state anxiety. In previous studies using the n-back task we showed that 1) threat of shock interferes with WM performance, and 2) increasing WM load reduces state anxiety (Vytal et al., 2012, 2013). Because the n-back requires the constant encoding, maintenance, and retrieval of information in WM, it was unclear whether the reduction in state anxiety was due to engagement of the central executive component of WM (Altamura et al., 2007; Fletcher & Henson, 2001; Rottschy et al., 2012). In the current study we used the Sternberg WM task, which separates the encoding, maintenance, and retrieval intervals (Sternberg, 1966). This allowed us to test the effects of WM maintenance alone, without requiring the engagement of the central executive component of WM (Altamura et al., 2007; Fletcher & Henson, 2001; Rottschy et al., 2012). Consistent with the previous n-back studies, these results confirm that increasing WM load reduces state anxiety and, additionally, show that this state anxiety down-regulation can be caused by tasks that engage WM components other than the central executive. However, unlike the previous n-back studies, threat of shock did not reduce performance, suggesting that the performance impairment may be caused by influence from anxiety on the executive component of WM. The remainder of this discussion will focus on integrating these two findings into the ACT.

According to Eysenck's ACT, anxiety interferes with the ability of the central executive to efficiently allocate attention (Eysenck et al., 2007). Support for the ACT comes primarily from studies of individuals with high trait anxiety, and can be summarized by the following two lines of evidence: 1) individuals with high trait anxiety often have trouble efficiently allocating attention during cognitive tasks (Basten, Stelzel, & Fiebach, 2011, 2012; Qi, Ding, & Li, 2014), and 2) these individuals also tend to show biases in attention toward threat-related stimuli (Peers, Simons, & Lawrence, 2013; Stout, Shackman, & Larson, 2013; Telzer et al., 2008).

As mentioned in the introduction, the ACT does not directly address the effect of cognition on anxiety. According to the implication of the ACT proposed in the introduction, the central executive component of WM should be necessary for state anxiety reduction; however, the results of the current study suggests that taxing the WM stores by increasing the number of items in WM is sufficient to reduce state anxiety, which argues against exclusive central executive necessity. Although we specifically studied working memory, these results are consistent with a number of studies showing that a wide variety of cognitively demanding tasks elicit both a reduction in emotional expression (King & Schaefer, 2010; Neumann, 2002; Van Dillen, Heslenfeld, & Koole, 2009; Vytal et al., 2012, 2013) and reduction in emotion-related brain activity (Clarke & Johnstone, 2013; Erk, Kleczar, & Walter, 2007; Van Dillen et al., 2009), suggesting that task difficulty may be a key factor in state anxiety disruption. For instance, King and Schaefer (2010) found a decrease in startle potentiation during an emotional picture viewing task when subjects were required to maintain items in working memory. Van Dillen et al. (2009) also found that subjects reported less negative affect during an emotional picture viewing task when required to engage in a concurrent mental arithmetic task. In addition, this study also found corresponding decreases in activity in regions of the brain known to be involved in emotional expression (Van Dillen et al.,

2009). Similarly, Erk et al. (2007) found decreases in amygdala activity to negative pictures presented during high load verbal WM trials. Finally, Clarke and Johnstone (2013) found negative prefrontal-amygdala interactions during a 3-back spatial WM task under threat of shock.

Unlike the findings with startle modulation, the findings with accuracy are not necessarily inconsistent with the ACT. Although the ACT predicts reduced performance efficiency, i.e., RT (Eysenck et al., 2007), rather than reduced performance efficacy, i.e., accuracy, we do indeed find decreases in accuracy using the n-back task (Vytal et al., 2012, 2013), but not during the Sternberg WM task. This is important because the former (n-back) requires the central executive, which is key to the ACT, while the latter (Sternberg) typically does not (Altamura et al., 2007; Fletcher & Henson, 2001; Rottschy et al., 2012). Thus although the ACT does not necessarily predict a performance effect even when central executive is needed, the fact that we see a performance deficit in a task that requires the central executive, but not in a task that does not require the central executive is consistent with the ACT. Similarly, others have shown that high trait anxious individuals perform worse than non-anxious individuals on a Sternberg task WM task, but only when there are competing demands for access to WM storage (Calvo & Eysenck, 1996). In addition, subjects recruit additional neural resources performing the Sternberg WM task under cold pressor stress than during periods of non-stress, suggesting that stress reduces WM processing efficiency (Porcelli et al., 2008). Finally, both verbal and spatial WM capacity is reduced following recall of negative autobiographical memories (Allen, Schaefer, & Falcon, 2014), suggesting that negative mood induction has a similar detrimental effect.

However it should be noted that there are other possible explanations for the lack of a performance effect in the current study. One possible explanation is that the effects of threat on WM performance are limited to spatial WM. This is consistent with two studies showing that threat interferes *selectively* with spatial 3-back performance (Lavric, Rippon, & Gray, 2003; Shackman et al., 2006). In line with these studies, we found selective deficits in spatial 3-back performance under threat of shock. However, deficits in both verbal and spatial n-back performance were found only at lower loads (i.e. 1-back and 2-back; Vytal et al., 2013). Given that we included both high and low load WM trials and found no effect on performance, it is unlikely that our lack of performance effect is due to our choice of a verbal WM task.

In order to fully explain the relationship between anxiety and WM, the ACT must be extended to address the effect of WM on state anxiety. In the following, we propose an extension to the ACT, along with a set of testable hypotheses. In short, we argue that engaging in a cognitively demanding task results in a prioritization of goal-directed attention over stimulus-driven attention, and that when these tasks take place during periods of elevated state anxiety, this prioritization leads to an overall reduction in anxiety (Vytal et al., 2012). In other words, the primary function of attention control when it comes to anxiety is to prevent anxious thoughts from occupying WM resources, and that this filtering reduces anxiety. Given that this anxiety reduction occurs even during tasks that do not require the central executive, our results suggest that this reduction could be due to a competition for resources at the level of WM stores (Elliman et al., 1997; Rapee, 1991). According to the

ACT, individuals with high trait anxiety primarily suffer from deficits in tasks that require the central executive (Eysenck et al., 2007), which suggests that, unless otherwise filtered, these anxious thoughts interfere with central executive processing. In other words, those who can flexibly control their attention are better able to reduce their anxiety, which then has a less profound effect on their WM. In contrast, those with weak attention control are unable to suppress their anxious thoughts, and therefore suffer WM deficits (Balderston et al., 2016). Consistent with this hypothesis, APS is negatively correlated with performance deficits on the n-back task (Vytal et al., 2013).

Although we show state anxiety reduction during a task that alone does not require central executive engagement, it is possible that cognitive load is sufficient to engage the central executive during periods of high state anxiety, resulting in top-down suppression of threat-related information processing (Clarke & Johnstone, 2013; Van Dillen et al., 2009). Indeed, according to Baddeley's multi-component model of WM, one of the major functions of the central executive is to protect items in WM from potential sources of distraction (Baddeley, 1992, 2001; Repovs & Baddeley, 2006). Although the current results suggest that WM load-related anxiety reduction results from competition for resources among WM stores, rather than top-down central executive processes, it is difficult to rule out the latter hypothesis based on the current results alone. In either case, one candidate source for anxiety reduction is the dlPFC, which has been repeatedly shown to play a role in WM (Altamura et al., 2007; Barbey, Koenigs, & Grafman, 2013; Curtis & D'Esposito, 2003; Feredoes, Heinen, Weiskopf, Ruff, & Driver, 2011; Geier, Garver, & Luna, 2007), anxiety (Bishop, 2009; Forster, Nunez Elizalde, Castle, Bishop, & Elizalde, 2015; Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2006; Peers et al., 2013; Shang et al., 2014), and emotional learning (Carter, O'Doherty, Seymour, Koch, & Dolan, 2006).

One way to distinguish between these hypotheses is to experimentally engage these prefrontal mechanisms in the absence of a cognitively demanding task. For instance, previous studies have shown that transcranial direct current stimulation (tDCS) over the dlPFC can improve WM performance (Fregni et al., 2005), however this effect has yet to be tested under threat of shock. According to the top-down inhibition hypothesis, driving dlPFC activity with tDCS during a low load WM task should reduce state anxiety because it activates the cognitive control system responsible for filtering out anxious thoughts. In contrast, according to the competition for resources hypothesis, this manipulation should have no effect on, or perhaps increase anxiety because it increases the resources available in WM, thereby allowing for the expression of anxious thought. In line with the cognitive-based treatments for anxiety, this technique (if successful) could prove to be one avenue toward treatment for anxiety disorders. Future studies should be conducted to explore this possibility.

In the current study we examined the role of WM load on state anxiety across two experiments. In the Experiment I we observed a moderate effect of WM load on state anxiety, while in Experiment II we observed a more robust effect. Although not the main focus of this work, there are a number of potential explanations for this difference in effect size. First, the larger effect size in Experiment II could have been due to the difference in trial order. In the first study we presented shuffled high and low load trials together in blocks

of safety and threat. Perhaps this unpredictability reduced the subjects' ability to engage proactive attentional control mechanisms (Braver, 2012). Another possible explanation for this difference in effect size could be the difference in the number of items used in the low load conditions. In Experiment I we used 5 items for the low load condition, while in Experiment II we used 4 items. Indeed we do see better accuracy and lower RTs in the low load condition in Experiment II, suggesting that the load manipulation was more successful in Experiment II. This result that more substantial differences in load lead to larger effects on state anxiety further support the conclusion that WM load is key for this state anxiety reduction. Although there were differences in the experimental designs of the studies, and slight differences in the results across studies, the basic pattern of results across studies is largely consistent.

Strengths and Limitations

In this study we show that cognitive load reduces state anxiety. Major strengths of this study include 1) actual manipulation of state anxiety, 2) a within-subject design, and 3) reliance on well-established methods of anxiety induction (shock threat) and online anxiety measurement (anxiety-potentiated startle). In addition, this study built on previous studies using the n-back WM task. Because we used the Sternberg WM task, our study allowed us to isolate the effects of WM maintenance from other overlapping cognitive operations such as manipulation, encoding, and responding that are inherent to n-back tasks, therefore extending the findings from the previous n-back studies. In addition, we were able to replicate this effect across two independent experiments, suggesting that our findings are robust.

Although we were able to demonstrate a clear, robust down regulation of state anxiety by WM, we did not find that anxiety impaired WM performance. It could be argued that anxiety would have interfered with WM storage at higher load. However, this seems unlikely as we found WM deficits at low but not high-load in our previous n-back (Vytal et al., 2012, 2013), and higher load could lead to floor performance (~50%). Based on these results, it could also be argued that our lack of an anxiety effect on performance is due to our choice of an intermediate level of load in the low load condition. However, this is an unlikely explanation for the lack of a threat effect on performance in the current study. In the Vytal et al. (2013) work, participants performed the verbal 2-back task at ~94% accuracy in the safe condition, but only ~87% accuracy in the threat condition. Although accuracy in the low load condition of Experiment II was similar to that of the verbal 2-back condition in Vytal et al. (2013), accuracy in the low load threat condition was not significantly reduced.

Another limitation of the current study was that we used APS, a proxy for state anxiety, as our primary outcome measure. Given that most of the support for the ACT comes from studies focusing on trait anxiety (Basten et al., 2011, 2012; Peers et al., 2013; Qi et al., 2014; Stout et al., 2013; Telzer et al., 2008), additional work is needed to determine how our findings fit within this framework.

Another limitation of this study is the use of ITI startle probes as a control condition for maintenance startle probes. Because we paired 50% of the trials with a maintenance probe, and 50% with an ITI probe, trials where a maintenance probe was not presented ended with

an ITI probe 100% of the time, making them potentially more predictable than the maintenance probe. This could explain why APS in the ITI was lower than in the low load condition. However, given that the primary focus of this study was APS during the maintenance period, this effect should be examined in future studies (See below).

Future studies

Our results show that APS during maintenance was larger in the low load compared to the high load task. In addition, during the low load task, APS was larger during maintenance compared to ITI, whereas there was no difference between maintenance and ITI during the high load task (Figure 2). A potential interpretation of the increased APS from ITI to maintenance during the low load task is that subjects were more aroused or vigilant during maintenance compared to ITI, and this increase in vigilance served to further potentiate startle. One possibility is that involvement in any task increases vigilance, leading to enhancement of attentional processing of external and internal stimuli, and possibly increasing emotional responses. One way to test this hypothesis would be to compare the magnitude of APS during a picture task where subjects engage in either free viewing or target detection. If task involvement increases vigilance and increases emotional responses, adding a low load target detection condition to a free viewing picture task should increase APS.

Although not specifically designed to differentiate between block and event-related designs, our results also show that state anxiety reduction was moderately larger in the version of the task with the blocked trials than the version of the task where trials were interspersed. Given the hypothesis that this anxiety reduction may be mediated by the dlPFC, this difference across the experiments may be related to the organization of the dlPFC. Some have hypothesized that the dlPFC is organized along an anterior-to-posterior gradient where increasingly abstract information is processed by the anterior portions of the dlPFC (Badre, 2008; Koechlin, Basso, Pietrini, Panzer, & Grafman, 1999; Koechlin & Summerfield, 2007), and this information informs activity in the posterior regions (Donoso, Collins, & Koechlin, 2014). Accordingly, it is possible that blocking trials according to load differentially affects different regions of the dlPFC. If this is the case, we should expect to see different patterns of dlPFC activity evoked by blocked vs. interspersed Sternberg WM trials under threat of shock.

Finally, our recent work suggests that individuals with anxiety disorders show a general deficit in n-back performance compared to healthy controls, and that this deficit is accompanied by reduced WM-related activation of the dlPFC (Balderston et al., 2016). These results suggest that dlPFC-mediated cognitive control is impaired in these individuals. Interestingly, the current results suggest that taxing WM prevents anxious thought from accessing WM stores, thereby reducing state anxiety. It is possible that this capacity for state anxiety reduction is diminished in individuals with anxiety disorders, which could be a mediating factor in the cognitive deficits seen in these individuals. Future studies should be conducted to explore this relationship.

Conclusions

In this study we tested WM during periods of safety and threat, and show that high WM load reduces state anxiety as measured by APS. Because we used the Sternberg WM paradigm, we were able to isolate WM maintenance, and show that it alone is sufficient to achieve this state anxiety reduction. Based on these results, we propose an extension of the ACT such that engaging WM suppresses state anxiety in a load-dependent manner. Furthermore, we hypothesize that the efficacy of this effect may moderate the effect of trait anxiety or threat on cognition.

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Figure 1. Schematic of experimental design and statistical comparisons

A) On each trial subjects saw a series of letters. Letter presentation was followed by a brief maintenance period, which was followed by a response window. During the response window, subjects were presented with a letter and a number. The letter was one from the series, while the number indicated a position in the series. Subjects had to indicate whether the number matched the position of the target letter in the series. Startle probes (arrows) were presented either during the maintenance period or the ITI (intertrial interval). **B)** During Experiment I, subjects were presented with shuffled trials of high and low load during blocks of safety and threat. During Experiment II, subjects were presented with blocks of high and low trials in either the safety or threat conditions.

Figure 2. Anxiety potentiated startle (APS) during Experiment I (shuffled) and Experiment II (blocked)

A) APS during Experiment I. Subjects show marginally larger APS during low load trials than the high load trials when the startle probe is presented during the maintenance period (not significant). **B)** APS during Experiment II. Subjects show significantly larger APS during the low load trials than the high load trials when the probe is presented during the maintenance period. (Bars represent the mean $\pm$ SEM. Asterisks represent significant differences.)

Figure 3. Accuracy (% correct) during Experiment I (shuffled) and Experiment II (blocked)
A,B) Subjects are significantly more accurate during the low load than the high load trials regardless of threat condition. **A)** Accuracy during Experiment I. **B)** Accuracy during Experiment II. (Bars represent the mean $\pm$ SEM. Asterisks represent significant differences.)

Figure 4. Reaction time (RT) during Experiment I (shuffled) and Experiment II (blocked)
A,B) Subjects are significantly faster during the low load than the high load trials. Additionally, subjects are significantly faster during the threat trials than during the safe trials; however there is not threat $\times$ load interaction. **A)** RT during Experiment I. **B)** RT during Experiment II. (Bars represent the mean $\pm$ SEM. Asterisks represent significant differences.)

Table 1

Affective rating scales.

Condition	Safe	Threat
Experiment I		
Anxious	2.61 (0.51)	6.47 (0.45)
Afraid	1.79 (0.37)	4.53 (0.56)
Happy	5.71 (0.59)	3.41 (0.45)
Shock Rating		7.81 (0.32)
Shock Intensity		3.42 (0.26)
Experiment II		
Anxious	2.89 (0.42)	6.09 (0.45)
Afraid	2.3 (0.37)	4.29 (0.56)
Happy	5.79 (0.59)	3.99 (0.52)
Shock Rating		8.1 (0.36)
Shock Intensity		2.76 (0.19)