The effects of acute psychological stress on working memory and verbal declarative memory

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ABSTRACT

Previous research has shown that stress can cause severe impairment in a number of different memory systems. This study aimed to investigate the effects of acute psychosocial stress on verbal declarative and working memory functioning. Declarative memory was tested by cued recall and recognition of a verbal paired-associates test; working memory was measured by an nback test with various difficulty levels. Forty-three undergraduate university students (22 females) were recruited to participate. They were required to take part in two sessions, 24 hours apart. Session One involved learning and cued recall of the word pairs, as well as a complete nback protocol. During Session Two, 21 of the participants (the Stress group) were exposed to a psychosocial stressor and the other 20 (the Relax group) were exposed to a relaxation period. Physiological and self-report measures of stress were taken at three different intervals pre- and post-exposure. Participants were then administered (a) cued recall and recognition tests for the previously-learned word pairs, and (b) the same *n*-back protocol as before. Data were analysed for participants who produced sufficient samples of salivary cortisol, and for participants in the Stress group who could be characterised as 'cortisol responders'. Results suggested some interesting trends (e.g., on the recognition task, participants in the Stress group displayed a positive response bias) that require further investigation in studies employing larger sample sizes.

Keywords: stress; cortisol; declarative memory; working memory; sex differences; menstrual cycle.

EFFECTS OF STRESS ON FUNCTIONING

One of the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition's (DSM-IV) symptoms for Posttraumatic Stress Disorder, which occurs in response to traumatic, stressful events such as rape or torture, is the "inability to recall an important aspect of the trauma" (American Psychiatric Association, [APA], 1994, p. 428). However, this memory impairment does not only occur in the face of major stressors. Almost all people have experienced the situation where they have studied diligently for an important test or exam, only to walk into the venue feeling stressed and anxious and to discover that when they look at the questions on the paper, they cannot remember the answers. Thus the experience of memory impairment as a result of stress is an everyday occurrence that has practical implications for understanding the working of cognition. As a result, this subject has been widely researched for a number of years, although there remains much to be discovered.

A stressor is an environmental event, or the anticipation of such an event, that results in one of two general stress responses (Kemeny, 2003; Sapolsky, 2004; Wolf, 2003). The first of these responses quickly results in major physiological manifestations that include sweating and an increasingly rapid heart beat. The second response is a slower cognitive reaction involving the functioning of the hypothalamus-pituitary-adrenal (HPA) axis (Sapolsky, 2004; Wolf, 2003). The HPA axis response leads to the secretion of hormones involved in increasing the ability of an individual to handle a crisis situation (Alderson & Novack, 2002; Kemeny, 2003). It is important to note that although these hormones are produced and sustained by the HPA axis response under normal circumstances too, at times of stress the amount produced is far greater (Alderson & Novack, 2002).

Basic HPA axis functioning progresses along the following paths: First, the detection of a stressor causes the hypothalamus to release corticotrophin-releasing hormone into the anterior pituitary, which in turn releases adrenocorticotrophin into the blood. This release results in the synthesis of hormones (viz., glucocorticoids; cortisol in humans) in the adrenal cortex. From here these hormones move through the blood-brain barrier into the brain where they impact on a number of different areas, including the hippocampus and prefrontal cortex (PFC; Alderson & Novack, 2002; Kemeny, 2003; Sapolsky, 2004; Wolf, 2003).

These two brain regions are of the most interest to this discussion because the hippocampus has been found to be critical for the functioning of declarative memory (DM) which involves the processing of specific information that is easily remembered and can be expressed verbally (Squire, 1992). The PFC has been implicated in working memory (WM; Wolf, 2003) which involves the short-term retention and handling of limited bits of information (Baddeley, 2001).

Effects of stress on memory

Research studies investigating the effects of stress on memory achieve an increase in participants' cortisol levels using several different methods. One way is through the direct administration of cortisol (e.g., orally in the form of cortisone) to participants (e.g., de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Lupien, Gillin, & Hauger, 1999; Wolf, Convit, et al., 2001). Another way is by means of exposing participants to a psychosocial stressor, such as a public speaking task (e.g., Elzinga & Roelofs, 2005; Kirschbaum et al., 1996; Kuhlmann, Piel, & Wolf, 2005; Oei, Everaerd, Elzinga, Van Well, & Bermond, 2006; Schoofs, Preuβ, & Wolf, 2008; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). The studies reviewed below each used one of these two forms of experimental manipulation of cortisol levels.

Declarative memory

Although a number of studies have investigated the effects of increased cortisol levels on DM through use of free and cued recall tasks, findings have been largely inconclusive. For instance, de Quervain et al. (2000) and Kuhlman et al. (2005) found that 24-hour delayed free recall of a word list was affected when cortisol levels were increased just before the retrieval phase. However, the former study did not find similar impairments when levels were raised at other points during the procedure and also found that immediate free recall remained unimpaired across all cortisone administration points. The latter study found that delayed cued recall remained unaffected by stress. Similarly, Wolf, Convit, et al. (2001) found that increased cortisol only affected 2-hour delayed free recall at the retrieval stage while increased levels at the encoding stage did not impair immediate or delayed free recall. Wolf, Schommer, et al. (2001) confirmed that free recall was not affected by stress experienced before encoding took place.

Lupien et al. (1999) found that elevated cortisol levels at the encoding phase did not affect delayed cued recall, although they did not investigate these effects at the retrieval stage. However, Kirschbaum et al. (1996) reported two investigations indicating that higher cortisol levels prior to encoding resulted in decreased delayed cued recall ability and de Quervain et al. (2003) also found that 24-hour delayed cued recall was impaired by increased cortisol levels at the retrieval stage.

On the other hand, the effects of stress on recognition have not been extensively examined. Of the abovementioned studies, only de Quervain et al. (2000) and de Quervain et al. (2003) included a recognition outcome measure of DM with both studies finding that recognition was unaffected by increased cortisol levels. However, Lupien et al. (1999) suggested that it might be advantageous in future research to test recognition in conjunction with free recall. They argued that if impairment in DM due to stress was found at the point of retrieval, then although free recall would be impaired, recognition would be intact because the words would have been properly encoded and would therefore be recognized even if not recalled. However, if the problem was at the level of encoding, then both free recall and recognition would be impaired because if the words had not been properly encoded, there would be nothing to recall or to recognise. Thus it may be necessary to measure recognition in addition to recall for a better understanding of the point (encoding or retrieval) at which memory processing is impaired by stress.

Working memory

The impact of stress on memory tends to be investigated in terms of effects on DM more often than in terms of effects on WM (Schoofs et al., 2008). Studies on the effects of increased stress and cortisol levels on WM use three main measures: digit span tests (using both forwards and backwards conditions), the *n*-back test and the 'Sternberg paradigm' (see Schoofs et al., 2008, for a detailed explanation of each). However, there is some debate about the efficacy of digit span tests as a measure of WM. Schoofs et al. (2008) argue that these tests do not include measurement of reaction times, a factor they believe to be important in investigating WM. They also state that digit span tests are shorter than either of the other two tasks used to measure WM, with each difficulty level typically being tested by only two trials. The combination of these two

factors suggests that digit span tests may not be as sensitive a measure of WM as, for instance, the *n*-back test.

Despite this debate, studies on the effects of stress on WM have reached more conclusive results than have studies on the effects of stress on DM. Lupien et al. (1999) and Wolf, Convit, et al. (2001) investigated the effects of increased cortisol levels on both DM and WM. The former study used the Sternberg paradigm as their measure of WM whereas the latter used the forward and backward digit span as theirs. In contrast to their findings regarding DM, basic results for both studies showed that increased cortisol levels resulted in a definite impairment of WM. However, while Lupien et al. (1999) explicitly and intentionally compared the effects of increase cortisol levels across WM and DM, Wolf, Convit, et al. (2001) did not. They admit that they "did not anticipate that cortisol would have much of an effect on frontal function" and as such, "Digit Span was being used as a control task" (p. 1008). They go on to state that other, more refined tests of WM should be used to investigate these effects more closely.

Most recent studies, with the exception of Kuhlmann et al. (2005) have supported these findings. For instance, Elzinga and Roelofs (2005) used a digit span task and found increased cortisol levels to be detrimental to WM functioning, specifically with regard to the forward condition. Oei et al. (2006), using the Sternberg paradigm, found that at greater WM loads, increased cortisol levels resulted in both greater WM impairments and slower reaction times.

Schoofs et al. (2008) found further evidence for the impairment of WM under stress. They used the *n*-back test (with two difficulty levels, a 2-back and a 3-back condition) to assess WM. Compared to unstressed participants, their stressed participants showed a significant decrease in WM ability (in terms of both correct responses given and reaction times) across both difficulty levels. Interestingly, however, the authors noted that the effects of stress on WM ability decreased during the duration of the task. This finding echoed the pattern detected by Elzinga and Roelofs (2005) who observed that for both stressed and non-stressed participant, WM improved as the task became more familiar. Schoofs et al. (2008) speculated that this finding may have arisen because WM tasks are more likely to be affected by stress when they are novel to the participant than when they are practiced. They also noted that it may be useful to include *n*-back conditions that range from easy (e.g., 1-back) to more difficult (e.g., 3-back) in order to identify at what difficulty level WM is affected. This suggestion is based on Sliwinski, Smyth, Hofer, and Stawski's (2006) finding that although impaired performance due to stress

could be seen on more difficult *n*-back conditions such as the 2-back condition, performance remained intact on easier conditions such as a 1-back condition.

RATIONALE FOR RESEARCH

The literature reviewed here indicates that there is largely conflicting evidence regarding the effects of stress on different memory processes. My study addressed many of the limitations found in previously conducted studies and improved and extended them in several ways, including the following:

- 1) Oei et al. (2006) state that the effects of a psychosocial stressor on WM and DM have generally not been looked at in the same study. My study investigated the impact of an acute psychosocial stressor on both WM and DM.
- 2) Although Kuhlmann et al. (2005) studied WM and DM in reaction to a psychosocial stressor, their test of WM, the digit span test, has been shown to be a potentially inefficacious WM task with which to investigate the effects of stress on memory. Thus, my study improved on this by following Schoofs et al. (2008) in using the *n*-back paradigm to measure WM. Additionally, I addressed the latter authors' concerns about their limited use of this task by including very easy *n*-back conditions alongside more difficult ones.
- 3) My study also aimed to extend Lupien et al.'s (1999) study. While they looked at how increased cortisol levels affected the *encoding* of the declarative memory material, my study investigated how stress affects the retrieval of previously learned pairs. In addition, my study followed these authors' suggestion that recognition should be tested in conjunction with recall to further investigate the effects of stress on DM.
- 4) Apart from de Quervain et al. (2000), de Quervain et al. (2003) and Kuhlmann et al. (2005), the majority of other studies in this field have been conducted in one day. My study was conducted over two days, allowing for (a) the establishment of a baseline WM measurement for each participant, and (b) for delayed cued recall to be tested. This meant I could study both between-group and within-group effects of stress on both DM and WM, thereby extending the literature in potentially interesting ways.

SPECIFIC AIMS AND HYPOTHESES

My study aimed to compare the effects of an acute psychosocial stressor on verbal declarative and working memory and in doing so, to address some of the limitations of previous studies in this field (see discussion above).

The hypotheses for the current study were that:

- 1) Twenty four hour delayed cued recall DM would be more impacted by stress than 24-hour delayed recognition, which would be unaffected by stress.
- 2) WM would be more negatively impacted by stress at higher levels of difficulty on the *n*-back than at lower levels of difficulty.
- 3) Although all participants would show some improvement on WM performance from Day 1 (when everyone was unstressed) to Day 2 (when on group was stressed) of testing, those participants who were administered the WM task after being stressed on Day 2 would show less improvement than those who continued to be unstressed.
- 4) Despite this improvement, WM would still be more influenced by the effects of stress than cued recall DM.

DESIGN AND METHODOLOGY

Design and Setting

Following Kuhlmann et al. (2005), my study took place over two consecutive days for each participant. Day 1 consisted of a learning and acquisition phase for the DM tests and established a baseline level for WM. On Day 2 the participants were pseudorandomly divided into either the stress condition or the control condition, and underwent the appropriate experimental manipulation before being administered the testing phase for both DM and WM.

Time of day has been found to be a possible confounding factor in studies of this nature because "cortisol levels show a circadian rhythm, in which levels increase dramatically on awakening and gradually decrease throughout the day, reaching lowest levels in the evening" (Dickerson & Kemeny, 2004, p. 359). Thus, cortisol changes due to acute stress are more easily identified in the afternoon as this is when they are most constant (Dickerson & Kemeny, 2004, p. 381). B. M. Kudielka (personal communication, June 5, 2008) concurs that studies in this area should be conducted in the afternoon, although she suggests that it would be optimal to run

participants after 16:00 as this is when "the circadian rhythm of cortisol is lower and therefore it is easier to elicit a stress response [and one] avoids any interference with the cortisol morning response". However, due to practical considerations, this was not possible, and I therefore conducted my study between 12:00 and 16:00 so as to minimise as many time of day effects as possible.

The independent variable in my study was psychological state (stressed or relaxed) of the participants when undergoing memory testing on Day 2. There were two dependent variables, namely the participants' performances on both a WM measure (as measured by the *n*-back test with different difficulty levels) and verbal DM (as measured by cued recall on a paired associates test and recognition test using the same pairs).

My study took place in the Department of Psychology at the University of Cape Town (UCT). All experimental procedures were approved by the Research Ethics Committees of both UCT's Department of Psychology Department and UCT's Faculty of Health Sciences. All participants freely gave their informed consent before participating in the study, signing a consent form that outlined the study, assured their anonymity and informed them that they could withdraw from the study at any point.

Participants

I recruited 43 volunteer undergraduate psychology students (22 females) between the ages of 18 and 35 years from the UCT Department of Psychology's Student Research Participation Programme (SRPP).

Sign-up procedures for males and females were different. All men wishing to participate in the study signed up on the SRPP notice board, supplying their name and contact details. However, due to research which has shown that women may respond differently to stress at different phases of their menstrual cycle, and that oral contraception may also affect the stress response (Kirschbaum, Kudielka, Gaab, Schommer, and Hellhammer, 1999; Kirschbaum, Pirke, and Hellhammer, 1995), these factors were controlled for. As a result, women wishing to participate in the study were asked to contact the researcher via email if they were not on oral contraception. They were then corresponded with via email to determine if they had a regular menstrual cycle and to obtain an estimate of when the first day of their next period was due. This ensured the female participants' privacy regarding their non-use of oral contraceptives.

Potential female participants were thus not taking any form of oral contraceptive and had a regular menstrual cycle. Female participants were also required to be in the late luteal phase of their menstrual cycle (the 6 days preceding the start of their menses; Bischof, 2003) during their participation in the second session of the study. This is due to research that has shown that women in this phase experience similar increases in levels of cortisol to men (Kirschbaum et al., 1999). I attempted to ensure that this criterion was met by having the women estimate when the first day of their next period would be and arranged to test them within the 6-day window preceding that date. All female participants were asked to contact me on the first day of their next period in order to verify the phase of the menstrual cycle in which they had been tested.

Men who had volunteered for participation were then contacted in order to fit them in around dates organised for the female participants. Participants were pseudorandomly assigned alternatively, according to their sex, to either the Stress or Relax group. Ultimately, there were 11 participants of each sex in the Stress group and 11 female and 10 male participants in the Relax group.

Exclusion criteria

Although 43 participants were recruited, there was an unfortunately high rate of attrition in my study. One female in each group only completed the first session and data from three female participants in the Stress group had to be disregarded because they were in the menses stage of their cycle on the day of their second session. One male in the Stress group was excluded due to the fact that he informed me that he had disregarded my instructions and smoked directly before his Day 2 session.

Because it is the increased cortisol levels that result from stress which cause memory impairments, the integrity of the study depended on the Stress group showing an increase in cortisol levels and the Relax group either remaining stable or decreasing in their cortisol levels after the respective manipulations. Therefore, only those participants who showed such effects were included in the final data analyses. Three females and one male from the Stress group and three females and one male from the Relax group were excluded because one or more of their saliva samples was insufficient for analysis of cortisol levels. In addition, four members of the Relax group (1 female, 3 males) were excluded on the basis that they showed increases in cortisol levels after that group's experimental manipulation and a further seven Stress group

participants (1 female, 6 males) were excluded on the basis that they were 'cortisol non-responders'. Elzinga and Roelofs (2005) made a similar distinction between cortisol responders and cortisol non-responders. When they analysed the data collected from the non-responders, they found that their WM performance was similar to that of the participants in the control group. Based on this convention, the data for non-responders in my study was not analysed because I was only interested in those participants who became stressed (as indicated by raised cortisol levels) as a result of the stress manipulation and those participants who remained stable or decreased in stress (as indicated by stable or lowered cortisol levels) as a result of the relax manipulation.

Final sample

As a result of the exclusion criteria outlined above, the final number of participants who went forward into the data analysis was 18 (Stress group: n = 6, 3 females; Relax group: n = 12; 6 females). Their ages ranged from 18 to 35 ($M = 20.22 \pm 3.70$) and there were no statistically significant between-group age differences (Stress: $M = 18.83 \pm 0.41$; Relax: $M = 20.92 \pm 4.42$; t(16) = -1.135, p = 0.273). None of these participants was on any form of steroid-based medication.

Post-experimental self-report verification showed that 4 of the female participants (2 in each group) were tested in the correct phase of the menstrual cycle while 4 of them (1 Stress and 3 Relax) were outside of the correct phase by only one day. The final female participant in the Relax group was 13 days outside of the correct phase. However, the fact that phase of menstrual cycle was being controlled for to ensure that males and females in the Stress group experienced a similar increase in cortisol levels from base rate after the psychosocial stressor (Kirschbaum et al., 1999), means that it was not of great importance that this Relax group participant was not in exactly the correct stage of her cycle as her cortisol levels did not need to increase.

All of the other female participants who completed both sessions and whose data were not used in the final analysis were tested in the correct phase of their menstrual cycle. This indicates that my method for controlling for this factor was generally reliable.

Materials

Participant self-report measurements

Beck Depression Inventory-II (BDI-II). The BDI-II (Beck, Steer, & Brown, 1996) is a self-report questionnaire consisting of 21 statements to which there are four possible responses, each indicating a different degree of possible depressive symptomatology. Participants are asked to choose the response that best suits how they have felt for the previous 2 weeks with higher scores indicating greater levels of depression.

The BDI-II has good psychometric properties; for instance, it is highly internally consistent ($\alpha = 0.91$; Dozois, Dobson, & Ahnber, 1998).

For the purposes of this study, the BDI-II was included as a potential basis for participant exclusion if a score of over 29 was obtained. None of the participants were excluded based on their BDI-II scores.

State-Trait Anxiety Inventory (STAI). The STAI (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) consists of two parts. Form Y-1 measures an individual's anxiety at a specific point in time (state anxiety) while Form Y-2 is an indicator of general levels of anxiety (trait anxiety). Each form consists of 20 statements. These are measured on a Likert-type scale with possible answers ranging from "not at all" (Y-1) or "almost never" (Y-2) to "very much so" (Y-1) or "almost always" (Y-2).

The STAI has been found to have a reliable factor structure, high validity and to be highly internally consistent (Spielberger & Vagg, 1984).

Physiological Measurements

Salivary cortisol collection and measurement. Cortisol measurements were collected by means of saliva samples. Such samples are an easy and effective way to collect cortisol, at least in part because they do not cause any stress for the individual from whom the sample is taken (Garde & Hansen, 2005).

BD VisispearTM (BD, Franklin Lakes, New Jersey) eye sponges were used to collect the sample. Participants were required to place these under their tongue for 1 minute. Two samples were taken at each of three points during the Day 2 protocol: 1) at the beginning of the session, 2) 5 minutes after the participants had finished the TSST or the relaxation period and 3) 5

minutes after cognitive testing concluded. Once the samples were collected they were immediately stored in a freezer until they could be transported to the laboratory for analysis.

Although additional physiological measures (i.e., heart rate and skin conductance measurements) were taken, they will not be analysed in this paper.

The Acute Psychosocial Stressor

The method used to induce stress in my study was a variant on the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). According to its developers, the TSST successfully induces increases in cortisol levels. Independent verification of these claims emerge from a meta-analytic study investigating under what conditions psychological stress results in increased cortisol levels (Dickerson & Kemeny, 2004) as well as recent individual empirical studies (e.g., Elzina & Roelofs, 2005; Oei et al., 2006)

The TSST variant I used was successfully employed by Bonito Attwood (2008). The participants in the Stress group were led into a dark room illuminated by a single bright lamp. The room contained a reflective sheet of glass that they were told was a one-way mirror, a videocamera and a microphone. They were led to believe that behind the 'one-way mirror' was a trained behavioural health expert who was going to evaluate their verbal and non-verbal behaviour and that a video recording was being made to help with this analysis. Participants were seated at a desk in front of the microphone and given 10 minutes to prepare, using pen and paper, a 5-minute speech in which they were required to present themselves to prospective employers for the job of their choosing. Participants were left alone during this preparation time. When the 10 minutes were over, the participants were told that they had to speak for a full 5 minutes without using their notes. The researcher, wearing a white laboratory coat and holding a clipboard and stopwatch, stood behind the participant. The reflection of the researcher in the mirror was visible to the participant. If the participant ceased talking before the allotted amount of time had elapsed, he or she was asked the questions set out by Kirschbaum et al. (1993, p. 77). Once the full 5 minutes had elapsed, the participants were required "to serially subtract the number 13 from 1,022 as fast and as accurately as possible" for another 5 minutes. Every time that a subtraction mistake was made the participant was required to restart as the researcher issued the following command: "Stop. 1,022" (Kirschbaum et al., 1993, p. 77).

Memory Tasks

Declarative memory task. Following Lupien et al. (1999), I used a verbal paired-associates (VPA) cued recall task to test DM. For this task I used Uttl, Graf and Richter's (2002) VPA15, which in turn is similar to the one presented in the Wechsler Memory Scale – Third Revision (WMS-III; Psychological Corporation, 1998). This VPA is described by Uttl, et al. (2002, p. 567) as being "among the most widely used instruments for assessing explicit episodic memory". The list contains 15 word pairs, of which four are regarded as 'related/easy' pairs (e.g., fruit-apple) and 11 are regarded as 'unrelated/difficult' pairs (e.g., bank-milk). The four related/easy word pairs and four of the unrelated/difficult pairs were taken directly from the Wechsler Memory Scale – Revised (Wechsler, 1987).

On Day 1 of the experimental protocol, the list was presented to each participant twice, using the relevant "study order" (1 or 2) provided by Uttl, et al. (2002, p. 573). This study phase began with the participants being instructed that they learn the pairs of words as the researcher read them aloud, one pair at a time with 3 seconds between each pair. After each presentation of the complete list of pairs, participants were required to engage in a cued recall task. The researcher, following the relevant "recall order" specified by Uttl, et al. (2002, p. 573) read the first word of each pair out loud, and the participant was required to give the second word of that relevant pair.

On Day 2 of the experimental protocol, there was no study phase, but the participants were again required to engage in a cued recall task. The researcher merely read out the first word of each pair (in an order randomly devised by the experimenter) and the participant was required to give the second word of that pair.

Following completion of that cued recall task, participants were also required to engage in a recognition task based on the word pairs studied on Day 1. Seventy-five word pairs were presented to the participant via a computer using E-prime software version 1.1 (Psychology Software Tools, 2002, Pittsburgh, Pennsylvania). These 75 pairs included all 15 of the studied pairs and 60 pairs constructed specifically for this study by finding an 'easy' and a 'difficult' partner for each of the first words in the original pairs (e.g., *fruit-vegetable* and *fruit-holiday*) as well as for each of the second words in the original pairs (e.g., *pear-apple* and *ship-apple*). The computer programme presented word pairs in an order randomly selected for each participant.

TARGET

NOT a Target

Participants were required to indicate, using the '1' or '2' keys on the computer keyboard, whether the presented pair was one of the studied 15 or not

The word pairs and order of presentation for both the study and testing phase on Day 1 and for the cued-recall testing phase on Day 2 can be found in Appendix A. The word pairs used in the Day 2 recognition task can be found in Appendix B.

Working Memory Task. I used an *n*-back test involving a 0-back condition, a 1-back condition and a 3-back condition. This protocol addressed the need, identified by Schoofs et al. (2008), for an easier difficulty level than those used in their study (a 2- and 3-back condition). This test was delivered via a computer using E-prime programming software version 1.1 (Psychology Software Tools, 2002, Pittsburgh, Pennsylvania) and was modified from a *n*-back test downloaded from http://step.psy.cmu.edu/scripts-plus/.

In this task a random series of letters were presented on the computer screen and participants were required to hit the 'j' key on the keyboard if the letter shown was not a 'target' and the 'f' key if it was a 'target'. In the 0-back condition, participants were told to consider a certain letter as a 'target' (in this test, the letter 'x'). In the 1- and 3-back conditions, a letter was a target if it was the same letter that was presented *n*-letters previously (i.e., 1 letter previously for the 1-back and 3 letters previously for the 3-back condition; see Figure 1 for a graphical explanation of these instructions).

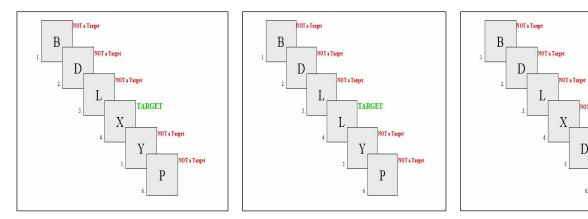


Figure 1. An example of the instructions for, from left to right, the 0-back, 1-back and 3-back conditions.

The initial 0-back section consisted of a practice set followed by the experimental set. Following this, the format was almost identical to that used by Schoofs et al. (2008). There were two further practice sets, one for the 1-back condition and one for the 3-back condition that were then followed by eight experimental sets that alternated between the two conditions. In order to make sure that participants understood what was required of them for each difficulty level, they were required to achieve at least 70% on a practice set in order to move forward in the test. Thus, they were required to repeat the practice set until they reached this goal.

In each practice set there were 20 stimulus presentations and in each experimental set there were 24 stimulus presentations. In the experimental sets, 33% of the letters presented were target stimuli although in each case the first three letters were non-targets and were not considered during data analysis. The letter 'x' was not used in any of the 1-back or 3-back sets. The time between the onset of one letter and the next was 3518ms, which is roughly the same as that in Schoofs et al. (2008).

Procedure

On Day 1 of the experimental protocol, the procedure for all participants was identical. The participants were met at the computer laboratory where the memory tests were to take place. Here they read and signed the consent form (Appendix C) and completed the BDI-II and the STAI - Trait questionnaires. The BDI-II was scored while the STAI - Trait was being completed so that participants would not need to complete the rest of the study if they met the exclusion criteria. After this, they completed the study and immediate cued recall phases of the VPA test as well as the *n*-back test. The VPA and *n*-back were administered in a counterbalanced order across participants to control for possible sequence effects.

At the end of the session, participants were reminded about their appointment the next day and were asked not to smoke or consume food or drink, chew gum or engage in physical exercise in the two hours before their session. This reminder is in line with protocols followed by other studies (e.g., Kirschbaum et al., 1993; Schoofs et al., 2008).

On Day 2 of the experimental protocol, participants were pseudorandomly assigned to either the Stress group or the Relax group. Participants in both groups arrived at the same venue where they had been tested the previous day. Here they completed the STAI - State test and gave a saliva sample. From this point, the procedure differed depending on the group to which the

participant belonged. Participants in both groups were led to the TSST room; there, participants in the Stress group were exposed to the 20-minute TSST procedure, whereas participants in the Relax group were seated in a comfortable chair and allowed to read magazines featuring bland content while listening to relaxing music. They were left alone in this environment for 20 minutes.

The second memory testing session (which consisted of 24-hour delayed cued recall and recognition testing of the VPA, as well as the *n*-back test, and which lasted about 50 minutes) then began. The memory tests were completed in the same order as they had been administered on Day 1.

After completing memory testing, the participants were instructed to relax for another 5 minutes before filling out a third STAI – State test and giving a third saliva sample. The participants were then debriefed as to the purpose of the study; in particular, participants in the Stress group had the TSST explained to them, and the researcher ensured that all participants were not distressed upon leaving the laboratory. If any participants had felt distressed, the contact details of a clinical psychologist were on hand for them to contact. Finally, participants were asked not to discuss any aspect of the study with anyone else so as to not confound the results.

Data Analysis

All statistical analyses were completed using STATISTICA version 8.0 (StatSoft, Inc., 2007). The design allowed for both within- and between-groups analysis. The level for statistical significance was set at $\alpha = 0.05$. Unless otherwise specified all required assumptions were upheld for all relevant statistical analyses.

Experimental manipulation

T-tests (both independent-samples for between-group analyses and dependent-samples for within-group analyses) were used to analyse results for the physiological and self-report stress measures (salivary cortisol and STAI data) for differences between the Relax and Stress groups.

Declarative Memory

Cued Recall. The cued recall DM task was scored based on how many word pairs were correctly recalled. Slight variations of words were scored as correct (e.g., 'cry' for 'cries'). In line with Kuhlmann et al. (2005, p. 2978), "[t]o account for possible within- and between-subject variance in initial learning" the score on the cued recall task on the Day 2 "was expressed as the percentage of words remembered in relation to the second (and last) learning trial on the [first] day". In order to compare the within- and between-group scores for the two groups, *t*-tests were conducted on the data.

Recognition. Performance on the recognition DM task was assessed using a d-prime (d') score as the dependent variable. This d' score was calculated based on the 'hit' (word pair correctly identified as on original VPA list) and 'false alarm' (FA; word pair incorrectly identified as on the original VPA list) rates for each participant. The d' statistic is calculated as d' = z(FA) - z(H) with bigger values indicating greater discrimination between the original and distracter stimuli. For perfect hit or FA rates (1 or 0 respectively), the formula 1 - 1/(2N) was used to calculate adjusted hit rates and 1/(2N) was used to calculate adjusted FA rates (see http://psy.ucsd.edu/~kang/sdt/sdt.htm for more details). In addition, t-tests compared the hit scores to those of the cued recall scores for both Day 1 and Day 2 in order to observe the differences between the effects of stress on these two forms of memory.

Working Memory

For the WM task, the number of 'hits' (targets correctly identified) and 'correct rejections' (non-targets correctly identified) were summed and a percentage of correct responses (CRs) for each set was thus obtained for each participant for both Day 1 and Day 2 testing. I also calculated the mean reaction times (RTs) for the CRs.

For the WM data from Day 1, 2 X 4 (Task Difficulty [1-back/3-back] X Set [time]) repeated-measures ANOVAs were run on both the percentage of CRs and the RTs for all participants together. For the WM data from Day 2, and following the analysis used by Schoofs et al. (2008), 2 X 2 X 4 (Experimental Condition [stress/control] X Task Difficulty X Set) repeated-measures ANOVAs were performed on the percentage of correct responses and mean RTs.

In addition, the difference between CRs and mean RTs for Day 1 and Day 2 were calculated for each group, and further 2 X 2 X 4 repeated-measures ANOVAs were run on these results. In all of the ANOVAs, the Experimental Condition was the between-group factor, while the Task Difficulty and Set variables were the within-group factors. Following Schoofs et al. (2008), I ran *t*-tests on the interactions for all of the ANOVAs conducted on the Day 1 (dependent-samples), Day 2, and the difference between Day 1 and Day 2 data (independent-samples).

RESULTS

Experimental manipulation

The following measurements allowed me to ensure that my assumption that the effects of my experimental manipulation could be fairly and easily compared across the two groups was valid. They also provided an indication of the effectiveness of the manipulation. Table 1 provides the descriptive statistics for each of these measurements.

BDI-II scores

BDI-II scores were relatively low for both groups, implying low levels of depression amongst my participants. A comparison of these scores across the two groups found that they were not statistically significantly different on their levels of depression, t(16) = -.153, p = .880, d = .08. These results implied that my results would not be confounded by pre-existing negative emotional states.

Self-report anxiety measures: STAI

STAI - Trait anxiety scores. An independent-samples t-test showed that the two groups did not score at a statistically significantly different level on their self-report trait anxiety, t(16) = -.09, p = .933, d = .04, thus indicating that the groups were equal in their general levels of anxiety. In addition, the sample seemed to be representative of the general population: When the male ($M = 37.11 \pm 5.07$) and female ($M = 38.44 \pm 0.78$) participants were compared to the normative data for college students (males: $M = 38.30 \pm 9.18$; females: $M = 40.40 \pm 10.15$) supplied by the STAI manual (Spielberger et al., 1983), the single-samples t-test showed that my groups did not differ

statistically significantly from this normative data set (males: t(8) = -0.37, p = .721, d = .13; females: t(8) = -.60; p = .567, d = .19).

STAI - State anxiety scores. As with the trait anxiety scores, independent-sample *t*-tests showed that the groups did not differ significantly statistically in their baseline state anxiety levels, t(16) = .48, p = .639, d = .24, thus indicating that they were feeling similar levels of anxiety at the start of the second session. In addition, as with the trait anxiety scores, single-samples *t*-tests showed that the male ($M = 32.78 \pm 7.68$) and female ($M = 36.67 \pm 9.95$) groups did not differ significantly statistically (males: t(9) = -1.44, p = .187, d = .37; females: t(9) = -.63, p = .546, d = .18) from the normative data supplied by the STAI manual (Spielberger et al, 1983) for state anxiety for college students (males: $M = 36.47 \pm 10.02$; females: $M = 38.76 \pm 11.95$). This again indicates that the current study's sample seemed to be representative of the general population and that the participants were not feeling excessively anxious at the start of the session.

In analysing the effect of the experimental manipulation on the groups, dependent-samples t-tests found that the Relax group experienced a statistically significant decrease in self-report anxiety, t(11) = 3.46, p = .003, d = .75 (one-tailed). However, although there was an increase in the self-report anxiety scores for the Stress group, this was not statistically significant, t(5) = -1.35, p = .117 d = .31 (one-tailed). This was not, however, considered to be a problem as it was the changes in cortisol levels that were important for my study. This small change in self-reported anxiety was interpreted as possibly being due to the influence of relief felt by the participants that the anticipated experimental manipulation was over.

Ethically, in order to check that the participants were not still in a stressed state at the end of the study, it was important to compare their baseline anxiety scores to their final anxiety scores. Dependent-samples t-tests indicated that the levels of anxiety for both groups were statistically significantly lower at the end of the session compared to the start of the session (Stress: t(5) = 4.83, p = .005, d = 1.17; Relax: t(11) = 3.33, p = .007, d = .92). Figure 2 shows the changes in participants' self-report anxiety.

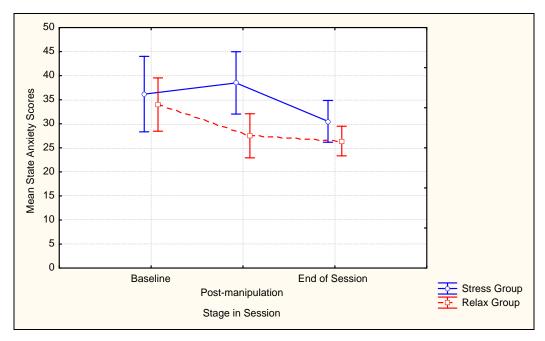


Figure 2. Change in self-reported state anxiety across the second session.

Physiological anxiety measures: Salivary cortisol levels

As with the state anxiety scores, the independent-samples t-tests showed that the Stress and Relax groups did not differ significantly statistically in their levels of cortisol at the baseline reading, t(16) = -.62, p = .545, d = .31. Thus, levels of cortisol for the two groups were equitable at the start of the second session.

Dependent-sample t-tests analysing the effect of the experimental manipulation on the two groups showed that the Relax group experienced a statistically significant decrease in cortisol levels, t(11) = 2.40, p = .018, d = .55. By comparison, the Stress group experienced a statistically significant increase in cortisol levels, t(5) = -2.50, p = .027, d = .79 (one-tailed). Thus, it appears that the experimental manipulation successfully caused a statistically significant increase in cortisol levels for the cortisol responders in the Stress group.

Again, independent-samples t-tests comparisons between the baseline and end of session cortisol levels for the Stress group indicated that they did not leave the session in a stressed state as the difference between these two measurements was not statistically significant, t(5) = 1.26, p = .263, d = .43, with cortisol levels at the end of the session being lower than at the start of the session. The cortisol levels for the Relax group were statistically significantly lower at the end of

the session compared with the baseline levels, t(11) = 3.66, p = .003, d = .41. Participants' changes in salivary cortisol levels can be seen in Figure 3.

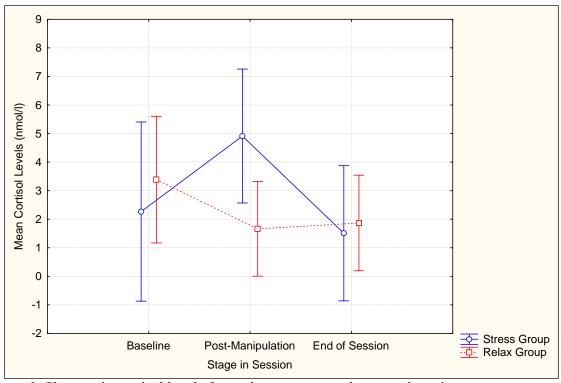


Figure 3. Changes in cortisol levels for each group across the second session.

Declarative Memory

Cued Recall results

Day 1. Results of a dependent-sample t-test showed that there was a statistically significant increase in the number of pairs remembered by participants between the first (VPA-IR-1) and second (VPA-IR-2) immediate cued recall tests on Day 1, t(17) = -4.87, p = .0001, d = .85, showing that the second presentation was of great benefit in terms of increasing their memory of the pairs.

Although participants had not yet been divided into groups at this stage of the experiment, between-groups analyses using independent-samples were conducted to judge the level of encoding for the two groups and to see if a difference existed before the start of Day 2 testing. Although the groups did not show a statistically significant difference in the number of

pairs remembered after VPA-IR-1, t(16) = -.85, p = .410, d = 0.43, there was a statistically significant difference between them after VPA-IR-2, t(16) = -2.53, p = .022, d = 1.27, with the Relax group remembering more pairs. This implied that participants in this group might do better on Day 2 in the absence of the experimental manipulation because they had better encoded the word pairs to begin with; this needed to be borne in mind when analysing the Day 2 data. However, any potential effects of differential Day 1 encoding were controlled for by using within-groups methods for analysis of the Day 2 data, including looking at a 'percentage savings' score.

Day 2. Although dependent-samples t-tests showed that participants in the Stress and Control group both remembered a statistically significant lower number of word pairs on Day 2 than they did on Day 1 after VPA-IR-2 (Stress: t(5) = 3.50, p = .017, d = .53; Relax: t(11) = 4.01, p = .002, d = 1.15), independent-samples t-tests showed that there was not a statistically significant difference between the number of words remembered by the two groups for delayed cued recall on Day 2 (VPA-24-DR), t(16) = -1.37, p = .189, d = .69. In addition, there was not a statistically significant difference between the two groups in terms of a percentage savings score (the percentage of encoded Day 1 word pairs that were also remembered on Day 2, i.e., (VPA-24-DR / VPA-IR-2) * 100), t(16) = 1.05, p = .310, d = .52. The Stress group did, however, remember a greater percentage of their word pairs from Day 1 than did the Relax group. These data imply that the decrease in memory for the Stress group from Day 1 to Day 2 is only attributable to the effects of time, and are not due to the experience of Stress. The groups' scores for the three cued recall tests are shown in Table 2 and in Figure 4.

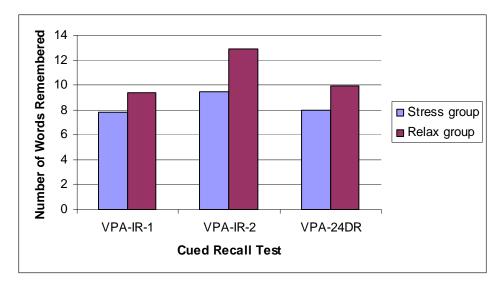


Figure 4. Average number of word pairs recalled by each group across the VPA test trials.

Recognition results

An independent-samples t-tests showed that the groups did not differ statistically significantly in terms of the ratio of word pairs identified during the recognition test to those correctly recalled during VPA-24DR, t(15) = .81, p = 0.43, d = .44. Both groups did, however, identify more word pairs on the recognition test than they had recalled during VPA-24DR (see Table 3 and Figure 5).

A second independent-samples t-test showed that the groups did differ statistically significantly in terms of the ratio of word pairs identified during the recognition test to those correctly recalled during VPA-IR-2, t(15) = 2.39; p = 0.03, d = 1.29. On this ratio score, the Stress group outperformed Relax group, although the Relax group also identified more word pairs on the recognition task than they had recalled during VPA-IR-2 (see Figure 5).

These latter results are somewhat misleading, however, as one does need to take into account that the Relax group was victim of a ceiling effect. That is to say, the maximum Recognition hits:VPA-IR-2 ratio that the Relax group could achieve was 1.16; in contrast, the Stress group could achieve a ratio of 1.57. Therefore, the Stress group had a greater opportunity for increased performance on the recognition test. In other words, both groups performed better on the recognition test than on the first day's cued recall test, and the experimental manipulation therefore had little effect on recognition performance.



Figure 5. Percentage difference between recognition hits and VPA scores for the two groups.

Interestingly, and in contradiction to the above results which imply that recognition is unaffected by stress, the d' analysis showed that the Relax group was statistically significantly better than the Stress group at discriminating between the original VPA word pairs and the new, distracter word pairs, t(15) = -2.98, p = .009, d = 1.61. This indicates a positive response bias from the Stress group, indicating that stress did have a negative impact on recognition memory.

Working Memory

For some of the ANOVAs conducted on the WM data, there appeared to be slight violations of the normality assumption. However, due to the small sample sizes, such violations were understandable and although these may impact results and render such analyses inappropriate, I performed the tests as they would have been conducted on larger sample sizes as will be the case in my future research.

0-back

Across both days, the accuracy rate for the 0-back task was 99.5%, with the mistakes on Day 2 being made by participants in the Relax group. This result shows that the participants were attending to the presented stimuli, and implies that they were motivated to perform well.

Day 1: 1- and 3-back comparison

On Day 1, the reaction time data for one of the Stress-group participants were lost from the third 3-back set onwards. All descriptive statistics for the performed ANOVAs are shown in Table 4.

Accuracy. The results showed a statistically significant main effect for task difficulty, F(1, 17) = 19.67, p = .0004, $\eta^2 = .54$, in the absence of a significant main effect for set or an interaction between task difficulty and set, p = .891 and p = .520, respectively. As can be seen in Figure 6, overall the 1-back yielded a greater percentage of correct responses (M = 94.84) than did the 3-back (M = 89.02).

In order to investigate the differences between the difficulty levels in each set, dependent-samples *t*-tests were conducted. The results, reported in Table 5, showed that the difference in accuracy between the difficulty levels was statistically significant for each set except for the final one. This piece of data suggests that performance across the difficulty levels evened out as the task progressed.

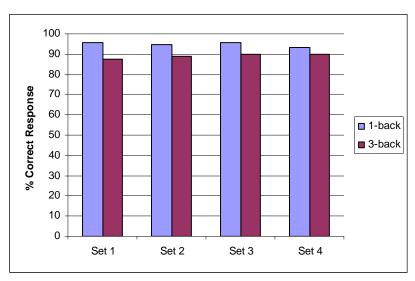


Figure 6. Percentage correct responses across both difficulties: Day 1.

Speed. As with accuracy, the results showed a statistically significant main effect for task difficulty, F(1,16) = 18.72, p = .001, $\eta^2 = .54$, in the absence of a significant main effect for set or a significant interaction, p = .232 and p = .958, respectively. Figure 7 clearly indicates that the 1-back yielded faster RTs (M = 549.96) than did the 3-back (M = 764.79).

As with the accuracy data, dependent-samples *t*-tests were conducted to analyse the differences in speed between difficulty levels in each set. Table 5 shows that there were statistically significant differences across all four sets. This piece of data suggests that participants responded to the 1-back condition at a significantly faster rate across the duration of the task.

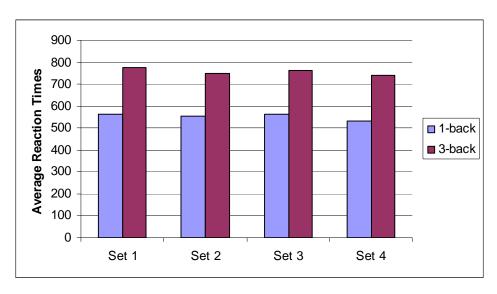


Figure 7. Average reaction times across both difficulties: Day 1.

Day 2: 1- and 3-back comparison

On Day 2, data were lost for one of the Stress group participants due to a hardware malfunction after the second 1-back set. Thus, the sample size varied across the tests conducted on this data. All descriptive statistics for the performed ANOVAs are in Table 4.

Accuracy. The results only showed a statistically significant main effect for task difficulty, F(1,15) = 5,414, p = 0.034, $\eta^2 = .27$, with participants performing more accurately on the 1-back task (M = 96.05) than on the 3-back task (M = 93.28) (see Figures 8 and 9). None of the other main effects or interactions were statistically significant.

Independent-samples *t*-tests were conducted between the groups within each set for each difficulty level. Table 7 shows that these analyses yielded no statistically significant results, although the difference between the groups for the first 3-back set approached significance. This

latter piece of data indicates that performance on this set may have been affected by the stress induction.

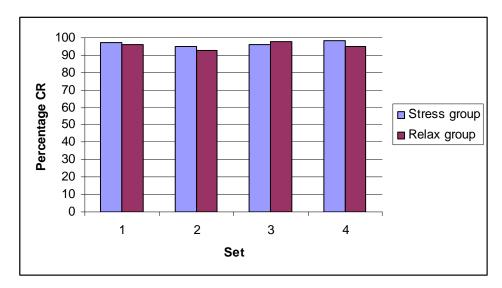


Figure 8. Percentage correct responses for both groups: 1-back, Day 2.

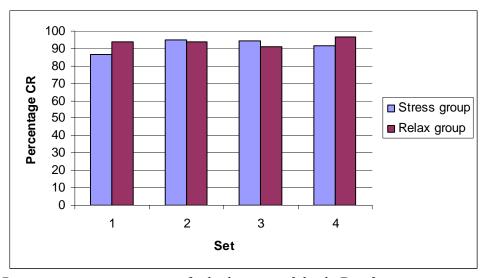


Figure 9. Percentage correct responses for both groups: 3-back, Day 2.

Speed. As with the accuracy data, the results for the average RTs only showed a significant effect for task difficulty, F(1,15) = 7.288, p = 0.016, $\eta^2 = .33$, with the 3-back task (M = 627.96) being performed at a slower rate than the 1-back task (M = 529.93). Figure 10 shows clearly, however, that participants in the Stress group, on average, reacted more quickly to the stimuli than did participants in the Relax group. This result stands in contrast to data reported by Schoofs et al.

(2008), who showed that their stressed participants reacted at a consistently slower rate than did their control participants (although the between-groups difference decreased as the study progressed).

Independent-samples *t*-tests were conducted between the groups within each set for each difficulty levels. Table 6 shows that there were no statistically significant differences in reaction times.

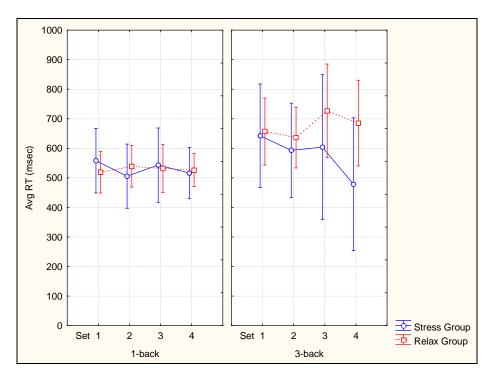


Figure 10. Average reaction time performances for both groups across task difficulty and set: Day 2.

Comparison of Day 1 and Day 2: 1-back and 3-back

All of the descriptive statistics for the performed ANOVAs are in Table 7.

Accuracy. The results for the analysis of the differences in accuracy between Day 1 and Day 2, represented graphically in Figure 11, showed that there was only a statistically significant main effect for the task difficulty, F(1, 15) = 5.73, p = .030, $\eta^2 = .28$. None of the other main effects or interactions were statistically significant.

Independent-samples *t*-tests were conducted between the groups within each set for each difficulty level. As shown in Table 8, there were no statistically significant differences, indicating that stress did not impact WM as predicted.

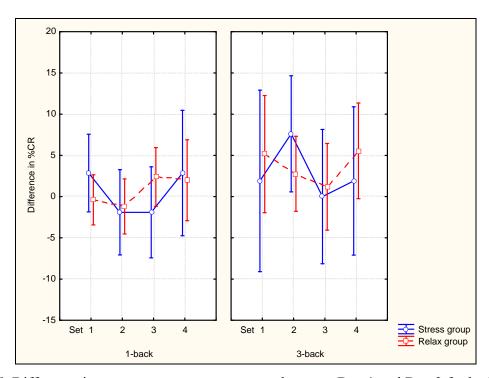


Figure 11. Difference in percentage correct responses between Day 1 and Day 2 for both groups.

Speed. The results for the analysis of the differences in RT between Day 1 and Day 2, represented graphically in Figure 12, showed that there was only a statistically significant main effect for task difficulty, F(1, 14) = 5.54, p = .034, $\eta^2 = .28$. None of the other main effects or interactions were statistically significant.

Independent-sample t-tests were conducted between the groups within each set for each difficulty levels. Table 8 shows that only one statistically significant difference was found. This was for the Set 2 1-back, on which the Stress group (M = -71.38) showed a much greater decrease in average RT from Day 1 than did the Relax group (M = 28.39). A similar result was found for the Set 4 1-back, where the Stress group performed at a quicker rate than the Relax group at a level that approached statistical significance. However, these results appear to be anomalies, most likely caused by extremes in the data for one of the groups: There is no other

plausible reason why significant (or near-significant) between-group differences should occur on only some sets and only in the 1-back condition.

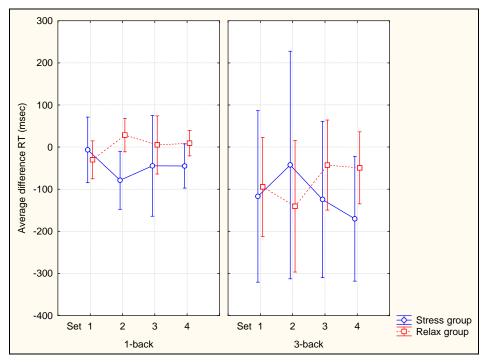


Figure 12. Difference in average reaction times between Day 1 and Day 2 for both groups.

DISCUSSION

Summary and Implications of Results

Experimental manipulation

From the analysis of the cortisol and STAI - State results, it appears that for those participants included in the final Stress group sample, the stress-induction procedure worked as predicted, significantly increasing cortisol levels from baseline levels. Similarly, the Relax group's manipulation significantly decreased their cortisol levels. Thus, one can conclude that at the start of the Day 2 memory testing, the two groups were experiencing different physiological states, with the Stress group having significantly higher levels of cortisol, implying that this group might be experiencing impairments in frontal- and hippocampal-based functions (i.e., WM and DM, respectively).

Declarative memory

The majority (66.67%) of the participants surpassed the maximum possible score on the WMS-III (Psychological Corporation, 1998) version of the Verbal Paired Associates (VPA) test after the first immediate cued recall test, with only 16.67% not doing so after the second such test. In addition, after the 24-hour delay, regardless of the experimental manipulation, 77.78% of the participants surpassed the WMS-III maximum. In contrast, only one participant in the current sample reached the maximum score on first immediate cued recall test of the current version of the VPA test, and only 22% of them reached it after the second such test. These data are consistent with those reported by Uttl et al. (2002), confirming the suggestion that the VPA test used in this study is a better option than that contained within the WMS-III for measuring hippocampal-dependent declarative memory.

Cued Recall. This study looked at the effects of stress on DM by investigating the retrieval of previously-learnt material as measured by a cued recall task and a recognition task. In terms of cued recall, I hypothesised that cued recall memory would be affected by stress. Although results showed that the number of word pairs recalled on Day 2 was lower than on Day 1, it did not appear that the psychosocial stressor significantly impacted this type of DM. On the contrary, it seemed that the Stress group actually performed better on Day 2 than the Relax group: When the Stress group's performance on Day 2 was compared to the scores obtained on Day 1, they remembered a greater percentage of the words that they had initially encoded than did the Relax group.

A number of different protocols have been employed in previous studies to test the effects of increased cortisol levels on DM. Although findings of those studies have been mixed, the results of this study are in line with those of Kuhlmann et al. (2005), who also employed a similar 2-day design and also investigated the effects of psychological stress on retrieval. They found that while free recall of words was impaired by stress, delayed cued recall was not. However, they did not do a within-subject analysis across the two days as they only tested cued recall on Day 2. In addition, Lupien et al. (1999) also found that cued recall was unaffected by increased cortisol levels, although they were investigating these effects on encoding of material and not on retrieval. However, de Quervain et al. (2003) found that delayed cued recall was

impaired by stress. Therefore, although this study seems to replicate previous findings of some studies regarding the effects of stress on delayed cued recall, results are still inconclusive. A larger sample size than that used here needs to be employed in order to fully investigate the within-group effects of stress on delayed recall.

Recognition. At a surface level, the results from the recognition test appear to support Lupien et al.'s (1999) hypothesis that recognition memory is not impaired by stress, a finding that would be consistent with de Quervain et al. (2000, 2003). Otherwise stated, the Stress group did not perform statistically significantly worse on the Day 2 VPA recognition test than the Relax group. It thus appears that the way in which the learnt material is encoded (from a free recall base, as in de Quervain (2000) or from a cued recall base, as in the current study) does not affect the recognition retrieval of these words.

Taking a look beyond the surface level, the Stress group, in comparison to the Relax group, recognised far more words on the recognition test than they had encoded. However, when one looks at the rate of hits to false alarms, it appears that this improvement may be due to a positive response bias rather than to actual knowledge-based recognition of the word pairs. In other words, stressed participants appeared to categorize the presented word pairs as having been on the original list more often the Relax group, whether they were or not. Thus, in contrast to de Quervain et al. (2000, 2003), my results indicate that stress may in fact have a negative impact on recognition memory: It causes false memories to occur.

This observation appears to only be noticeable when taking all responses into account (as opposed to just hit responses, or, as in de Quervain et al. (2003), hit responses and false alarm responses compared separately). My result is in line with that of Payne, Nadel, Allen, Thomas, and Jacobs (2002), who also found that stressed participants produced more false alarm responses than did non-stressed participants. The production of false memories in this way is a promising direction for future studies.

Working memory

The results for the *n*-back task from Day 1 confirm the expectation that the 1-back would be the easier task with participants scoring both with more accuracy and at a faster rate than for the 3-back task.

Results for Day 2 and (Day 2-Day 1) difference data did not confirm the hypothesis that WM would be adversely affected by stress. There were no statistically significant between-group differences on either the 1-back or the 3-back condition on Day 2. However, the results did show that the Stress group performed at a generally faster level on Day 2 compared to Day 1, whereas the Relax group did not. These results stand in contrast to Schoofs et al.'s (2008) finding that reaction times were impaired by stress. Additionally, the Stress group tended towards slightly less of an increase in accuracy from their performance on Day 1 than the Relax group did. This indicates that the experience of stress may have had a slight effect on WM performance, although a larger sample size would need to be employed.

However, it is possible that my findings that stress did not seem to impair WM could have been due to practice effects from Day 1. Participants may have become too familiar with the task for the stress induction to have noticeable effects on performance on Day 2. This hypothesis is in line with the observation by Schoofs et al. (2008) that as stressed participants became more familiar with the WM task, so their performance improved. Thus the practice effects from Day 1 may have negated the effects of the experimental manipulation on the stressed participants.

Due to the small sample sizes and largely inconclusive results, it is difficult to establish which of DM or WM was more impacted by stress. Although an initial prediction was that WM would be more impaired than cued recall DM, my results indicate that it may in fact have been recognition DM that was most impaired. This result obviously needs further investigation with larger sample sizes.

Limitations and Directions for Future Research

Small sample size

A number of participants in both groups had to be excluded from my analysis due to cortisol changes in the wrong direction. Although previous studies (e.g., Elzinga & Roelofs, 2005; Kirschbaum et al., 1996) also included participants who did not respond as expected (but who were also not included in the final data analysis), this study had an additional problem in that while it did find a significant increase in cortisol levels in the Stress group after the TSST procedure, three (i.e., half) of participants in this group had cortisol increases of less than 1 nmol/l with the average increase being 2.65 nmol/l. This is far less than most other studies,

including Elzinga and Roelofs (2005), Kuhlmann et al. (2005) and Schoofs et al. (2008), who all had average increases of over 4 nmol/l.

In addition, the baseline salivary cortisol levels for participants in this study ($M = 3.01 \pm 3.56 \text{ nmol/1}$) appeared to be lower than those in previous studies utilising the TSST (e.g., Elzinga & Roelofs, 2005, M = 8.89; Kirschbaum et al., 1993 M = 4-9; Kuhlmann et al., 2005, M = 7; Schoofs et al., 2008, M = 12). Bonito Atwood (2008), who ran her study in the same laboratory as I did and used a similar population, experienced the same problems in her study and noted that the differences in baseline levels of cortisol is less easily explainable than the smaller increases in cortisol. However, my study was conducted between 12h00-16h00, while the other studies mentioned above were conducted between 10h00-13h00 and 16h00-19h00, which as previously discussed could possibly have an effect on cortisol levels (e.g., Dickerson & Kemeny, 2004). However, it has been noted that higher baseline levels of cortisol may actually result in smaller cortisol increases to stress (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Therefore, it appears that my study should actually have benefited from these lower basal levels, although this does not appear to be the case.

Thus it appears that the variation of the TSST used in the current study may not have been as efficacious as other versions for inducing stress and increasing cortisol levels, which, in turn, may have led to less impairment of hippocampal and frontal lobe functioning than was anticipated. For instance, a number of participants voiced their suspicion that there was in reality no-one watching them from behind the 'one-way mirror'. In addition, because it was often the case that the same experimenter oversaw both sessions for a particular participant, the participant may have become comfortable with the experimenter on Day 1, decreasing the anxiety experienced when performing in front of them on Day 2. In future studies, researchers should adhere more closely to the TSST protocol originally outlined by Kirschbaum et al. (1993). Specifically, confederates should be employed as an audience for the participants' speeches, and the experimenter should not be present in the room while these presentations occur.

The generally small number of participants and uneven groups that I was left with for data analysis obviously affects the generalizability of my results and leaves the data vulnerable to extreme scores and outliers. In future studies, a much larger sample size should be obtained to allow for attrition, and methods to ensure sufficient saliva sample collection should be employed.

Sex differences

An important consideration for future research is the investigation of sex differences in the effects of stress on memory. Few studies have examined this thoroughly, mainly due to the difficulties inherent in this investigation.

Sex differences in HPA axis functioning. It has been found that in response to an acute psychosocial stressor (e.g., the TSST), men exhibit greater HPA axis responses (i.e., greater increases in cortisol levels) compared to women (Kirschbaum, Wüst, & Hellhammer, 1992; Uhart, Chong, Oswald, Lin, & Wand, 2006). However, in the Uhart et al. (2006) study, the women involved were in the follicular phase of the menstrual cycle, while in the Kirschbaum et al. (1992) study female participants were in different stages of the menstrual cycle and the sample also included women who were using oral contraceptives. This calls into question the validity of these studies as it has been found that the use of oral contraception and the phase of the menstrual cycle can influence stress-induced cortisol levels.

Specifically, Kirschbaum et al. (1995) found that women using oral contraception exhibited smaller increases in cortisol compared to men and women not using oral contraception. In addition, Kirschbaum et al. (1999) investigated the effects of the use of oral contraception and phase of menstrual cycle (namely the follicular and luteal phases) on HPA axis functioning. The results indicated that although under normal circumstances there are scarcely noticeable differences between the two sexes in terms of HPA axis functioning, this is not the case in instances of stress. Specifically, women in the luteal phase were closely matched with men in terms of stress-induced salivary cortisol levels, although as with the Kirschbaum et al. (1992) and Uhart et al. (2006) studies, men did exhibit a slightly greater cortisol increase. The levels for these two groups were higher than for the women taking oral contraceptives and the women in the follicular phase of their cycle.

Thus, it appears that sex differences in HPA axis functioning in reaction to stress do exist and should be investigated in studies involving memory. Additionally, the use of oral contraceptives and stage of menstrual cycle should also be controlled for in these studies as these can also impact stress-induced cortisol level increases. However, studies controlling for the differential effects of stress on HPA axis activity in the sexes are generally lacking. More than half of the studies reviewed in this paper (the second study by Kirschbaum et al., 1996;

Kuhlmann et al., 2005; Lupien et al., 1999; Oei et al., 2006; Schoofs et al., 2008; Wolf, Convit, et al., 2001) only used male participants. Although both de Quervain et al. (2000) and the first Kirschbaum et al. (1996) study included both male and female participants, neither explicitly controlled for the use of oral contraceptives or stage of the menstrual cycle. However, the latter found that male participants experienced greater cortisol increases than female participants.

Only Elzinga and Roelofs (2005) and Wolf, Schommer, et al. (2001) attempted to control for the abovementioned variable, excluding females who were using oral contraceptives or who were not in the late luteal phase, defined in these studies as being days 21 to 25, of their menstrual cycles. The former study only did so in an attempt to reduce any differences in the results that may have arisen due to gender. It is thus clear that even if studies do not specifically investigate sex differences in the effects of stress on memory, studies including both sexes and controlling for the relevant extraneous variables, as my study did, are necessary to give a well rounded account of this area. However, in future, the investigation of actual differences between the two sexes, controlling for all of the mentioned variables, should take place.

My study controlled for the use of oral contraception and phase of the menstrual cycle. It was found that my method for estimating the late luteal phase in my participants was generally successful, with the majority of participants falling within the correct phase. This sets the framework for investigating sex differences in the effects of stress on DM and WM in future studies.

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APPENDIX A

Declarative memory cued recall task: VPA word lists

Day 1

Trial 1:

Study Order:Recall order:Frog-neckRose (flower)Metal-ironFruit (apple)Foot-treeRoom (face)School-groceryCoal (year)Fruit-appleMetal (iron)

Hill-ring School (grocery)

Baby-cries Hill (ring) Obey-inch Frog (neck) Crush-dark Cabbage (pen) Girl-sign Bank (milk) Girl (sign) Coal-year Obey (inch) Room-face Rose-flower Foot (tree) Cabbage-pen Baby (cries) Bank-milk Crush (dark)

Trial 2:

Study Order: Recall order: Obey (inch) Bank-milk Bank (milk) Frog-neck Room-face Hill (ring) Crush (dark) School-grocery Coal-year Coal (year) Room (face) Girl-sign Rose-flower Foot (tree) Obey-inch Girl (sign) Baby (cries) Baby-cries Fruit-apple Metal (iron) Metal-iron Frog (neck) Fruit (apple) Cabbage-pen Rose (flower) Foot-tree Crush-dark School (grocery) Cabbage (pen) Hill-ring

Day 2:

Recall Order:

Bank-milk

Frog-neck

Room-face

School-grocery

Coal-year

Girl-sign

Rose-flower

Obey-inch

Baby-cries

Fruit-apple

Metal-iron

Cabbage-pen

Foot-tree

Crush-dark

Hill-ring

APPENDIX B

Declarative memory recognition task: VPA word list

FROG – NECK	SCHOOL – GROCERY

Frog – Pond School – Children
Throat – Neck Shop – Grocery
Frog – House School – Garden
Wall – Neck Hair – Grocery

METAL – IRON FRUIT – APPLE

Metal – SteelFruit – VegetableOre – IronPear – AppleMetal – PillowFruit – HolidayShoe – IronShip – Apple

FOOT – TREE BANK – MILK

 $Foot-Shoe & Bank-Money \\ Leaf-Tree & Cow-Milk \\ Foot-Window & Bank-Cow \\ Chair-Tree & Sofa-Milk \\ \\ \end{array}$

<u>CABBAGE – PEN</u> <u>HILL – RING</u>

 $\begin{tabular}{ll} Cabbage - Boil & Hill - Climb \\ Ink - Pen & Jewel - Ring \\ Cabbage - Mat & Hill - Scissors \\ Bed - Pen & Car - Ring \\ \end{tabular}$

OBEY - INCH

Obey - Command

Ruler – Inch Obey – Green

Cinema – Inch

GIRL - SIGN

Girl – Boy

Traffic - Sign

Girl - Plastic

Razor – Sign

ROOM - FACE

Room-House

Eyes-Face

Room-Dart

Basket-Face

BABY - CRIES

Baby – Dummy

Tears – Cries

Baby – Iron

Muscle – Cries

CRUSH - DARK

Crush – Ice

Night – Dark

Crush – Key

Hammer – Dark

COAL - YEAR

Coal - Fire

Month – Year

Coal – Book

Bonnet - Year

ROSE – FLOWER

Rose – Thorn

Vase – Flower

Rose – Alarm

Club – Flower

APPENDIX C

Consent form

Consent Form

Informed Consent to Participate in Research and Authorization for Collection, Use, and Disclosure of Protected Health Information

This form provides you with information about the study and seeks your authorization for the collection, use and disclosure of your protected health information necessary for the study. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. By participating in this study you will not be penalized or lose any benefits to which you would otherwise be entitled.

1. Name of Participant ("Study Subject")

2. Title of Research Study

The impact of acute psychological stress on cognitive functioning

3. Principal Investigator and Telephone Number(s)

Kevin G. F. Thomas, Ph.D. Department of Psychology University of Cape Town 021-650-4608

Robyn Human, B.A. Honours Candidate Department of Psychology University of Cape Town 021-788-5536

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4. What is the purpose of this research study?

The purpose of this research study is to better understand how exposure to acute psychological stress affects cognitive functioning. More specifically, we are interested in individual differences in cognitive responses to acute psychological stress.

5. What will be done if you take part in this research study?

This study requires you to be take part in two research sessions on two consecutive days. On the first day you will be required to complete a number of memory-based tasks. On the second day you may be required to complete a 20-minute presentation which will be followed by another series of memory based tasks. Throughout the study your levels of stress will be assessed through the collection of heart rate measurements and saliva samples with the aid of a cotton swab.

6. What are the possible discomforts and risks?

If you are one of the participants selected to complete the 20-minute presentation, you may be placed in a mildly stressful situation involving public speaking. There are no other discomforts and risks associated with participation in the study.

7. What are the possible benefits of this study?

One major benefit of this study is that scientists, and society in general, will have better understanding of the effects of acute psychological stress on cognitive functioning. This knowledge can then be applied to many different individuals and situations, including students who are taking exams, business managers who have to present to their boards, and so on.

8. Can you withdraw from this research study and if you withdraw, can information about you still be used and/or collected?

You may withdraw your consent and stop participation in this study at any time. Information already collected may be used.

9. Once personal information is collected, how will it be kept confidential in order to protect your privacy and what protected health information about you may be collected, used and shared with others?

Information collected will be stored in locked filing cabinets or in computers with security passwords. Only certain people - the researchers for this study and certain University of Cape Town officials - have the legal right to review these research records. Your research records will not be released without your permission unless required by law or a court order.

If you agree to be in this research study, it is possible that some of the information collected might be copied into a "limited data set" to be used for other research purposes. If so, the limited data set may only include information that does not directly identify you.

10. Signatures

Table 1

Descriptive Statistics for Self-Report and Physiological Measures

	Group			
Measure	Stress	Relax		
	n=6	n=12		
BDI	8.50 (7.45)	8.92 (4.21)		
STAI - Trait	37.50 (11.34)	37.92 (8.95)		
STAI - State				
Baseline	36.17 (5.53)	34.00 (10.27)		
Post-manipulation	38.50 (9.01)	27.5 (6.68)		
End of Session	30.50 (4.09)	26.42 (5.38)		
Cortisol Level				
Baseline	2.27 (2.30)	3.39 (4.09)		
Post-manipulation	4.91 (4.15)	1.66 (1.68)		
End of Session	1.51 (0.92)	1.87 (3.24)		

Note. Mean scores are provided with standard deviations in parentheses. Cortisol levels are measured in nanomoles per litre (nmol/l). Where cortisol levels for a participant were indicated to be <0.50nmol/l, 0.45nmol/l was used as an estimate.

Table 2

Descriptive Statistics for Cued Recall DM Test Scores

	Group				
Measure -	Stress $n = 6$	Relax $n = 12$	Combined ^a $n = 18$		
VPA-IR-1	7.83 (4.79)	9.42 (3.15)	8.89 (3.71)		
VPA-IR-2	9.50 (3.21)	12.92 (2.43)	11.78 (3.10)		
VPA-24DR	8.00 (2.37)	9.92 (2.97)			
Percentage Savings: VPA-24DR and VPA-IR-2	85.30 (8.12)	77.23 (17.74)			

Note. Data presented are means with standard deviations in parentheses. ^aCombined data only relevant for Day 1 analyses.

Table 3

Descriptive Statistics for Recognition DM Test Scores

Measure	Stress Group $n = 5^{a}$	Relax Group $n = 12$
Hits ^b	11.80 (1.30)	13.50 (1.31)
False Alarms ^c	5.00 (3.16)	2.17 (2.86)
Hits / VPA-IR-2	1.44 (0.19)	1.09 (0.30)
Hits / VPA-24DR	1.67 (0.24)	1.48 (0.50)
d	2.27 (0.41)	3.30 (0.72)

Notes. Mean scores are provided with standard deviations in parentheses. ^aData for the Stress group only for 5 participants due to experimenter error. ^bMaximum possible hits is 15. ^cMaximum possible false alarms is 60.

Table 4

Descriptive Statistics for WM Percentage CR and Average RTs: Day 1 and Day 2^a

	Group				
	Measure		Day 1	Da	y 2
	Wicasuic		Combined $n = 18/17^{b}$	Stress $n = 5$	Relax $n = 12$
Set 1:					
	1-back	CR	95.77 (5.14)	97.14 (2.61)	96.03 (3.98)
		RT	561.74 (124.52)	558.00 (147.27)	519.42 (100.07)
	3-back	CR	87.30 (10.96)	86.52 (7.82)	94.05 (5.42)
		RT	776.25 (286.81)	642.51 (140.60)	656.87 (197.36)
Set 2:					
	1-back	CR	94.71 (5.39)	95.24 (3.37)	92.86 (7.18)
		RT	544.23 (126. 91)	505.79 (81.39)	538.89 (123.75)
	3-back	CR	89.15 (8.61)	95.24 (8.25)	94.05 (5.42)
		RT	750.09 (251.62)	593.34 (127.76)	636.52 (179.73)
Set 3:					
	1-back	CR	95.50 (6.21)	96.19 (3.98)	97.62 (3.21)
		RT	561.18 (140.24)	543.20 (122.16)	531.69 (135.22)
	3-back	CR	89.68 (10.23)	94.29 (6.21)	91.27 (7.27)
		RT	791.06 (277.27)	603.94 (158.34)	726.80 (284.73)
Set 4:					
	1-back	CR	93.39 (5.92)	98.10 (2.61)	95.24 (4.54)
		RT	532.67 (101.47)	516.23 (77.46)	526.23 (95.56)
	3-back	CR	89.95 (9.08)	91.43 (9.16)	96.43 (5.42)
		RT	741.75 (271.21)	478.30 (52.05)	685.37 (273.54)

Note. Mean scores are provided with standard deviations in parentheses. CR = Percentage correct response, RT = Average reaction times. Average reaction times are measured in milliseconds. ^aThese values are for the 2 x 4 and 2 x 2 x 4 ANOVAs, *t*-tests factored in participants for whom some data was lost, thus altering the number of participants in some cases and altering mean and standard deviation values. ^bData for percentage CRs based on 18 participants, data for average RTs based on 17 participants. ^cData for percentage CRs based on 5 participants, data for average RTs based on 4 participants.

Table 5.

Descriptive Statistics and Results for WM Dependent-Sample t-tests: Day 1

		Task di	fficulty				Cohen's
Meas	sure	1-back	3-back	t	df	p	d
		n = 18	n = 18				и
Set 1:							
	CR	95.77 (5.14)	87.30 (10.96)	3.19	17	.005	.99
	RT	562.70 (120.87)	763.72 (283.28)	-3.69	17	.002	.92
Set 2:							
	CR	94.71 (5.39)	89.15 (8.61)	2.62	17	.018	.77
	RT	543.23 (123. 13)	740.59 (247.42)	-3.55	17	.002	1.01
Set 3:							
	CR	95.50 (6.21)	89.68 (10.23)	2.22	17	.041	.70
	RT ^a	561.18 (140.24)	791.06 (277.27)	-3.65	16	.002	1.05
Set 4:							
	CR	93.39 (5.92)	89.95 (9.08)	1.51	17	.148	.45
	RT a	532.67 (101.47)	741.75 (271.21)	-4.02	16	.001	1.02

Note. Means presented with standard deviations in parentheses. CR = Percentage correct response, RT = Average reaction times. Average reaction times are measured in milliseconds. ^aData for 17 participants.

Table 6

Descriptive Statistics and Results for WM Independent-Samples t-tests: Day 2

		Gro	oup				Cohen's
Measu	re	Stress $n = 6$	Relax $n = 12$	t	df	p	d
Set 1:							
1-back	CR	96.03 (3.58)	96.03 (3.98)	.00	16	1.000	.00
	RT	582.72 (144.98)	519.42 (100.07)	1.09	16	.291	.51
3-back	CR	86.51 (10.18)	94.05 (5.42)	-2.08	16	.054	.92
	RT	653.16 (128.43)	656.87 (197.36)	04	16	.967	.02
Set 2:							
1-back	CR	95.24 (3.01)	92.86 (7.18)	.77	16	.453	.43
	RT	539.42 (109.93)	538.89 (123.75)	.01	16	.993	.00
3-back	CR	95.24 (8.25) ^a	94.05 (5.42)	.36	15	.728	.17
	RT	593.34 (127.76) ^a	636.52 (179.73)	48	15	.635	.28
Set 3:							
1-back	CR	96.19 (3.98) ^a	97.62 (3.21)	78	15	.447	.40
	RT	543.20 (122.16) ^a	531.69 (135.22)	.16	15	.872	.09
3-back	CR	94.29 (6.21) ^a	91.27 (7.27)	.81	15	.431	.45
	RT	603.94 (158.34) ^a	726.80 (284.73)	90	15	.384	.53
Set 4:							
1-back	CR	98.10 (2.61) ^a	95.24 (4.54)	1.30	15	.212	.77
	RT	516.23 (77.46) ^a	526.23 (95.56)	21	15	.839	.11
3-back	CR	91.43 (9.16) ^a	96.43 (5.42)	-1.42	15	.177	.66
	RT	478.30 (52.05) ^a	685.37 (273.54)	-1.65	15	.120	1.05

Note. Means presented with standard deviations in parentheses. CR = Percentage correct response, RT = Average reaction times. Average reaction times are measured in milliseconds. ^aData for 5 participants.

Table 7
Descriptive Statistics for WM Percentage CR and Average RTs: Difference Between Day 1 and Day 2^a

ruy 2			Day 2 – Day 1		
	Measure			roup	
	1/1000001		Stress $n = 5/4^{b}$	Relax $n = 12$	
Set 1:					
	1-back	CR	2.86 (4.26)	-0.40 (5.16)	
		RT	-6.74 (15.00)	-30.23 (81.61)	
	3-back	CR	1.90 (9.28)	5.16 (12.26)	
		RT	-117.22 (223.22)	-94.71 (179.85)	
Set 2:					
	1-back	CR	-1.90 (2.61)	-1.19 (6.13)	
		RT	-78.80 (39.70)	28.38 (69.37)	
	3-back	CR	7.62 (5.43)	2.78 (7.98)	
		RT	-42.56 (203.88)	-140.66 (263.64)	
Set 3:					
	1-back	CR	-1.90 (4.26)	2.38 (6.26)	
		RT	-44.68 (42.39)	5.20 (123.76)	
	3-back	CR	0.00 (8.91)	1.19 (8.40)	
		RT	-124.76 (97.67)	-42.69 (188.22)	
Set 4:					
	1-back	CR	2.86 (4.26)	1.98 (8.96)	
		RT	-44.86 (34.75)	9.45 (52.17)	
	3-back	CR	1.90 (9.87)	5.56 (9.27)	
		RT	-170.27 (119.98)	-49.34 (142.82)	

Note. Means presented with standard deviations in parentheses. CR = Percentage correct response, RT = Average reaction times. Average reaction times are measured in milliseconds. ^aThese values are for the 2 x 2 x 4 ANOVAs, *t*-tests factored in participants for whom some data was lost, thus altering the number of participants in some cases and altering mean and standard deviation values. ^bData for percentage CRs based on 5 participants, data for average RTs based on 4 participants.

Table 8
Descriptive Statistics and Results for WM Independent-Samples t-tests: Difference Between Day 1 and Day 2

Measure		Gr	oup				Cohen's
		Stress	Relax	t	df	p	d
Q + 1		<i>n</i> = 6	<i>n</i> = 12				
Set 1:							
1-back	CR	1.59 (4.92)	-0.40 (5.16)	.78	16	.447	.39
	RT	-6.09 (22.37)	-30.23 (81.61)	.70	16	.493	.40
3-back	CR	2.38 (8.38)	5.16 (12.26)	50	16	.626	.26
	RT	-134.85 (225.57)	-94.71 (179.85)	41	16	.686	.20
Set 2:							
1-back	CR	-0.79 (3.58)	-1.19 (6.13)	.15	16	.886	.08
	RT	-71.38 (43.43)	28.38 (69.37)	-3.2	16	.006	1.72
3-back	CR	7.62 (5.43) ^a	2.78 (7.98)	1.23	15	.237	.71
	RT	-31.51 (178.29) ^a	-140.66 (263.64)	.84	15	.414	.49
Set 3:							
1-back	CR	-1.90 (4.26) ^a	2.38 (6.26)	-1.39	15	.185	.8
	RT	-30.98 (47.81)a	5.20 (123.76)	62	15	.541	.39
3-back	CR	$0.00(8.91)^a$	1.19 (8.40)	26	15	.797	.14
	RT	-124.76 (97.67) ^b	-42.69 (188.22)	82	14	.425	.55
Set 4:							
1-back	CR	2.86 (4.26) ^a	1.98 (8.96)	.21	15	.840	.13
	RT	-44.86 (34.75) ^b	9.45 (52.17)	-1.92	14	.075	1.23
3-back	CR	1.90 (9.87) ^a	5.56 (9.27)	73	15	.478	.38
	RT	-170.27 (119.98) ^b	-49.34 (142.82)	-1.52	14	.152	.92

Note. Means presented with standard deviations in parentheses. CR = Percentage correct response, RT = Average reaction times. Average reaction times are measured in milliseconds. ^aData for 5 participants. ^bData for 4 participants.

University of Cape Town

Faculty of Humanities

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