Stress-induced sex differences in spatial navigation: A pilot study.

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Abstract

Spatial navigation is the ability to direct oneself to a set destination in the most practical way possible, and to recognise that one is approaching said destination. Certain forms of spatial navigation are centred, neuroanatomically, on the hippocampal formation, a brain structure also vulnerable to increased levels of the stress hormone cortisol. Although empirical studies have identified a substantial sex difference, in favour of males, on laboratory-based spatial navigation tasks, little research has investigated whether, and how, these sex differences manifest under conditions of stress. Furthermore, studies have failed to report physiological measurements to confirm that stress inductions actually generate the predicted and sought-for physiological responses. The current pilot study aimed to provide data supporting methodological improvements that would provide the foundation for a research programme aimed at creating a clearer idea of whether, stress-induced sex differences in spatial navigation do exist. Using a within-subjects design in which males and females were tested on two separate days (the first day under control conditions and the second under stressful conditions), I hypothesised that (a) the stress induction paradigm would produce both HPA-axis and ANS activity in males and females, (b) that the spatial navigation environment created would allow for cue usage of both landmarks and gradients, and (c) stress would affect spatial navigation performance in females more than in males. Results suggested that (a) the stressor used was effective in eliciting appropriate responses in males but not females, (b) the spatial environment created showed a bias toward proximal cue utilisation, and (c) there were no sex differences evident under stress conditions. This study provides a basis for future research on stress-induced sex differences in spatial navigation. Specifically, it identifies the factors that need to be addressed in order to effectively conduct research such a study.

Keywords: stress, spatial navigation, sex differences, FFST, CG Arena, cortisol

Stress-induced sex differences in spatial navigation: A pilot study.

We use cognitive navigation systems constantly in our daily lives. These systems allow us to move from one destination to another following familiar routes, and they are particularly active when we learn routes to new places. They also allow us to locate, both visually and mentally, objects in space. Without these systems, many activities we complete effortlessly every day would not be possible.

Stress affects particular brain regions linked to certain types of spatial navigation. Thus, one might predict that stress would impair performance on certain spatial navigation tasks. This impairment might manifest differently in men and women, however, given the well-established sex differences in spatial abilities. The pilot study described here aimed to provide the foundation for a research programme that seeks to add to current knowledge about stress-induced sex differences in spatial navigation.

Spatial Navigation

Spatial navigation is the ability to (a) direct oneself to a set destination in the most practical way possible, and (b) be able to recognise that one is approaching said destination (O'Keefe & Nadel, 1978). From a purely cognitive perspective, spatial navigation is a complex process. Mental rotation, visual-spatial attention, and numerous other basic cognitive processes are integrated in the service of spatial navigation (Barkley & Gabriel, 2007; Chen, Chang, & Chang, 2008). *Mental rotation* refers to the ability "to maintain an active representation of all the parts, and interrelations of all the parts" in order to manipulate objects mentally (Kaufman, 2007, p. 212). When we navigate, we need to maintain a constant representation of our position in relation to our surroundings. Mental rotation assists in maintaining this mental representation of the environment and the resulting execution of movement within it (Garden, Cornoldi, & Logie, 2002; Gramann, Muller, Eick, & Schönebeck, 2005).

O'Keefe and Nadel (1978) were first to distinguish between cognitive systems responsible for map-guided and stimulus-response navigations. Their theory and subsequent empirical work by others (e.g., Banquet, Gaussier, Quoy, Revel & Burnod, 2005; Munzer, Zimmer, Schwalm, Baus & Haus, 2006) has led to the conclusion that spatial knowledge is divided into two main types: route and survey. *Route knowledge* depends on an egocentric wayfinding strategy, which involves using memories about various landmarks in order to

direct oneself to a location from a particular starting point, keeping in mind one's position in relation to the target location (Gramann et al., 2005; Hund & Minarik, 2006). *Survey knowledge*, in contrast, depends on an allocentric wayfinding strategy, which involves using memories about the geometric relationships between different locations (Gramann et al., 2005).

Although research into human spatial navigation dates almost to the beginning of the psychological enterprise, real impetus for understanding the neural substrates of this process came with the early-1970s discovery of place cells (i.e., cells with location-specific activity) in the rat hippocampus (O'Keefe & Dostrovsky, 1971). Subsequent work, in rodents, monkeys, and humans, has described a complex network of navigational neurocircuitry, centred on the hippocampal formation but also including other medial temporal lobe structures and regions of the parietal and frontal lobes (Bohbot, Giuseppe & Petrides, 2004; Burgess, Maguire, Spiers, & O'Keefe, 2001; Roche, Mangaong, Commins, & O'Mara, 2005).

Recent neuropsychological and neuroimaging studies have confirmed that different forms of human spatial navigation are associated with activity in different brain regions. Specifically, it appears that the hippocampal formation, particularly in the right cerebral hemisphere, is important for encoding spatial associations that utilise cognitive map-based navigation (Astur, Tropp, Sava, Constable, & Markus, 2004; Banner, Bhat, Etchamendy, Joober, & Bohbot, 2011). In contrast, the caudate nucleus is linked to stimulus-response learning, such as memory of a series of turns from different and discrete points in the environment (Baumann, Chan, & Mattingley, 2009; Miyoshi et al., 2011).

Sex Differences in Spatial Navigation

Empirical studies regarding performance on spatial navigation tasks have identified a substantial sex difference in favour of males (Barkley & Gabriel, 2007; Picucci, Caffö, & Bosco, 2011). For instance, Astur, Ortiz, and Sutherland (1998) showed that males were significantly faster than females at finding a platform in a human analogue of the Morris water maze (Morris, 1981, 1984). An elaboration of these results demonstrated, using a similar virtual environment (VE) navigation task, that although both males and females were capable of learning a target location, their efficacy was altered when the availability of distal cues was changed: Females performed better if landmark cues were made available than if they were not (Sandstrom, Kaufmann, & Huettel, 1998).

Thus, a crucial point is that sex differences in spatial navigation may be attributable, at least in part, to the types of cues available in navigation tasks. In most experimental

navigation tasks, the types of cues available appear to favour males rather than females. If one accepts that two critical features of navigation-aiding cues are (a) their distance to the person (proximal or distal), and (b) their physical features (landmarks or gradients), then males prefer distal gradient cues (e.g., distant skylines with varying high and low points), whereas females prefer proximal landmark cues (e.g., a tree in the foreground; Barkley & Gabriel, 2007; Gabriel, Hong, Chandra, Longborg, & Barkley, 2011). Therefore, when the environment features more distal cues (or distal cues exclusively, as is the case in many VE tasks), then skewed results in favour of males ought to be expected.

However, even when this bias in cue availability is controlled, males still outperform females on spatial navigation tasks. For instance, Picucci et al. (2011) reported that females are as good as males at identifying allocentric cues, but that they appear to overlook these if landmark cues are present. On the other hand, males are able to combine information from both types of cues when both are available.

Effects of Stress on Spatial Navigation

In humans, the experience of a stressor results in the activation of two biological systems: The autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. The ANS is responsible for increasing heart rate and blood pressure when the organism is under stress. The HPS axis response results in increased levels of circulating glucocorticoids (specifically, of the hormone cortisol) when the organism is under stress. These increased cortisol levels have effects on the hippocampus, amygdala, and prefrontal cortex (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Putman & Roelofs, 2011).

As mentioned previously, the hippocampal formation is critical for certain forms of spatial navigation (Astur et al., 2004; Burgess, Maguire, & O'Keefe, 2002). Hence, one might predict that the experience of stress and a subsequent increase in cortisol levels would disrupt map-based navigation (i.e., navigation utilising the hippocampus) but leave intact route-based navigation (i.e., navigation utilising the caudate nucleus). Several studies have found results consistent with this prediction (e.g., McDonald et al., 2010; Schwabe et al., 2007; Schwabe, Oitzl, Richter, & Schachinger, 2009). However, some studies have not confirmed this hypothesis, and some have even found that the experience of acute stress has a positive impact on spatial navigation performance (see, e.g., Duncko, Cornwell, Cui, Merikangas, and Grillon, 2007).

These inconsistent findings might be attributed to the fact that the studies cited above differed in their methods and measures. For example, the different types of stress inductions might have played a role. For example, the Star Mirror Tracing Task and the cold-pressor test

(CPT; Hines & Brown, 1932 as used by Richard and Tomasulo (2010) and Duncko et al. (2007), respectively) are only effective at increasing the ANS aspect of the physiological stress response (i.e., they raise heart rate and blood pressure) but not at increasing the HPA axis arm (i.e., they do not raise cortisol levels; Schwabe, Haddad, & Schachinger, 2008). On the other hand, the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) increases cortisol levels significantly and reliably. Pharmacological induction of increased corticosterone levels (as used by Schwabe et al. (2009)) is effective in doing the same.

Otherwise stated, only studies that produce increases in cortisol/corticosterone that are of a magnitude sufficient to impair hippocampal function can be compared directly to one another if one is interested in the effects of stress on (map-based) navigation.

Stress-Induced Sex Differences in Spatial Navigation

Even though males and females differ in their spatial navigation ability, and stress has impairing effects on spatial navigation, few studies have addressed the question of what effects stress has on sex differences in human spatial navigation. In animals, Snihur, Hampson, and Cain (2007) found that when corticosterone levels were increased in male and female rats, spatial navigational ability declined significantly in both sexes.

In humans, Richard and Tomasulo (2010) found no significant difference in accuracy on general non-navigational spatial tasks, in both men and women, before and after inducing acute stress using the Star Mirror Tracing Task. They did note, however, that participants had slower reaction times after stress induction, and that males were more accurate than females. Of importance here, however, is that salivary cortisol levels showed no increase after stress induction, which suggests there was no effect of the induction method on hippocampal activity.

Thomas, Laurence, Nadel, and Jacobs (2010) found that exposure to the TSST increased sex differences in spatial navigation. Specifically, females exposed to the stressor performed poorly on cognitive map-guided tasks compared to a no-stress control group; males exposed to the same stressor showed no such impairments. In contrast, Gabriel et al. (2011) found that, after application of the CPT, sex differences that were apparent under normal conditions decreased (i.e., there was an increase in women's spatial ability under stress).

The differences in results reported by Thomas et al. (2010) and Gabriel et al. (2011) may be accounted for by methodological variations. For instance, the type of stress induction was different. The TSST involves placing the participant under psychological stress through a series of interviews/tasks, whereas the CPT involves placing the participant under

physiological stress by requiring him to place his hand in a bucket of ice water. Although both methods produce reliable stress responses (von Dawans, Kirshbaum, & Heinrichs, 2011; Schwabe et al., 2008), only the TSST raises cortisol levels and subsequently impairs hippocampal function (Dickerson & Kemeny, 2004; Schoofs, Preuβ, & Wolf, 2007; Kirschbaum, Pirke, & Hellhammer, 1993).

Furthermore, the tasks used to measure spatial performance differed across these studies. Thomas et al. (2010) used a VE task, based on the Morris water maze, in which participants had to find first a visible and then an invisible target across a series of trials. In that task, the environment featured distal cues only. In contrast, Gabriel et al. (2011) used a non-navigational task that involved a series of paired photographs containing various combinations of proximal or distal and landmark or gradient cues. The participants had to identify whether the object (i.e., the cue) in the second photograph had appeared in the first. This task allowed the researchers to assess which cue type participants depended on more heavily. Although both of these tasks provide a procedure in which cue strategies play an important role in success, it is difficult to compare their results accurately because one assesses navigation directly while the other does not. With the exception of these two studies, there is no other published research on sex differences in the effect of stress on spatial navigation.

Summary, Rationale, and Hypotheses

The above review has highlighted some important points about performance on spatial navigation tasks. One point is that performance on map-guided spatial navigation tasks depends on brain regions affected by cortisol increases. Another point is that there is notable methodological inconsistency in this area of research. Overall, however, it seems, both empirically and from the viewpoint of neurobiological prediction, that stress affects the use of map-based spatial strategies more than it does landmark-based strategies. This is because the HPA-axis response to stress t affects hippocampal functioning (an area associated with only map-based strategies of spatial navigation).

The current study aimed to improve upon the methodology of the studies conducted by Thomas et al. (2010) and Gabriel et al. (2011). These methodological improvements will help resolve the inconsistencies in the results reported by those studies, and will thus help the field reach firmer conclusions about the possibility of stress-induced sex differences in spatial navigation.

One area of methodological improvement centres on the fact that neither of those studies took or reported physiological measurements (e.g., cortisol levels and heart rate

measurements). Hence, neither study provides psychophysiological or neuroendocrinological confirmation of a provoked physiological stress response. If one is to study the effects of raised stress on hippocampus-centered, map-based spatial navigation in humans, one has to show that an experimental manipulation is effective in raising cortisol levels.

A second, related, area of methodological improvement is that, if one is to study the effects of raised stress on spatial navigation, one has to use a stress-induction procedure that can provoke activity in both arms of the physiological stress response. As noted above, commonly-used laboratory-based stressors tend to produce different effects on ANS and HPA-axis physiology.

A third area of methodological improvement relates to the fact that males and females appear to react differently to stressors. One reason for this difference is that men and women are challenged differently by different environmental events. Males respond more to achievement (or challenge) stressors that involve needing to prove intellectual or physical superiority, whereas females show a greater response to social stressors that involve interpersonal concerns (Stroud, Salovey, & Epel, 2002). The TSST is more of an achievement than an interpersonal social stressor, which is why it tends to produce a greater response in males than in females (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008). Therefore, again, any study of stress-induced sex differences in spatial navigation (or in any domain of cognitive functioning) must use a stress-induction method that produces physiological responses reliably in both males and females.

A final area of methodological improvement relates to the task used in studies of stress-induced sex differences in spatial navigation. Specifically, the task must be a navigation task, and it must contain both landmark and gradient cues. Although Gabriel et al. (2011) attempted to assess the use of both types of cues, their task did not assess navigation directly. In contrast, although Thomas et al. (2010) used a non-immersive desktop VE navigation task, it is not clear that that task assessed the preferential use of one type of cue over another, or whether, in fact, both types of cues were present in that environment.

Hence, the overall purpose of the pilot study described here was to provide data supporting methodological improvements and innovations that would provide the foundation for a research programme aimed at creating a clearer idea of whether, in fact, stress-induced sex differences in spatial navigation do exist. Specifically, in the current study I (a) took physiological measurements to confirm that the stress induction was in fact generating the predicted and sought-for physiological responses, (b) used a stress-induction method that sought to minimise the potential sex differences present in other such methods, and (c)

created and used a spatial navigation task that contained both landmark and gradient cues, and that allowed the participant to use either in the service of efficient navigation.

Those steps allowed me to test these specific hypotheses in a small sample of males and females:

1. In an unstressed condition, males will perform better on spatial tasks than females.
2. Stress will impair spatial performance in both males and females, but more so in females.

Furthermore, the current study adopted a within-subjects design, rather than the between-subjects designs typical of previous research in this field. The within-subjects design allowed for baseline measures and for the effects of stress on each individual's ability to be observed. In this way, I sought to rule out the effects of potentially confounding individual difference factors (e.g., stressor reactions, environment of the spatial navigation task) that often complicate interpretation of data in this field (Hegarty, Montello, Richardson, Ishikawa & Lovelace, 2006; Hegarty, Smallman, & Stull, 2012).

The results of these hypothesis tests, because they are based on a small sample, must be interpreted with caution. The methodological elements of this pilot study are perhaps more critical, however: If this methodology appears useful and viable, then the stage is set for subsequent, larger research programmes to resolve important questions about stress-induced sex differences in spatial navigation.

Methods

Design and Setting

The hypothesis-testing aspect of the study followed a 2 x 2 repeated-measures factorial design. The first predictor variable was the participant's sex (i.e., male or female). The second was the psychological state of the participant (i.e., stressed or relaxed). Outcome variables were derived from the participant's scores on two spatial cognitive tasks: the Computer-Generated (CG) Arena and a mental rotation (MR) task. Each participant was tested on two separate occasions, over 2 days. The first day's testing was under the relaxed/control condition; the second was under the stressed/experimental condition.

On each day, experimental procedures took place between 14h30 and 18h30 to control for cortisol's diurnal cycle (Kudielka et al., 2004; Maheu et al., 2005). The study ran in two venues at the Department of Psychology at the University of Cape Town (UCT). One venue was a computer laboratory where the cognitive testing, physiological measures, and

questionnaire completion took place. The second venue was the room where the participants underwent the experimental manipulation.

Participants

Fourteen volunteers (9 males, 5 females) between the ages of 18 and 25 were enrolled. Because this study forms part of a larger data collection effort that utilises only White participants, I was bound by this criteria in my recruiting. The participants were recruited from undergraduate psychology classes at UCT by means of the Student Research and Participation Project (SRPP). Potential participants were notified via the SRPP website of the study's availability and the relevant inclusion and exclusion criteria. They signed up for sessions via that website.

Exclusion criteria. Participants were screened for the presence of some of these exclusion criteria via a questionnaire administered prior to the onset of experimental procedures. General exclusion criteria included (a) smoking, (b) presence of any DSM-IV-TR (APA, 2000) Axis I or II disorders, (c) the use of any steroid-based medication, and (d) a body mass index (BMI) of more than 27 or less than 19. These exclusion criteria have been identified as potentially confounding variables in research investigating the effects of psychosocial stress on cognitive performance (Kudielka et al., 2009), and are in line with those criteria used in previous research (e.g., Schoofs et al., 2008; Schwabe & Wolf, 2010).

Materials and Procedure

All self-report measures listed below have good psychometric properties in that they are highly internally consistent and have high levels of validity (Beck, Steer, & Brown, 1996; Dozois et al., 1998; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; Watson et al., 1988). Furthermore, their usefulness in characterizing South African individuals has been demonstrated (Rieckert & Moller, 2000; Ward, Flisher, Zissis, Muller, & Lombard, 2001).

Figures 1 and 2 illustrates the timeline of procedures described below.

Day 1. All procedures took place in the computer laboratory. Each participant was tested individually by one of two female postgraduate researchers (AA or RH).

First, participants read through and signed a consent form (see Appendix A). Thereafter, the researcher asked the participant to complete the Beck Depression Inventory-II (BDI-II; Beck et al., 1996) and the Trait form of the State-Trate Anxiety Inventory (STAI; Spielberger et al., 1983). These measures were used to ensure that, across groups, participants were experiencing similar levels of depression and anxiety in their everyday lives, as well as to screen out individuals who reported experiencing high levels of depression (BDI-II scores > 20).

Every BDI-II item has four possible responses, with each indicating a different degree of possible depressive symptomatology. Respondents 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 STAI-Trait form is an indicator of general levels of anxiety and is measured on a 20-item Likert-type scale. The BDI-II was scored while participants completed the STAI-Trait, so that those who met the depression exclusion criterion would not have to continue with the rest of the experiment.

Following completion of these questionnaires, the researcher measured the participant's weight and height in order to calculate Body Mass Index (BMI). Thereafter, she administered the MR and CG Arena tasks.

Measures of spatial navigation. The Card Rotations Test (CRT) from the Kit of Factor-Referenced Cognitive Tests battery (Ekstrom, French, Harman, & Derman, 1976) assessed mental rotation ability. The CRT is presented across two separate pages, each of which contains 10 target items. Each target item consists of a drawing of an irregularly-shaped card. Eight other drawings of the same card are presented to the right of it, with each drawing a version of the target that is either rotated or turned over to its other side. The participant must indicate whether each of these eight is a rotated or flipped representation of the target card. The participant is given 6 minutes (3 minutes per page) to complete the 80 items on each page. This test is considered a reliable measure of mental rotation (Spearman-Brown coefficient = .86). For the purposes of this study, the test was split so that the problems on one page were presented on Day1 and those on the other were presented on Day 2. We counter-balanced presentation order to remove potential effects of between-page differences (see Appendix B).

The CG Arena (Jacobs, Laurance, & Thomas, 1997; Jacobs, Thomas, Laurance & Nadel, 1998; Thomas et al., 2001) is a non-immersive desktop VE navigation task. In tasks such as these, an individual is able to use representations of distal cues, and the multiple spatial relations between them, to form a cognitive map of the virtual space. This map can then be used to relocate specific places within the space (Burgess et al., 2002; Maguire et al., 1999). There appears to be a good transfer of spatial information from a VE to a real environment, and learning in such an environment allows humans to make accurate judgements about metrics in real space (Astur et al., 2002; Loomis, Lippa, Klatzky, & Golledge, 2002; Thomas, 2003; Worsley et al., 2001).

The participants were read a set of standardised instructions describing the general characteristics of the two Arena rooms (a waiting room and an experimental room; see Figure

3) and were given instructions on how to navigate using the arrow keys on the keyboard. The *waiting room* was designed to allow the participant to become familiar with navigation in the Arena. The walls and floor of this room were texture-less (with each wall being distinguishable only by its colour). The walls of the *experimental room* featured a panoramic picture that ran across the four walls. Different pictures were used for the Day 1 and Day 2 arenas (see Figure 3). The ceiling of these rooms was not visible and the floor was colourless and texture-less.

Two objects (3-dimensional cubes placed at different locations within the arena) served as proximal landmark cues, whereas the walls served as distal cues containing both landmarks (e.g., people, lakes) and gradients (e.g., mountain lines). To facilitate data analysis and interpretation, the Arena was divided into four quadrants (northeast, southeast, northwest, and southwest) by lines not visible to participants.

On Day 1, the participant was required to first complete a set of 4 experimental room trials, each of which featured a visible target (a large coloured square on the floor of the experimental rooms; see Figure 3, panel b). The target could be located easily following a basic visual scan of the room. The participant was required to find, move toward, and stand on the visible target. While standing on the target, the Arena software played a clicking sound. To complete the trial, the participant was required to press the space bar on the keyboard while standing on the target. Doing so led the software to move the participant back to the waiting room. The target was in a different location for each visible-target trial, and the starting point for each was different. These trials were conducted in a separate arena to that of the rest of Day 1's experimental manipulations. The purpose of the visible target trials was to ensure that the participant understood the instructions and was able to move around efficiently in the arena (Jacobs et al., 1997).

On both Day 1 and Day 2, participants completed 34 separate trials in the experimental room (see Table 1). On each of these trials, the target (a large blue square) was hidden until the participant stood on it. It remained in a fixed location across trials. As noted above, the panoramic picture that spanned the walls of that room differed from Day 1 to Day 2.

The 8 acquisition trials mirrored those contained in previous Arena designs (see Jacobs et al., 1997, 1998). These trials served as learning set as to where the hidden target was located, as previous studies have shown that learning should occur within the first 8 trials provided all aspects (dimensions, proximal cues, relations between distal cues) remain unaltered.

On each odd-numbered trial from 9-33, walls of the arena (distal cues) and objects within the arena (proximal cues) were either eliminated (removed) or swapped (moved around). For example, trial 19 involved swapping the pictures on the north, east, south and west walls in an anticlockwise direction so that a different picture was on the wall closest to the target. Similarly, on trial 15 objects were moved around within the arena so that they were no longer in the same quadrant as before. Both rodent and human studies have shown that removal of any subset of distal stimuli will leave intact performance about a well-learned target, whereas changing the relations among stimuli will disrupt this performance (Fenton, Arolfo, Nerad, & Bures, 1994; Jacobs et al., 1998; Suzuki, Augerinos, & Black, 1980).

At the end of the Day 1 session, participants were reminded about their session for the next day. They were also asked to refrain from eating or drinking anything (except water), and from taking part in any form of exercise, for at least 2 hours prior to their sessions.

Day 2. The researcher met the participants at the same venue in which their previous session had taken place.

To measure heart rate, we used the Vrije Universiteit Ambulatory Monitoring system, version 5fs (VU-AMs; Vrije Universiteit, Amsterdam, Holland). This non-invasive device is portable, and participants were thus able to move around and walk between the two study venues while wearing it. The device was attached at the beginning of the Day 2 session, and measured heart rate continuously until it was removed at the end of the session. After the device was fitted, a 5-min rest period was allowed for the device to normalise to the participants' heart rate, following which a 2-min baseline reading was taken (HR_B). A second 5-min reading was then taken directly following the stress manipulation (HR_1) and a final 5-min reading was taken 40 minutes after the manipulation ended (HR_2).

Participants rated their current level of general negative affect at three different times using the appropriate scale from the Positive and Negative Affect Schedule (PANAS; (Watson et al., 1988): the first served as a baseline measurement (NA_B) and was reported shortly after entering the laboratory; the second was reported 5 minutes following the end of the stress manipulation (NA_1), and the third 45 minutes after the manipulation ended (NA_2). The NA scale, but not the PA scale, is related to self-reported stress and coping as it measures the extent to which the respondent feels unpleasant and distressed. Intra-subject fluctuations in self-reported stress are highly correlated with fluctuations in NA (Watson et al., 1988). Thus, this scale is most relevant to stress-manipulation studies.

Participants also rated their current level of anxiety at three different times using the STAI-State form. This form measures an individual's anxiety at a specific point in time and

is measured on a 20-item Likert-type scale. The three STAI-State reports were given at the same time as the PANAS-NA reports: the first (a baseline measurement) shortly after entering the laboratory ($STAI_B$), the second 5 minutes following the end of the stress manipulation ($STAI_1$), and the third 45 minutes after the manipulation ended ($STAI_2$).

We collected cortisol three times by means of saliva samples using SARSTEDT Salivette® Cortisol swabs (Sarstedt, Nümbrecht, Germany): the first (a baseline measurement) shortly after entering the laboratory ($CORT_B$), the second 5 minutes following the end of the stress manipulation ($CORT_1$), and the third 45 minutes after the manipulation ended ($CORT_2$). These samples are an easy, effective, and non-intrusive way to collect cortisol and do not cause any distress for the participant (Garde & Hansen, 2005). Once the samples were collected, they were stored immediately in individual, labelled tubes and then frozen until they were transported to the National Health Services Laboratory at Groote Schuur Hospital, where they were analysed.

The researcher administered the second page of the CRT after the stress induction, which is described below. Thereafter, participants completed the Day 2 CG Arena procedures. After that, participants were debriefed as to the purpose of the study. They were asked not to divulge any aspect of this study with anyone else so as to not confound the results.

Experimental manipulation. Participants were exposed to the *Fear-Factor Stress Test* (*FFST*), a stress induction procedure developed in our laboratory (du Plooy, Thomas, Henry, Human, & Jacobs, under review). The FFST combines procedures from the TSST (Kirschbaum et al., 1993) and the CPT (Hines & Brown, 1932). The room in which the stressor occurred had bright lights, a video-camera, and a two-person (one male, one female) judging panel. Both judges were undergraduate research assistants.

The researcher instructed participants that they were to audition for the reality television show *Fear Factor.* She then read a set of standardised instructions introducing the task. The participant was asked to imagine s/he was undergoing an audition for *Fear Factor,* and that s/he must therefore convince a panel of two judges that s/he is a suitable person to be on the show. The researcher told participants that the judges were behavioural health experts who would analyse verbal and nonverbal behaviour with the aid of a video recording.

The participant was told the audition would comprise three tasks: 1) a 5-min free motivational speech as to why s/he should be a *Fear Factor* contestant; 2) a 5-min mental arithmetic task, demonstrating that s/he is able to think under pressure; and 3) a 2-min

submersion of the dominant arm in cold water, demonstrating that s/he is able to withstand the physical demands of the television show.

The participant was given 10 minutes to prepare the speech. After that preparation period, the researcher took him/her to the room in which the rest of the task was completed. The participant then presented the speech extemporaneously. If s/he stopped speaking before 5 minutes elapsed, the judge of the opposite sex to the participant asked a set of standard prompting questions (e.g., "What is your ultimate fear and how do you think you will be able to overcome it in front of the camera?"). Following the speech, judges asked the participant to perform the mental arithmetic task (serial subtractions of 17 starting from 2043). If the participant performed an incorrect subtraction, s/he was asked to re-start the task from the beginning. Finally, the participant submerged his/her arm in cold water (between 0 and 4 ºC) for as long as possible (up to a maximum of 2 minutes). The participant remained standing for all three of the tasks.

Ethical Considerations

This study followed the ethical guidelines for research with human subjects outlined by the Health Professions Council of South Africa (HPCSA) and the University of Cape Town (UCT) Codes for Research. We received ethical approval for the study from the Human Research Ethics Committees of the UCT Department of Psychology and the UCT Faculty of Health Sciences.

Participation was voluntary. On Day 1, participants were presented with an informed consent document (see Appendix A) that outlined the study clearly, detailing what was to be expected of them, and noting that their confidentiality was ensured and upheld. It also informed them of their right to terminate participation at any point. They were reminded of this fact at the start of the Day 2 session.

All participants were debriefed at the end of Day 2. The researcher informed them that they had not been videotaped or were not evaluated in any way on their performance in the 'interview' section of the stressor. The researcher then explained to them that it was necessary to have them believe so in order for the psychosocial stressor to be of maximum effect.

The risks involved in participation included being placed in a mildly stressful situation involving public speaking. Furthermore, participants were required to place their hands in very cold water. There were no other discomforts and risks associated with participation. Should an individual have been excluded based on the BDI-II criterion, or if s/he showed any signs of distress at the end of the Day 2 session, s/he would have been given

the contact details for the UCT Student Wellness Centre so that counselling could be initiated, if so desired.

Participants received no financial compensation. They did receive 3 credits to serve toward their SRPP total, however.

Data Management and Statistical Analysis

Outcome variables. The MR task was scored by subtracting the number of incorrect responses from the number of correct responses. The maximum total score, for each page, was 80. The CG Arena software generated a number of outcome variables for each trial. I used total path length to the target as my primary Arena outcome variable, where shorter lengths indicate better performance.

Descriptive and inferential analyses. All analyses were completed using SPSS version 20. The threshold for statistical significance was set at alpha = .05, unless otherwise noted. Before starting inferential analyses, I ensured the data met the assumptions underlying each proposed parametric test. If an assumption was violated, I either used the non-parametric equivalent or used other means to ensure validity of the analysis (e.g., for repeated-measures ANOVAs where Mauchly's test indicated that the assumption of sphericity had been violated, I used Greenhouse-Geisser estimates for corrected degrees of freedom). For each analysis, I calculated the appropriate effect size estimate. More details of each specific analysis are provided at the appropriate place in the Results section.

Results

Final Sample Characteristics

One male participant (aged 19 years) was excluded because, after enrolling, the research team discovered he was on steroid-based medication. One female participant (aged 18 years) was excluded because her BMI (34.6) fell outside the required range. As Table 2 shows, independent-sample *t*-tests detected, for the final sample of 12 participants, no significant between-sex differences regarding age, BMI, BDI-II scores and STAI-Trait scores.

Regarding BMI scores, the average value across the entire sample (and the average within each group) was within the defined "normal" range of 19-25. This variable is important to control for because of the positive association between cortisol secretion rate and BMI, particularly in obese individuals (Fraser et al., 1999).

Regarding BDI-II scores, these were relatively low for both groups, with the mean for each falling with the range conventionally described as 'minimally depressed' (0-13). There

were no significant differences between the mean of this sample and normative data for college students in the United States (M = 12.26, SD = 9.93) supplied by the test manual (Beck et al., 1996), $t(11) = -1.50$, $p = .08$.

Regarding STAI-Trait scores, the sample appeared representative of the general population: When compared to the normative data for college students in the US (males: M = 38.30, SD = 9.18; females: M = 40.4, SD = 10.15) supplied by the test manual (Spielberger et al., 1983), a single-sample t-test was not significant for males, $t(7) = 0.19$, $p = .43$, or for females, $t(3) = 0.20$, $p = .43$.

Effectiveness of the Stress Induction Method: Day 2 data

The analyses described below tested the effectiveness of the FSST, and examined whether the level of stress induction on Day 2 was equal in males and females. For each variable listed in Table 3, I conducted a 2 x 3 (Sex x Testing Stage) repeated-measures ANOVA and ran planned comparisons to test pre-existing hypotheses about where within-group differences existed.

Participant self-report measures.

STAI-State. There was a significant main effect of Testing Stage, $F(2, 20) = 3.71$, $p = .04$, partial $\eta^2 = .37$. There was no significant main effect of Sex, $F(1, 10) = 2.68$, $p = .13$, partial $\eta^2 = .21$, and no significant interaction effect, $F(2, 20) = 0.59$, $p = .56$, partial $\eta^2 = .09$. This pattern of data suggests that male and female scores on this instrument were not significantly different overall, and that the change across time was no different in males and females. It does suggest, however, that there were overall changes in self-reported anxiety across the stress-induction procedure.

Planned pairwise comparisons revealed that, for the entire sample, there was a significant increase in STAI-State scores from baseline to time 1 ($p = .04$), and that there was no significant difference between baseline and time 2 scores ($p = .12$). Figure 4 illustrates this pattern of data.

PANAS-NA. There was a significant main effect of Testing Stage, $F(2, 20) = 5.04$, $p = .02$, partial $\eta^2 = .34$. There was no significant main effect of Sex, $F(1, 10) = 1.81$, $p = .21$, partial $\eta^2 = .15$, and there was no significant interaction effect, $F(2, 20) = 0.60$, $p = .56$, partial $\eta^2 = .06$. Again, this pattern of data suggests that male and female scores on this instrument were not significantly different overall, and that the change across time was no different in males and females. It does suggest, however, that there were overall changes in self-reported negative affect across the stress-induction procedure.

Planned pairwise comparisons revealed that, for the entire sample, there was a significant increase in PANAS-NA scores from baseline to time 1 ($p = .04$), and that there was no significant difference between baseline and time 2 scores ($p = .31$). Figure 5 illustrates this pattern of data.

STAI-State and PANAS-NA scores were highly correlated at each measurement point: $r = .79$ at baseline, $r = .68$ at time 1, and $r = .79$ at time 2, $p < .05$ in each case. This pattern of data confirms that participants' self-reports of changed anxiety and negative affect were consistent across measures.

Physiological measurements.

Heart rate. Due to hardware malfunctions, complete sets of heart rate were only available for 5 males and 3 females. Hence, the analyses described below pertain to those individuals only.

There was a significant main effect of Testing Stage, $F(1.05, 6.32) = 13.66$, $p = .009$, partial $\eta^2 = .69$. There was no significant main effect of Sex, $F(1, 6) = 0.20$, $p = .67$, partial $\eta^2 = .03$, and there was no significant interaction effect, $F(1.05, 6.32) = 0.26$, $p = .64$, partial $\eta^2 = .04$. Again, this pattern of data suggests that heart rates in males and females were not significantly different overall, and that the change across time was no different in males and females. It does suggest, however, that there were overall changes in heart rate across the stress-induction procedure.

Planned pairwise comparisons revealed that, for the entire sample, there was a significant increase in heart rate from baseline to time 1 ($p = .047$), but that there was no significant difference between baseline and time 2 scores ($p = .99$). Figure 6 illustrates this pattern of data.

Salivary cortisol. There was a significant main effect of Testing Stage, $F(2, 20) = 5.25$, $p = .02$, partial $\eta^2 = .34$. There was no significant main effect of Sex, $F(1, 10) = 3.59$, $p = .09$, partial $\eta^2 = .26$, but there was a significant interaction effect, $F(2, 20) = 4.03$, $p = .03$, partial $\eta^2 = .29$. This pattern of data suggests that male and female cortisol levels were not significantly different overall, but that the change across time was different in males and females, and that there were overall changes in cortisol levels across the stress-induction procedure.

Planned pairwise comparisons detected, for the entire sample, no significant differences between cortisol levels at baseline, time 1, and time 2. For the male sample only, however, similar planned pairwise comparisons revealed that there was a significant increase

in salivary cortisol levels from baseline to time 1 (p = .02), and that that increase over baseline persisted at time 2 (p = .02). Figure 7 illustrates this pattern of data.

Qualities of the CG Arena: Day 1 data

Visible target trials. The analyses described here sought to confirm that (a) there were no motor, processing speed, or other deficits that impacted on participants' navigation in the computer-generated environment, and (b) male and female participants were able to use landmark-based navigation strategies equally well in that environment.

I ran a repeated-measures ANOVA to ensure that there were no significant between-group differences in spatial performance and that no acquisition curve was evident. Results showed that there was a main effect for Trials, $F(3, 30) = 143.75$, $p < .001$, partial $\eta^2 = .94$; however there was no main effect for sex, $F(1, 10) = 1.28$, $p = .29$, partial $\eta^2 = .11$, or an interaction effect, $F(3, 30) = 3.55$, $p = .06$, partial $\eta^2 = .26$. This indicates that participants were equally capable of navigating and locating the visible target in the Arena. Figure 8 below shows no learning curve is evident from performance on these trials; hence, the main effect of Trials detected by the above analysis can be accounted for by participants probably starting farther away from the target on later trials.

Acquisition and test trials. The analyses described here sought to demonstrate that orderly place learning occurred during the acquisition trials (invisible target trials 1-8) of the Day 1 Arena. If such orderly learning occurred, as it did in previous CG Arena preparations (e.g., Jacobs et al., 1997, 1998), then one can assume that the participants in this study were using spatial navigation strategies in this panoramic room as they were in the rooms described in those previous studies

I ran a repeated-measures ANOVA on path length data for the acquisition trials, across all participants. There was a significant main effect of Trials, $F(2.46, 27.03) = 10.57$, $p < .001$, partial $\eta^2 = .49$. A linear trend analysis was also statistically significant, indicating that there was an orderly learning curve from trials 1 through trial 8, $F(1, 11) = 60.3$, $p < .001$, $\eta^2 = .85$. Figure 9 illustrates this acquisition curve.

As Figure 9 illustrates, place learning appeared reasonably complete at trials 4-5. For this reason, I took the average path length on trials 6-8 as a baseline measurement against which to compare performance on the test trials (i.e., those trials where walls or objects were removed or swapped). This was done to identify the effect each test trial had on performance, which indicated the effect that proximal and distal cues had on spatial performance.

A series of paired samples t-tests compared performance on each test trial to baseline performance. Table 5 shows the results of those comparisons. As can be seen, those test trials

involving object removal or elimination had the most impact on path length to the target. Specifically, when the object closest to the target was removed from the Arena entirely or swapped to another place, participants tended to take much longer path lengths, relative to baseline, to relocate the target. Figures 10 and 11 also illustrate this pattern of data.

Sex Differences in Spatial Performance under Unstressed Conditions: Day 1 data

A series of one-way ANOVAs were conducted on the Day 1 data from the MR and CG Arena tasks to identify whether males evinced better spatial performance than females under unstressed conditions. For the MR task, there were no statistically significant between-group differences, $F(1,11) = 1.14$, $p = .31$. For the CG Arena, I grouped trials according to their general test conditions (object or wall removals, and object or wall swaps). Table 6 shows the results of the analyses conducted on those data. Again, no sex differences were evident, although a power analysis revealed that an effect would have been seen had there been a greater number of participants.

Stress-Induced Sex Differences in Spatial Performance: Day 2 versus Day 1

MR task. Figure 13 illustrates male and female MR performance across days. A 2 (Testing Occasion: day 1 versus day 2) x 2 (Sex: male versus female) repeated-measures ANOVA revealed a significant main effect for Testing Occasion, $F(1, 10) = 7.47$, $p = .02$, partial $\eta^2 = .43$, but no significant main effect for Sex, $F(1, 10) = 3.41$, $p = .25$, partial $\eta^2 = .94$, and no significant interaction effect, $F(1, 10) = 1.80$, $p = .20$, partial $\eta^2 = .15$.

To analyse these data further, I calculated difference scores from day 1 to day 2 (i.e., I subtracted day 1 scores from day 2 scores to get an indication of the amount of improvement from the first testing occasion to the second). An independent samples *t*-test revealed no significant between-sex differences with regard to those difference scores, $t(1, 12) = 1.34$, $p = .10$, $d = 0.82$. The large effect size suggests, however, that a significant sex difference, in favour of males, would have been found had the sample size been larger.

CG Arena. A repeated-measures ANOVA conducted on the Day 2 acquisition trials data indicated that learning had occurred in the Day 2 Arena: There was a significant main effect of Trials on path length to find the target, $F(7, 77) = 3.74$, $p = .05$, partial $\eta^2 = .25$. However, a linear trend analysis was not statistically significant, $F(1, 11) = 3.33$, $p = .09$, partial $\eta^2 = .23$ (see Figure 11).

Several repeated-measures ANOVAs were run to compare the performance on Day 1 to performance on Day 2. Each ANOVA compared performance of a test trial or group of test trials across days. For elimination trials, all wall eliminations except trial 17 (removal of the west wall) were averaged together. Removal of Trials 11 and 25 were grouped together.

Trials 17 and 21 were compared as individual trials because, as shown in the analysis of Day 1 data, the test manipulations on those trials (p = .23 and p = .07, respectively) had the largest impact on path length to the target relative to baseline (see Figure 10). All swap trials were compared individually.

Table 7 shows the results of these analyses. Results showed that there were no significant main effects for the relaxed versus stress conditions (Table 7). Wall Removal 1 and Swap A showed almost significant results with large effect sizes, p (Cohen's d) = .07 (0.74), and .06 (0.74), respectively. However, a significant interaction effect was found between sex and trial 15 (object swap), $F(1, 10) = 3.90$, p = .03 η^2 = .41. Figure 12 illustrates this interaction and shows that males performed better on Day 1 than females, however, on Day 2 their performance increased whereas females' performance decreased. There was also a significant main effect for sex on trial 31 (object switch; p (Cohen's d) = .18 (0.62)), $F(1, 10) = 5.14$, p = .05, partial η^2 = .34. Further analysis revealed that females performed significantly better on the object switch trial than did males.

Discussion

The purpose of this pilot study was to lay the groundwork for a research programme that would investigate thoroughly the effects of acute psychosocial stress on spatial navigation performance. Specifically, I aimed to develop and describe (a) a stress-induction method that would activate both ANS and HPA-axis responses in both males and females, and (b) a spatial navigation task that featured both landmark and gradient cue usage, and that could be used to observe potential sex differences under stress. The hypothesis-testing aspect of the study consisted of predictions that (a) under unstressed conditions, males would perform better on the spatial tasks than females, (b) stress would impair spatial performance in both males and females, and (c) stress would impair spatial performance in females more than in males.

Analysis of responses to the Fear Factor Stress Test method indicated that it raised self-reported negative affect and anxiety, as well as heart rate, significantly and successfully in both males and females. Furthermore, participants entered and left Day 2 of the study in the same state of relative calm, with a significant increase in subjective and some physiological experiences of stress occurring in the middle phase procedure. The same pattern was found for salivary cortisol in males; females, however, exhibited no significant increases in salivary cortisol levels. Hence, the results from this study, in combination with those from previous studies in our lab (du Plooy et al., in review; Thomas, Dreyer, Amod,

Wolf & Human, in review), show that the FFST induces at least some elements of the stress response successfully, and raises cortisol levels consistently in males. The results also show, however, that the FFST was successful in increasing ANS activity only in females, whereas in males both ANS and HPA-axis activity were increased.

Although this pattern of data points towards a differential response to the FFST by males and females, previous studies investigating HPA-axis activity due to stressors (e.g., TSST) have shown that it is not necessarily or only the stressor that is limited in producing reliable HPA-axis response, but rather that the modulated female menstrual cycle must be taken into account (Kirschbaum et al, 1999). More specifically, it appears that when females are in the luteal phase, they exhibit similar cortisol response patterns to those of males; however, when they are in the follicular phase or on oral contraceptives, their responses are significantly lessened. In the current study, I did not take into account the menstrual cycle of female participants. It is therefore unclear whether menstrual cycle phase had an effect on HPA-axis response in the current female sample.

Furthermore, some studies have found no discriminable difference between young adult males and females with regard to free salivary cortisol responses (Kelly et al., 2008; Kirschbaum, Kudielka, Gaab, Schommer & Hellhammer, 1999). This means that with regard to this study, the sex differences found in HPA-axis responses are not attributable to flaws in the FFST. Therefore the FFST can be considered a reliable stressor for future stress-related research.

Regarding the general characteristics of the CG Arena, analysis of the Day 1 visible-target trials found no sex differences in performance as measured by path length to target. Similarly, analysis of the Day 1 acquisition trials showed that all participants were able to place learn adequately. The linear trend observed across the acquisition trials was similar to that found by Jacobs et al. (1998) in their development of a similar type of CG Arena (see also Thomas et al., 2010). These results indicate that once the location of the target was acquired, participants could relocate it easily and consistently, as long as the crucial aspects of the room remained unchanged.

Further analysis of the Day 1 CG Arena data suggested that participants relied primarily on proximal landmark cues (objects located within the Arena) to locate and relocate the target. Task performance declined markedly when the position of those objects was changed, or when they were removed from the Arena. On the other hand, the elimination or swapping of walls (distal cues) appeared to have no significant effect on performance,

although there was a trend toward significance in the elimination of the West wall (perhaps because that wall contained both landmark and gradient cues and was closest to the target).

Analysis of hidden target trial performance on the Day 1 CG Arena task showed no significant sex differences. However, a power analysis indicated that a larger sample size would have revealed a significant sex difference, in favour of males, in general spatial navigation performance. This finding would have been consistent with previous research (Astur et al., 1998; Barkley & Gabriel, 2007). Previous research has also shown, however, that females prefer using landmark during spatial navigation tasks. When more such cues are present, female performance on spatial navigation increases to almost match that of males (Gabriel et al., 2011; Sandstrom et al., 1998). Hence, with regard to the current findings, it may be the specific features of the task that attenuate the usual bias toward geometric cue usage found in previous VE studies (Picucci et al., 2011).

Analysis of the mental rotation data also revealed that there were no sex differences in performance in unstressed conditions. Mental rotation ability assists in holding a mental representation of the environment in which one is navigating, and it assists with the resulting execution of movement within that environment (Garden et al., 2002).

The hypothesis that stress would impair spatial navigation performance overall was not confirmed. Results showed, for instance, that increasing stress did not impair CG Arena performance. A power analysis revealed that there were too few participants in this study for the effect to be detected..

Contrary to the stated hypothesis, analysis of the MR task data indicated that performance actually increased under stressful conditions for both males and females. Although this is not the general finding with regard to stress effects on spatial navigation, the results may be due to the U-shaped function of cortisol: too much and too little cortisol impairs function, however a moderate amount may facilitate functioning (de Kloet, Oitzl & Joels, 1999; Maheu, Collicutt, Kornick, Moszkowski & Lupien, 2005). This is consistent with the level of cortisol increase induced in this study, and the positive effect of stress on navigation has been seen in some previous studies (e.g., Duncko et al., 2007)

There were some significant results with regard to individual test trials involving switching or swapping the landmark objects around in the CG Arena, however. Contrary to the prediction that stress would impair female but not male performance, on Day 2 females performed better than males on the test trial in which objects were switched around. Although previous literature has found that females generally perform more poorly than males under stress (e.g., Thomas et al., 2010), there has been some evidence consistent with

an improvement in female navigation performance under stress (e.g., Gabriel et al., 2011). Reasons for this increase may also be due to the fact that the current spatial navigation task was predominantly landmark-based, which has been shown to be of more benefit to females than males (Barkley & Gabriel, 2007).

Limitations and Considerations for Future Research

This pilot study shows that, in order to observe any effects of stress on spatial navigation performance (and to observe sex differences in those effects), the sample size needed is about 30 males and 30 females. By increasing sample size to those levels, one might be able to gain better insight into the nature stress-induced sex differences in spatial navigation, and could begin to explore mechanisms behind the observed associations.

Physiological data. One limitation of this study was the lack of heart rate data available for final analysis. Due to hardware malfunctioning, several data sets were lost from an already small sample. Hence, the reliability of the patterns observed must be questioned. Furthermore, I did not take any other measures of ANS activity. Many studies in this field use salivary alpha amylase as a measure of such activity (Dickerson & Kemeny, 2004; Schoofs et al., 2008) future research in that field should follow that lead so that multiple sources of data on the ANS stress response might be obtained.

FFST and HPA-axis activity. The FFST was not successful in eliciting HPA-axis responses in females. However, the study did not consider female menstrual cycle, which has been found to modulate increases in cortisol levels, during recruitment. Future research should aim to recruit only females who are in the luteal phase. In so doing, one would be able to determine if the FFST is a successful stress induction method in terms of increasing both HPA-axis and ANS activity in both males and females.

Test of spatial navigation. The current spatial navigation task appears to be primarily a test of proximal cue usage. Cue availability is one of the main variables that has contributed to sex differences in previous research. Results showed that the CG Arena created for this study did not adequately allow for interaction with both proximal and distal cues. In order to obtain reliable sex differences, the cue availability bias needs to be diminished. According to the literature, landmark-based navigation relies on the caudate nucleus. Thus, in order to identify stress effects on spatial navigation, an Arena that better integrates the usage of both gradient and landmark cues is needed. Therefore future research needs to take these factors into account when creating a spatial navigation task to test the effects of stress on spatial navigation (as well as underlying sex differences as a result of stress).

Summary and Conclusions

The lack of methodologically sound research into stress-induced sex differences in spatial navigation leaves open area for exploration, which this study aimed to begin to fill. The purpose of the current pilot study was to lay the foundation for further research that can clarify the effects of acute psychosocial stress on sex differences in spatial navigation, using the stress-induction procedure and spatial navigation task described here. This pilot study showed that the FFST is an adequate stress induction method that, particularly in males, produces reliable ANS and HPA-axis responses of the kind needed in this field. The study also provided a first step toward creating a CG Arena that contains both landmark (proximal) and gradient (distal) cues and that therefore can be used for testing spatial navigation in future research. Furthermore, the trends toward stress-induced sex differences in spatial navigation performance seen in this small sample tested here indicate that, with some of the modifications mentioned above taken into account, there is rich promise in launching a research programme exploring the mechanisms underlying such differences.

Hence, the significance of this study is that it points the way toward a potentially fruitful avenue of research into the nature and mechanisms of human spatial navigation. Research into spatial navigation is important as it allows us to identify the variables that affect our everyday navigation in the real world. This allows us to determine ways in which to improve on potential inhibitory variables so that we may effectively navigate in our environment.

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Appendix A

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**

Effects of Acute Psychosocial Stress on Visuo-Spatial Memory Performance in Healthy Humans

3. **Principal Investigators, Ethics Committee, and Telephone Numbers**

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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 performance. More specifically, we are interested in how the acute psychosocial stressor affects visuo-spatial memory performance.

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

During this study, you will be required to complete a number of memory based tasks and may be required to complete a 20-minute presentation. Your levels of stress will be assessed through the collection of self-report data, heart rate measurements, skin conductance measurements and saliva samples with the aid of a cotton swab. These saliva samples will be used to analyse levels of cortisol, a stress hormone.

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. Furthermore, you may be asked to place your hand in very cold water. 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 performance, and what variables moderate this relationship. 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.

Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternatives to being in the study; and how the participant's protected health information will be collected, used, and shared with others:

_____ _____

Signature of Person Obtaining Consent and Authorization Date

You have been informed about this study's purpose, procedures, and risks; how your protected health information will be collected, used and shared with others. You have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your protected health information. By signing this form, you are not waiving any of your legal rights.

_____ _____

Signature of Person Consenting and Authorizing Date

Please indicate below if you would like to be notified of future research projects conducted by our research group:

_____ (initial) Yes, I would like to be added to your research participation pool and be notified of research projects in which I might participate in the future.

Method of contact:

Phone number: _____

E-mail address: ________________________________

Mailing address: ________________________________

Appendix B

CRT: Comparison of Page 1 and Page 2

A paired-samples *t*-test was run to compare page 1 and page two of the CRT. This was done to ensure that both pages were of equal difficulty and that no differences existed between them. Results indicated that there was no difference between page 1, $M = 45.90$ ($SD = 14.48$) and page 2 $M = 45.55$ ($SD = 17.63$), $t(1, 19) = 0.159$, $p = .44$. This indicated that the pages could be counter-balanced without any differences in performance. Therefore, the page completed did not affect results in performance on the CRT.

Table 1

CG Arena Experimental Room: Trial Descriptions

Trial	Starting location	Trial Type	Test Trial Action
1	South	Acquisition	None
2	West	Acquisition	None
3	East	Acquisition	None
4	North	Acquisition	None
5	South	Acquisition	None
6	East	Acquisition	None
7	West	Acquisition	None
8	North	Acquisition	None
9	West	Test	Remove 1 wall (North)
10	East	Normal	None
11	South	Test	Remove 1 object
12	West	Normal	None
13	South	Test	Remove 1 corner (SW)
14	East	Normal	None
15	North	Test	Swap objects (to other side)
16	South	Normal	None
17	West	Test	Remove 1 wall (West)
18	East	Normal	None
19	North	Test	Swap A (anticlockwise rotation of wall pictures)
20	South	Normal	None
21	East	Test	Remove 1 object
22	West	Normal	None
23	North	Test	Remove 1 corner (Northeast)
24	West	Normal	None
25	East	Test	Remove both objects
26	South	Normal	None
27	North	Test	Swap B (clockwise rotation of wall pictures)
28	West	Normal	None
29	South	Test	Remove all walls
30	East	Normal	None
31	North	Test	Switch Objects
32	South	Normal	None
33	West	Test	Remove everything
34	East	Normal	None

Note. On Day 1, the target was located in the Northeast quadrant. On Day 2, it was located in the southeast quadrant. *Start location* refers to the place in which the participant began the trial in question. On each trial, the participant began at a point close to (within 2 units of) the arena wall.

Table 2

Sample Characteristics: Descriptive statistics and between-group comparisons (N = 12)

	Group				
Measure	Male (n = 8)	Female (n = 4)	t	p	Cohen's d
Age	19.50 (1.60)	20.25 (1.26)	-0.81	.22	0.50
BMI	24.08 (1.76)	22.53 (2.15)	1.34	.11	0.76
BDI-II	10.13 (4.88)	10.50 (7.00)	-0.11	.46	0.06
STAI -Trait	39.00 (10.66)	42.00 (15.90)	-0.39	.35	0.21

Note. In the second and third columns, means are reported with standard deviations in parentheses. BMI = body mass index; BDI-II = Beck Depression Inventory-II; STAI = State-Trait Anxiety Inventory. Degrees of freedom for each between-group comparison were (1, 10).

Table 3

Self-Reported and Physiological Stress: Descriptive statistics (N = 12)

	Group	
Measure	Male (n = 8)	Female (n = 4)
STAI-State		
Baseline	37.25 (11.41)	43.50 (6.56)
Time 1	41.63 (11.49)	53.25 (13.67)
Time 2	39.50 (8.88)	50.00 (10.42)
PANAS-NA		
Baseline	12.88 (2.59)	15.75 (1.71)
Time 1	17.75 (6.96)	20.50 (8.74)
Time 2	13.00 (3.55)	18.75 (7.68)
Heart rate[a]		
Baseline	76.15 (16.68)[b]	79.88 (4.31)[d]
Time 1	94.58 (8.50)[c]	103.43 (28.46)[d]
Time 2	77.09 (10.96)[c]	78.04 (6.66)[d]
Salivary cortisol[e]		
Baseline	4.74 (1.78)	5.45 (5.70)
Time 1	13.91 (6.68)	6.07 (3.87)
Time 2	11.53 (5.01)	5.65 (2.17)

Note. Means are presented with standard deviations in parentheses. STAI = State-Trait Anxiety Inventory; PANAS = Positive and Negative Affect Scale. [a]Measured in beats per minute (bpm). [b]n = 5; [c]n = 6; [d] n = 3. [e]Measured in nanomoles per litre (nmol/l).

Table 5

CG Arena Day 1: Comparison of performance on test trials to baseline performance (N = 12)

	Comparison Baseline versus:	*t*	*p*	Cohen's *d*	Observed power	Required *N*[a]
Elimination trials	Trial 9 (North wall removal)	-0.06	.48	0.02	0.05	4
	Trial 17 (West wall removal)	-0.52	.31	0.21	0.13	26
	Trial 13 (opposite corner removal)	-0.08	.47	0.03	0.06	38
	Trial 23 (critical corner removal)	0.77	.23	0.32	0.19	26
	Trial 29 (all walls removed)	-0.12	.45	0.05	0.06	16
	Trial 11 (near object removal)	-1.24	.12	0.54	0.36	26
	Trial 21 (far object removal)	1.61	.07	0.63	0.44	24
	Trial 25 (all objects removed)	-0.73	.24	0.27	0.16	26
Swap trials						
	Trial 19 (Swap A - anticlockwise)	-1.37	.099	0.56	0.38	26
	Trial 27 (Swap B - clockwise)	-0.60	.281	0.25	0.15	26
	Trial 15 (Swap objects - opposite side)	-4.02	.001**†	1.21	0.89	26
	Trial 31 (Switch objects)	-3.65	.002**†	0.91	0.70	26

Note. Degrees of freedom = (1, 11) for each comparison. Bonferroni-corrected *p*-value = .05/12 = .004. [a]*N* that is needed to detect a significant effect, given *p* = .05 and the currently-estimated effect size. On average, the analyses suggested that an *N* of 30 would be adequate.
*$p < .05$. **$p < .01$. ***$p < .001$. †Significant at Bonferroni-corrected *p*-value.

Table 6

CG Arena Day 1: Analysis of sex differences in path length across test trials

	Group				
	Males	Females			
Trial Group	(*n* = 8)	(*n* = 4)	*F*	*p*	ESE
Wall Removal	159.44 (34.38)	181.79 (69.81)	0.53	.49	0.39
Object Removal	215.88 (182.59)	177.36 (61.01)	0.16	.70	0.27
Wall Swap	462.30 (585.24)	319.34 (465.87)	0.17	.69	0.26
Object Swap	536.56 (387.97)	770.47 (750.22)	0.48	.50	0.38

Note: The second and third columns display means, with standard deviation in parentheses. ESE = effect size estimate; in this case, Cohen's *d*. *Wall Removal* = Trials 9, 13, 17, 23, 29; *Object Removal* = Trials 11, 21, 25; *Wall Swap* = Trials 19, 27; *Object Swap* = 15, 31

Table 7

CG Arena Day 1 vs. Day 2: Within-subject effects

Group	*M (SD)*	*F*	*p*	Cohen's *d*
Wall removal 1: All removals except w		4.10	.07	0.74
Day 1	155.32 (32.43)			
Day 2	227.78 (129.61)			
Wall removal 2: West wall		3.35	.10	0.49
Day 1	206.41 (172.84)			
Day 2	482.74 (752.07)			
Object removal 1: All + near		1.07	.33	0.53
Day 1	258.42 (212.23)			
Day 2	167.86 (101.02)			
Object removal 2: Far		1.59	.24	0.42
Day 1	68.68 (5.17)			
Day 2	217.90 (488.50)			
Swap A (anticlockwise)		0.82	.39	0.16
Day 1	561.80 (949.38)			
Day 2	712.94 (893.72)			
Swap B (clockwise)		4.59	.06	0.74
Day 1	222.49 (243.64)			
Day 2	459.45 (362.63)			
Object swap		0.98	.35	0.09
Day 1	783.94 (666.62)			
Day 2	849.42 (801.74)			
Object switch		2.06	.18	0.62
Day 1	468.16 (408.05)			
Day 2	256.08 (226.28)			

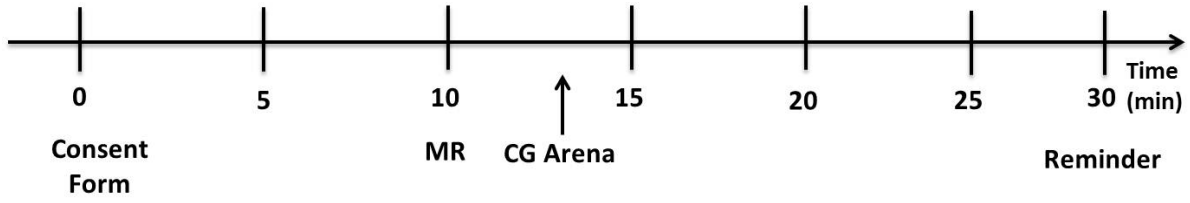

Figure 1. Day 1 study procedures.

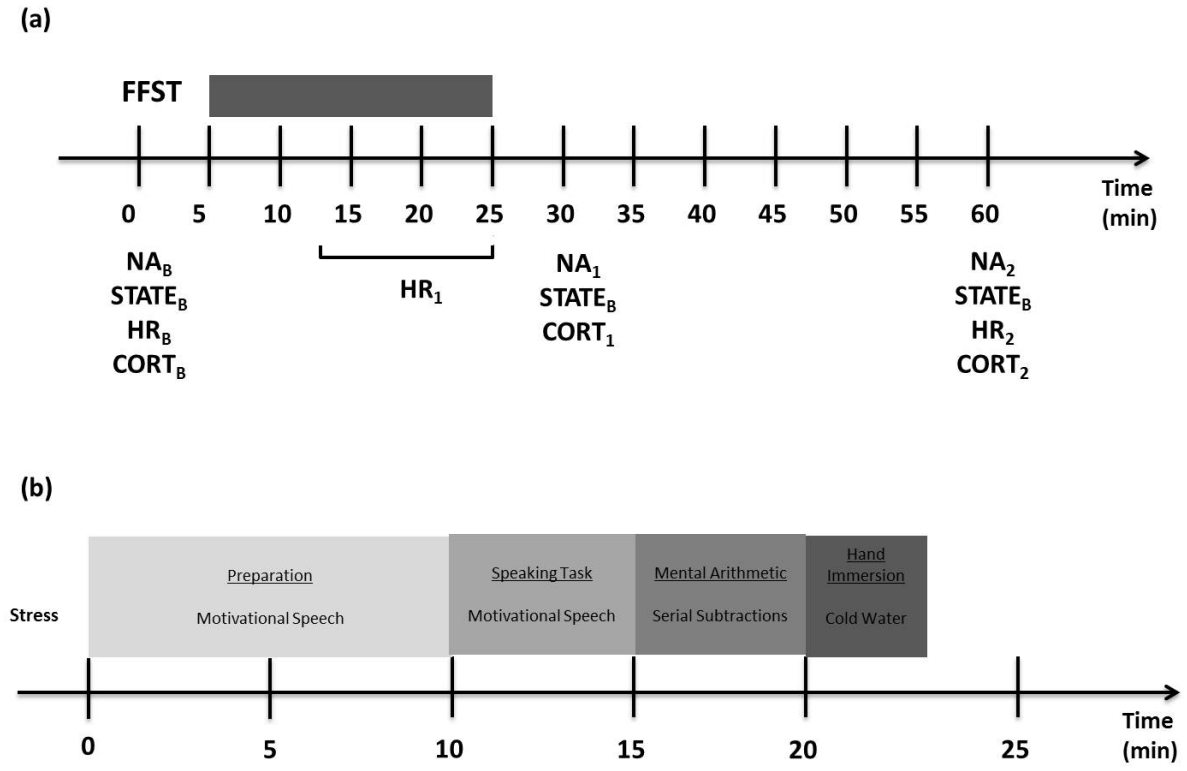

Figure 2. Day 2 study procedures.

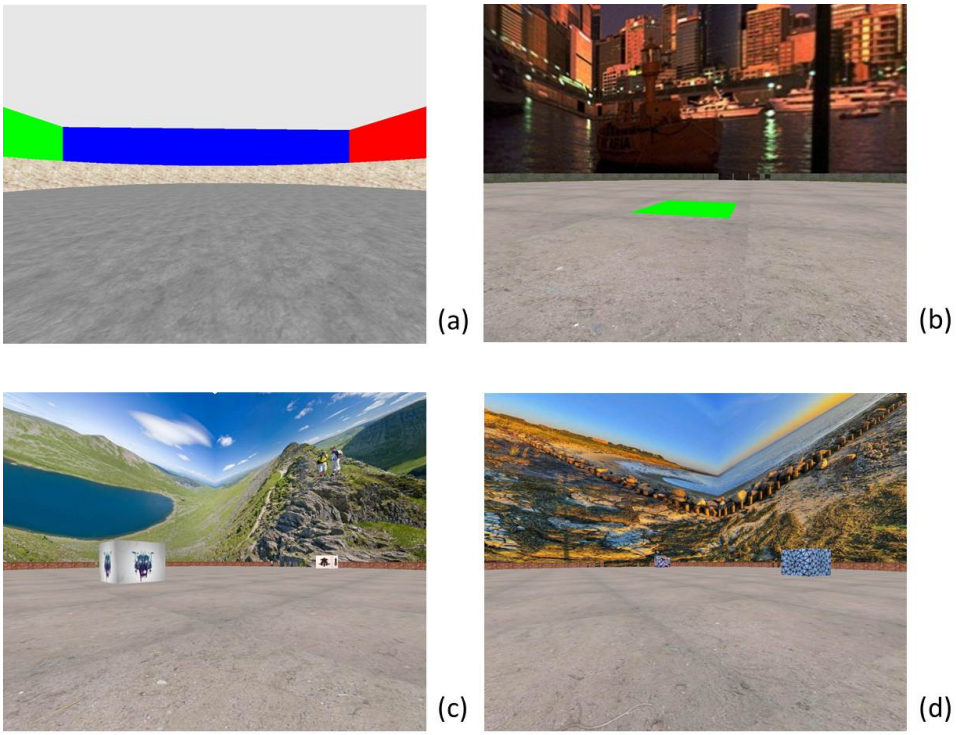

Figure 3. (a) Waiting room; (b) Visible target trial; (c) Day 1 experimental room; (d) Day 2 experimental room.

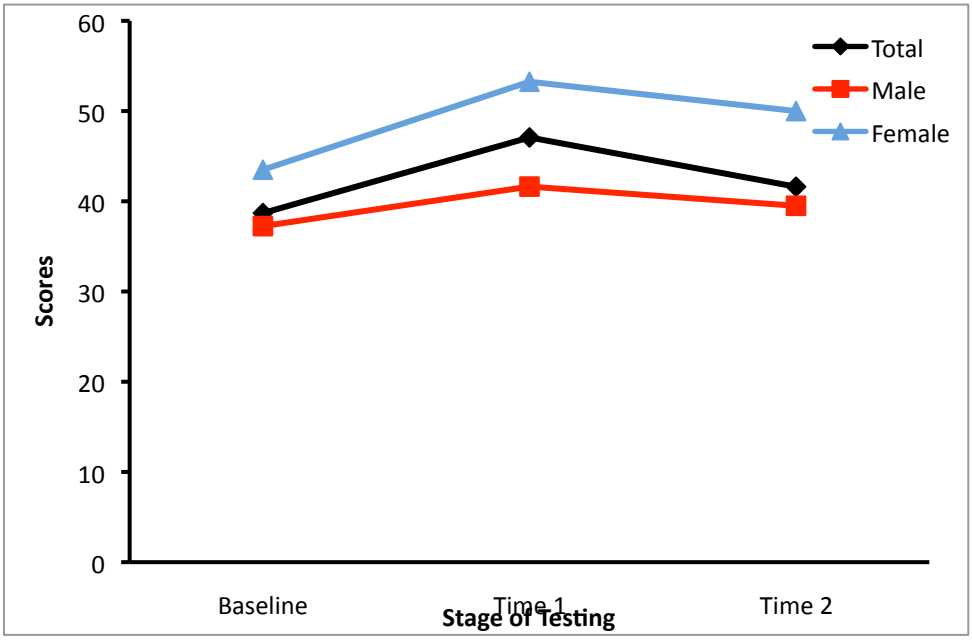

Figure 4. Fluctuations in STAI-State responses during the stress-induction procedure (*N* = 12). Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 2.94, 3.73, 2.98; Males = 4.03, 4.06, 3.14; Females = 3.28, 6.83, 5.21.

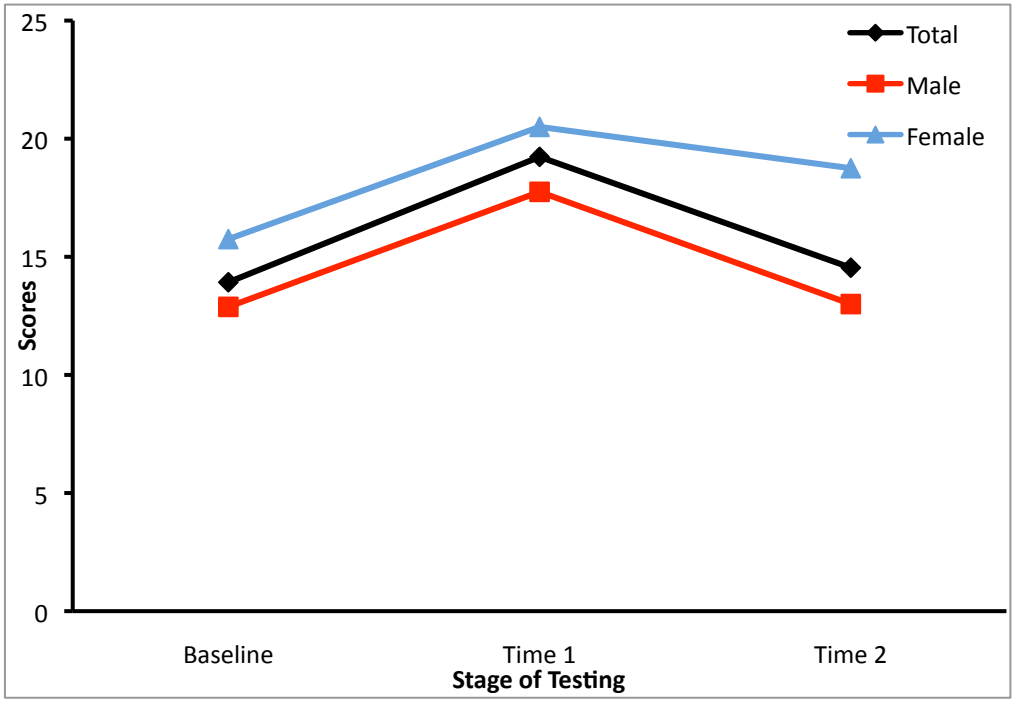

Figure 5. Fluctuations in PANAS-NA responses during the stress-induction procedure (*N* = 12). Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 0.76, 2.11, 1.64; Males = 0.91, 2.46, 1.25; Females = 0.85, 437, 3.84.

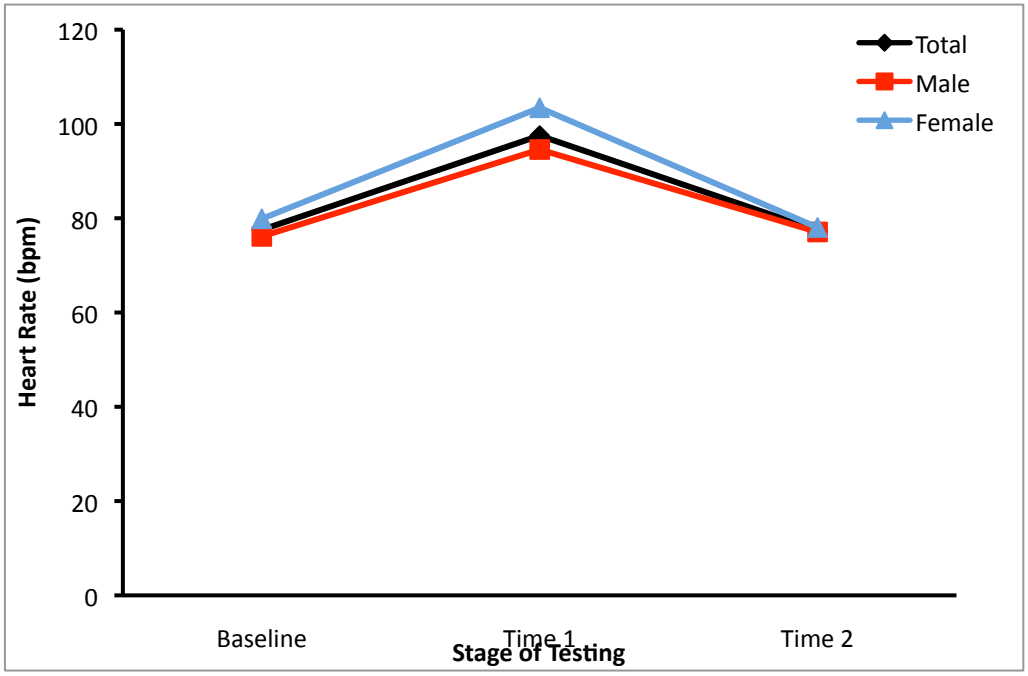

Figure 6. Fluctuations in heart rate responses during the stress induction procedure. Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 4.58, 5.45, 3.10; Males = 7.46, 3.47, 4.47; Females = 2.49, 16.43, 3.85.

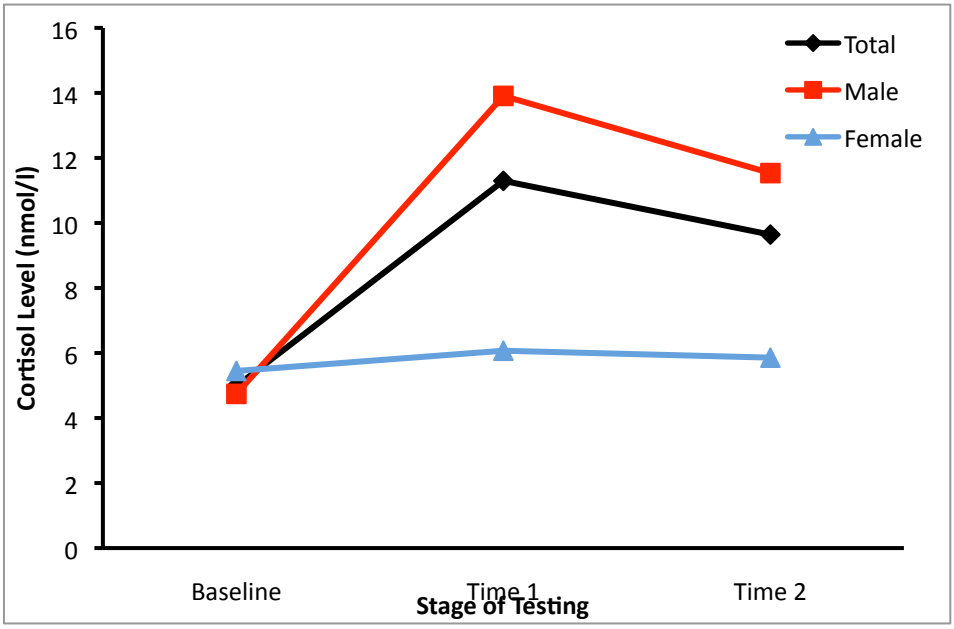

Figure 7. Fluctuations in cortisol levels during stress induction procedure. Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 0.96, 1.99, 1.45; Males = 0.45, 0.97, 0.66; Females = 0.55, 1.16, 0.95.

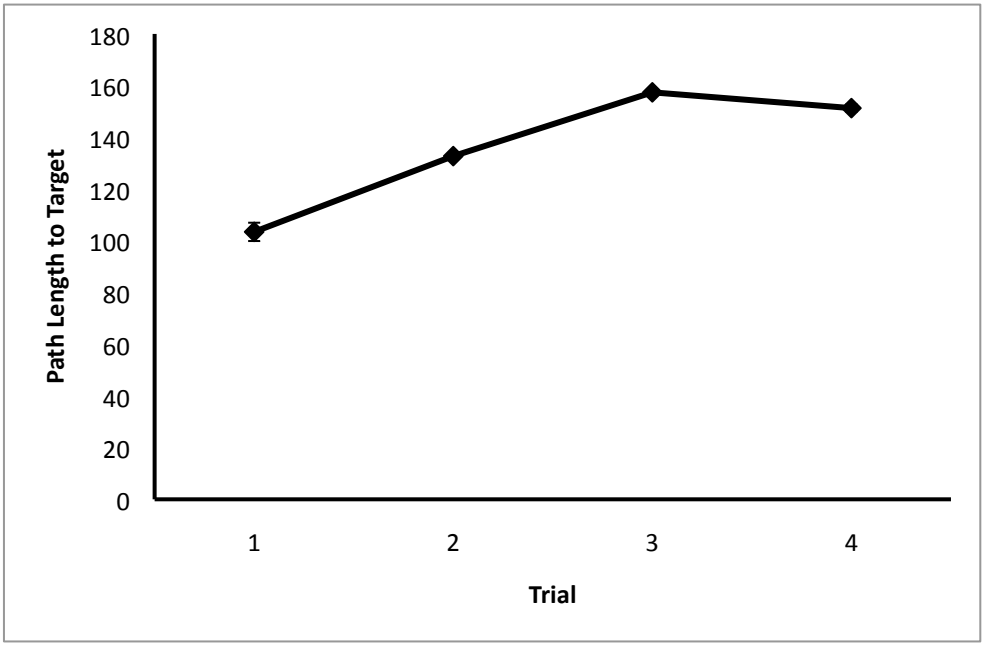

Figure 8. Average path length to target over visible target trials.

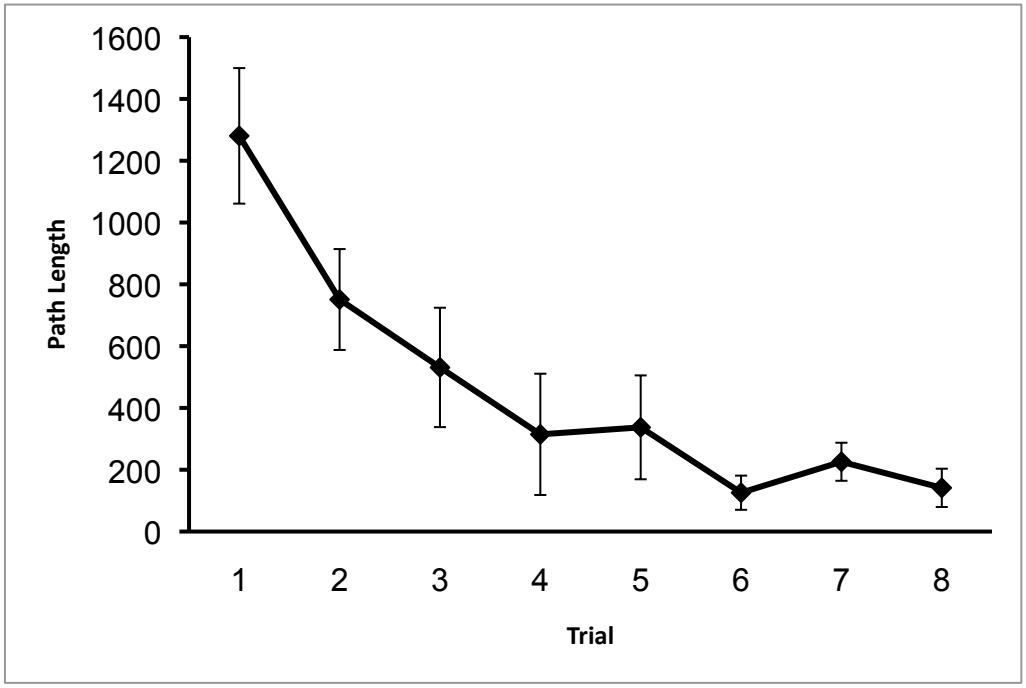

Figure 9. Average path length to target over Day 1 acquisition trials. Error bars indicate standard error of mean with 95% confidence interval.

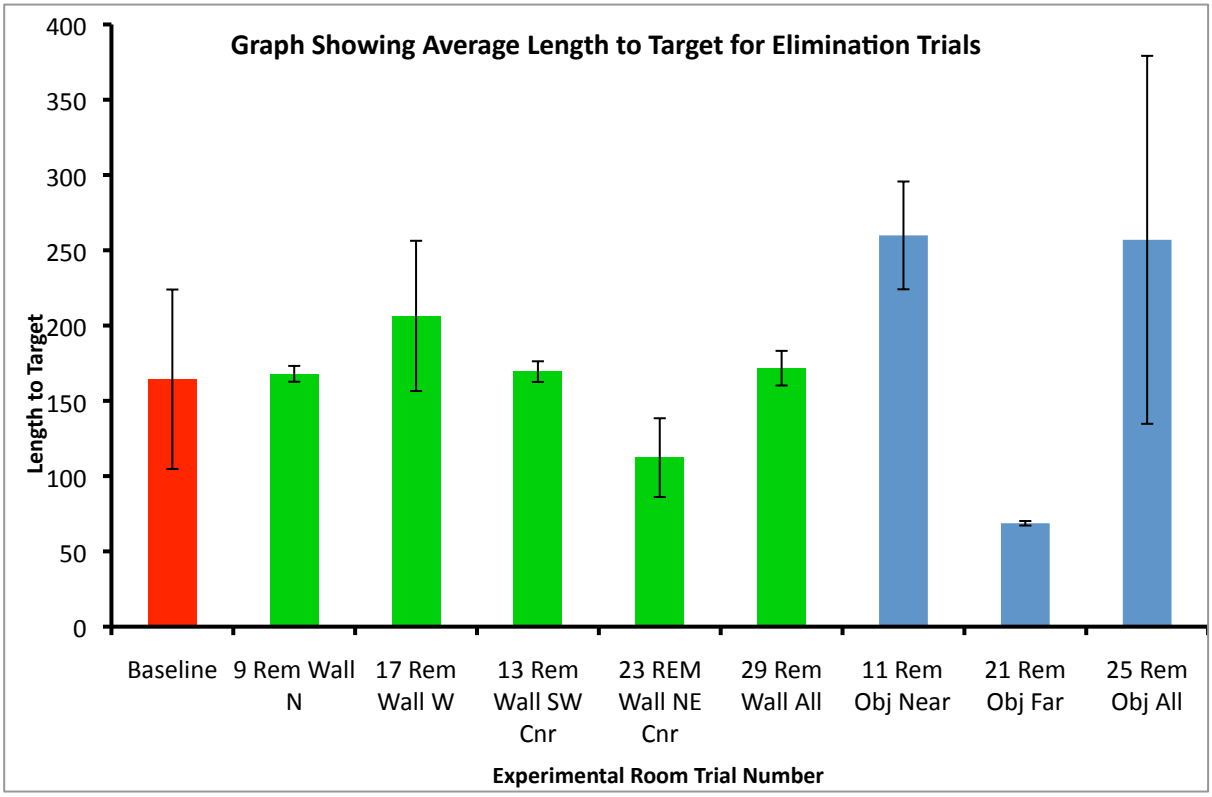

Figure 10. Average path length to target for elimination trials. Error bars indicate standard error of mean with 95% confidence interval.

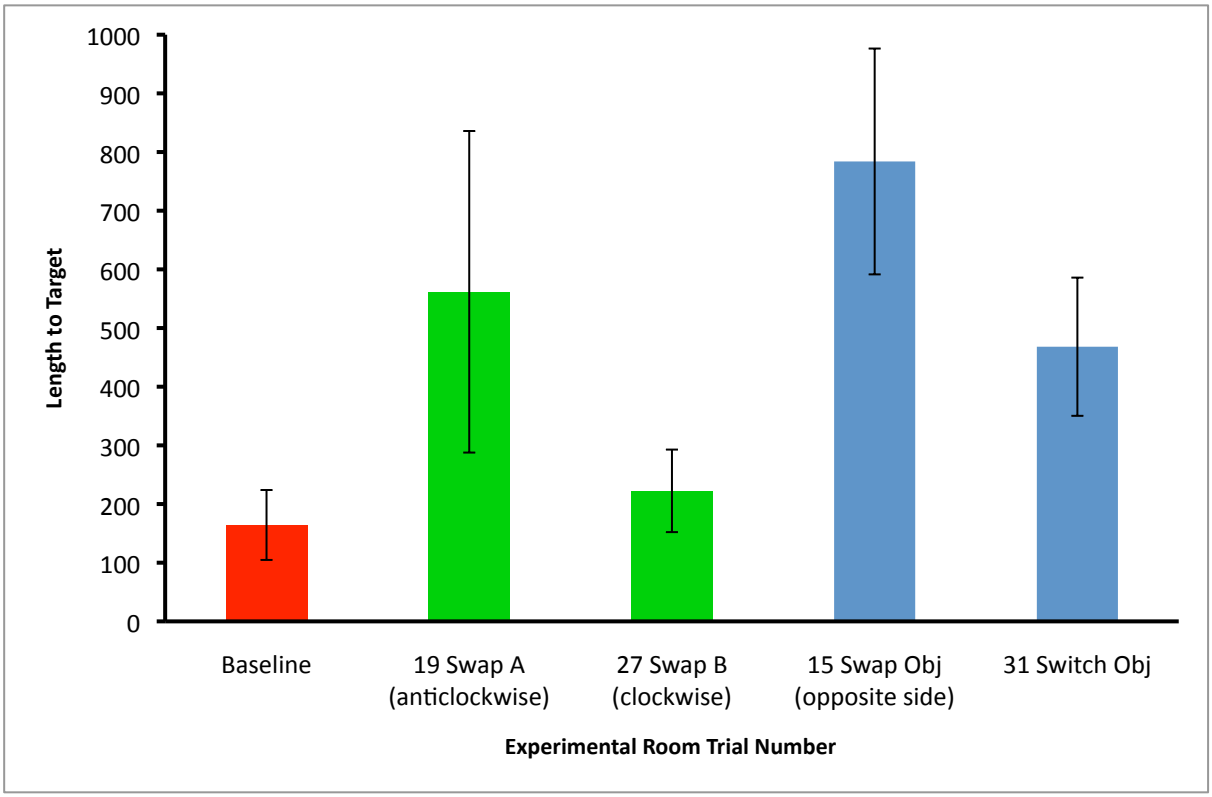

Figure 11. Average path length to target for swap trials. Error bars indicate standard error of mean with 95% confidence interval.

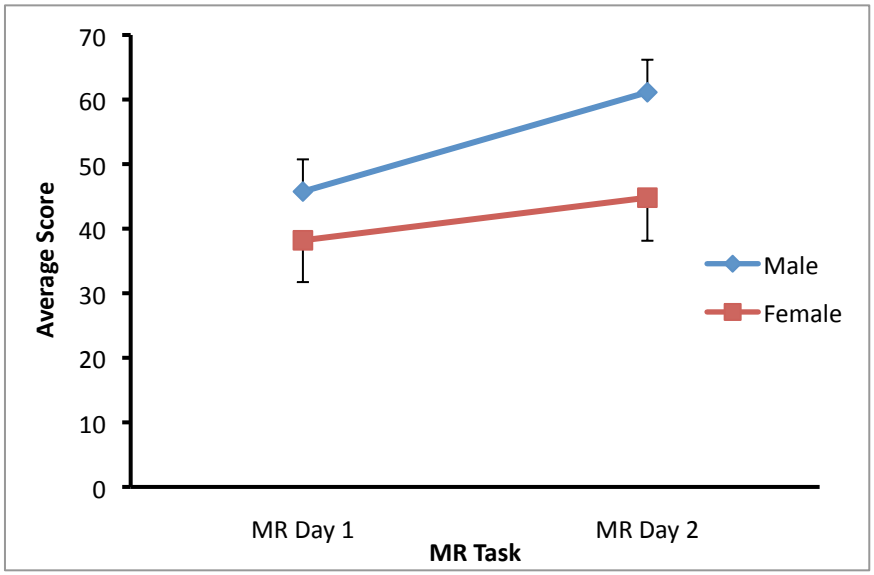

Figure 13. Stress effects on MR performance. Descriptive statistics for MR task: Day 1: males $M = 45.75$ ($SD = 14.10$), females $M = 36.75$ ($SD = 12.95$); Day 2: males $M = 61.12$ ($SD = 14.26$), females $M = 42.00$ ($SD = 13.34$). Error bars indicate standard error of mean with 95% confidence interval.

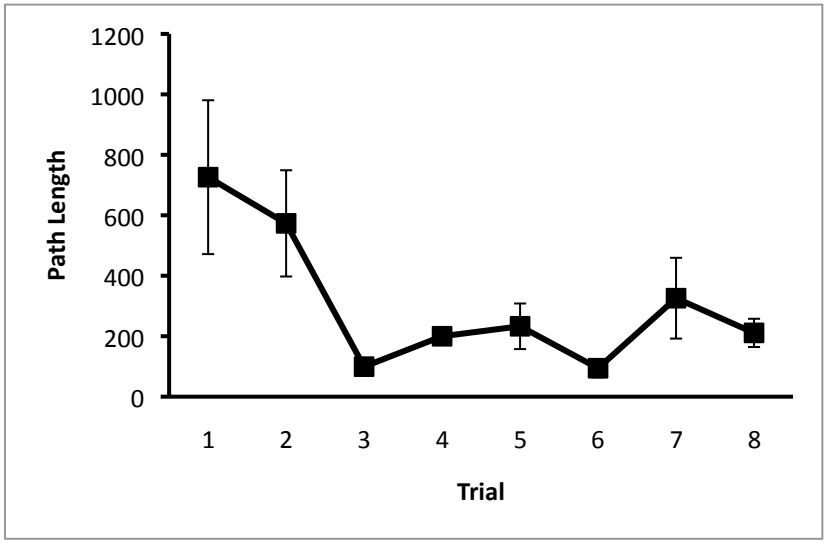

Figure 11. Average path length target over Day 2 acquisition trials. Error bars indicate standard error of mean with 95% confidence interval.

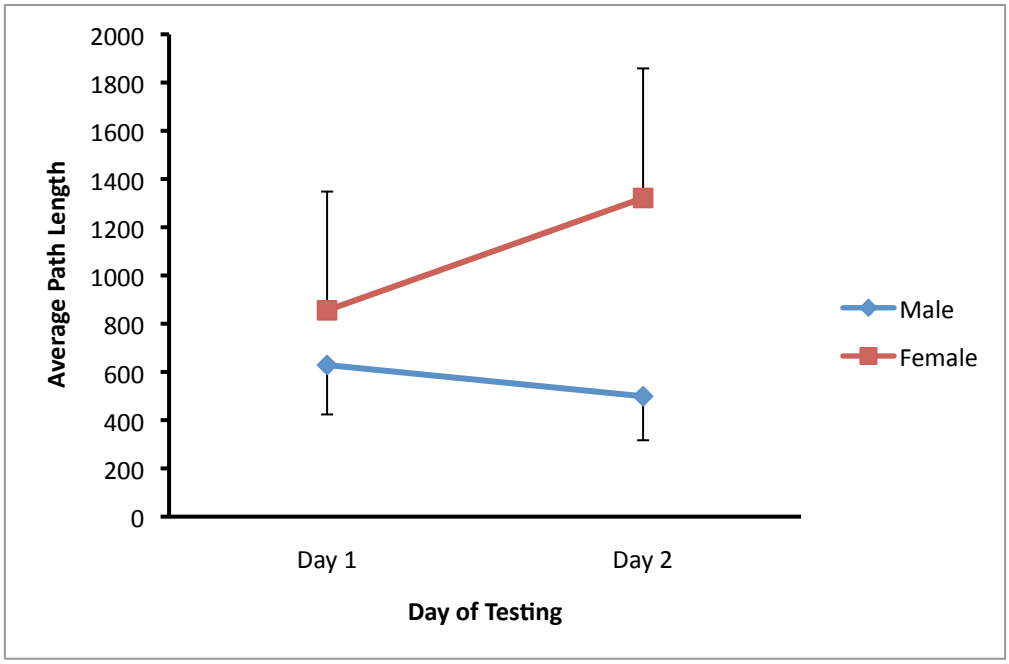

Figure 12. Interaction effect of object swap trial with sex. Error bars indicate standard error of mean with 95% confidence interval.

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