Stress-Induced Sex Differences in Spatial Navigation

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AMDALY001

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University of Cape Town
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COMPULSORY DECLARATION

This work has not been previously submitted in whole, or in part, for the award of any degree. It is my own work. Each significant contribution to, and quotation in, this dissertation from the work, or works, of other people has been attributed, and has been cited and referenced.

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<th>Full Form</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormones</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic Nervous System</td>
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<tr>
<td>AVP</td>
<td>Arginine Vasopressin</td>
</tr>
<tr>
<td>BDI-II</td>
<td>Beck Depression Inventory-II</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CG</td>
<td>Computer-Generated</td>
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<tr>
<td>CPT</td>
<td>Cold-Pressor Test</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-Releasing Hormone</td>
</tr>
<tr>
<td>CRT</td>
<td>Card Rotations Test</td>
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<tr>
<td>EMM</td>
<td>Estimated marginal means</td>
</tr>
<tr>
<td>FFST</td>
<td>Fear Factor Stress Test</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal</td>
</tr>
<tr>
<td>HPCSA</td>
<td>Health Professions Council of South Africa</td>
</tr>
<tr>
<td>MR</td>
<td>Mineralocorticoid</td>
</tr>
<tr>
<td>NA</td>
<td>Negative Affect</td>
</tr>
<tr>
<td>PA</td>
<td>Positive Affect</td>
</tr>
<tr>
<td>PANAS</td>
<td>Positive and Negative Affect Schedule</td>
</tr>
<tr>
<td>SECPT</td>
<td>Socially Evaluated Cold Pressor Test</td>
</tr>
<tr>
<td>SMTT</td>
<td>Star Mirror Tracing Task</td>
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<tr>
<td>SNS</td>
<td>Sympathetic Nervous System</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SRPP</td>
<td>Student Research Participation Program</td>
</tr>
<tr>
<td>S-R</td>
<td>Stimulus response</td>
</tr>
<tr>
<td>STAI</td>
<td>State-Trait Anxiety Inventory</td>
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<tr>
<td>TSST</td>
<td>Trier Social Stress Test</td>
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<tr>
<td>UCT</td>
<td>University of Cape Town</td>
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<tr>
<td>VE</td>
<td>Virtual Environment</td>
</tr>
<tr>
<td>VU-AMS</td>
<td>Vrije Universiteit Ambulatory Monitoring system</td>
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<tr>
<td>Allocentric wayfinding strategy</td>
<td>formation of spatial memories using inter-relationships between both perceived and non-visible cues to navigate.</td>
</tr>
<tr>
<td>Cognitive-map based navigation</td>
<td>navigation based on acquired mental representation of one’s environment (i.e., locations and attributes that one interacts with)</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>a group of steroid hormones produced in the adrenal cortex that plays a role in the body’s control of homeostasis (e.g., cortisol in humans and corticosterone in rats)</td>
</tr>
<tr>
<td>Cue</td>
<td>navigation aiding information in the environment</td>
</tr>
<tr>
<td>Distal cue</td>
<td>information that is not directly associated with the object</td>
</tr>
<tr>
<td>Egocentric wayfinding strategy</td>
<td>formation of spatial memories using the organism’s position in relation to perceptions of the environment.</td>
</tr>
<tr>
<td>Gradient cue</td>
<td>geometrical information like angles and shapes</td>
</tr>
<tr>
<td>HPA-axis</td>
<td>a response involving the stimulation of three endocrine glands and feedback between them</td>
</tr>
<tr>
<td>Landmark cue</td>
<td>a local object that can be used to deduce position</td>
</tr>
<tr>
<td>Mental Rotation</td>
<td>ability to maintain an active representation of all the parts, and interrelations of all the parts, in order to manipulate objects mentally.</td>
</tr>
<tr>
<td>Proximal cue</td>
<td>local information that must spatially co-occur with the goal object</td>
</tr>
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</table>
Route knowledge: any information pertaining to the environment that is acquired as a result of physical navigation through the environment (e.g., stimulus-response learning).

Spatial navigation: ability to direct oneself to a set destination in the most practical way possible, and recognize when one is approaching said destination.

Stimulus-response learning: forming memory for a series of turns from different and discrete points in the environment (see route knowledge).

Stressor: a circumstance that threatens one’s physical integrity or psychological well-being.

Survey knowledge: any information obtained through an external perspective, such as an aerial or map-like view, allowing direct access to the global spatial layout.

Wayfinding: encompasses all of the ways in which people (and animals) orient themselves in physical space and navigate from place to place.
Abstract

Certain forms of spatial navigation are centered, neuroanatomically, on the hippocampal formation, a brain structure vulnerable to increased levels of the stress hormone cortisol. Although empirical studies have identified a substantial sex difference, in favor of males, on laboratory-based spatial navigation tasks, little research has investigated whether, and how, these sex differences manifest under conditions of psychological or physiological stress. The current study aimed to resolve some of the inconsistencies in the literature, and to investigate the relations between stress and performance in male and female participants. The current study followed a mixed quasi-experimental pretest-posttest design in which men ($n = 23$) and women ($n = 23$) were tested on two separate days (the first day under control conditions and the second under stressful conditions). I utilized a novel stress induction paradigm (the Fear Factor Stress Test) that would produce both hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in men and women, and created a spatial navigation virtual environment task that would allow for cue usage of both landmarks and gradients. Participants also completed the Card Rotations Test as an assessment of their mental rotation abilities. I hypothesized that (a) men would perform better on spatial navigation tasks than women on Day 1 (i.e., the control condition) despite the availability of landmark cues, and (b) stress would affect spatial navigation performance in women more than in men. Results suggested that the stressor used was effective in eliciting appropriate responses in both men and women, however women showed smaller cortisol increases than men, relative to baseline. Regarding the navigation task, under unstressed conditions men showed a steeper learning curve than women in an unchanged environment, and performed better than women only when a proximal landmark cue was removed from the environment. Furthermore, findings suggested that acute psychosocial stress enhanced navigational performance in men, but impaired such performance in women. Regarding the mental rotation task, no sex
differences were observed under unstressed conditions; however, under the stressed condition men improved in their performance whereas women were relatively unaffected. It appears then that men’s spatial ability might, under particular conditions and on particular tasks, be enhanced following exposure to a stressor. Furthermore, the pattern of results observed in the spatial navigation task suggests that the types of navigation-aiding cues in an environment (as well as location of these cues relative to the target) play a significant role in eliciting sex differences in navigational performance following exposure to a psychosocial stressor.

*Keywords*: stress, spatial navigation, sex differences, FFST, CG Arena, cortisol
We use cognitive navigation systems constantly in our daily lives. These systems allow us to move from one destination to another, either by following familiar routes or by learning 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 impairs performance on some, but not all, spatial navigation tasks. This impairment might manifest differently in men and women, however, given that there are sex differences in spatial abilities. The proposed study aims to add to the current knowledge about stress-induced sex differences in spatial navigation.

**Literature Overview**

**Spatial Navigation: Definition and types**

Spatial navigation is the ability to (a) direct oneself to a set destination in the most practical way possible, and (b) recognize that one is approaching said destination (O’Keefe & Nadel, 1978). Even from a purely cognitive perspective, and ignoring neural substrates, 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, 2009). *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 internal representation of the environment and in the resulting execution of movement within it (Garden, Cornoldi, & Logie, 2002; Gramann, Müller, Eick, & Schönebeck, 2005).
O’Keefe and Nadel (1978) distinguished between cognitive systems responsible for map-guided and stimulus-response forms of spatial navigation. Their theory and subsequent empirical work by others (e.g., Banquet, Gaussier, Quoy, Revel, & Burnod, 2005; Boccia, Nemmi, & Guariglia, 2014; Münzer, Zimmer, Schwalm, Baus, & Aslan, 2006) has led to the proposition that spatial knowledge is divided into two main types: route and survey. To understand the different ways in which one accumulates these types of knowledge, the concepts of different wayfinding strategies need to be understood. In general, wayfinding strategies allow organisms to orient themselves in an environment, as well as to gather and encode new spatial information about the environment. Hence, information from the environment and schemata previously encoded within the brain are integrated to form spatial knowledge (Roche, Mangaong, Commins, & O’Mara, 2005).

Two main types of wayfinding strategies allow one to navigate within an environment: egocentric and allocentric. An egocentric wayfinding strategy refers to the use of spatial memories involving the organism’s position in relation to perceptions of the environment, which alter significantly each time movement takes place. Such a strategy might involve using memories about various landmarks in order to direct oneself to a location from a particular starting point (Gramann et al., 2005; Hund & Minarik, 2006). An allocentric wayfinding strategy, in contrast, refers to the use of spatial memories involving inter-relationships between both perceived and non-visible cues to navigate; these cues allow the organism to anchor its cognitive map. Such a strategy might involve using memories about the geometric relationships between different locations (Boccia et al., 2014; Gramann et al., 2005).

Both these strategies are required to facilitate the acquisition of route and survey spatial knowledge. Roche et al. (2005, p. 624) define route knowledge as “any information pertaining to the environment that is acquired as a result of physical navigation through the
environment.” Hence, such knowledge includes visual and auditory information, as well as information that pertains to distances, angles, and proprioception. In contrast, survey knowledge is defined as any information obtained through “an external perspective, such as an aerial or map-like view, allowing direct access to the global spatial layout” (Roche et al., 2005, p. 625). So, to build survey knowledge the construct of the environment is formed independent of the location of the viewer (Lawton, 1994; Gramann et al., 2005), and information about the relations between points in the environment is inferred through the viewer’s exploration within the environment (i.e., as the viewer’s knowledge of route-based information accumulates, s/he is able to construct, via inferential reasoning, an allocentric map composed of survey knowledge). Thus, the following general conclusions can be made regarding the two types of knowledge involved in navigating through an environment: route knowledge depends on an egocentric wayfinding strategy (as well as path integration; see Roche et al., 2005), whereas survey knowledge depends on an allocentric wayfinding strategy.

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, centered on the hippocampal formation but also including other medial temporal lobe structures and regions of the parietal and frontal lobes (Boccia et al., 2014; Bohbot, Iaria, & Petrides, 2004; Burgess, Maguire, Spiers, & O’Keefe, 2001; Roche et al., 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 utilize cognitive map-based navigation (Astur, Tropp, Sava, Constable, & Markus, 2004; Banner, Bhat, Etchamendy, Jioober, & Bohbot, 2011). In contrast, the caudate nucleus is linked to stimulus-response learning, such as memory for a series of turns from different and discrete points in the environment (Baumann, Chan, & Mattingley, 2010; Miyoshi et al., 2012).

**Sex Differences in Spatial Navigation**

Some empirical studies regarding performance on spatial navigation tasks have identified a substantial sex difference in favor of men (e.g., Barkley & Gabriel, 2007; Picucci, Caffo, & Bosco, 2011). For instance, Astur, Ortiz, and Sutherland (1998) showed that men were significantly faster than women at finding a platform in a human analog of the Morris water task (Morris, 1981, 1984). Similarly, Lövdén et al. (2007) demonstrated that men covered shorter distances and were more accurate than women in their navigation of a virtual maze-like museum projected in front of a treadmill.

Such findings are not reported consistently, however. Coluccia and Louse’s (2004) review noted that some studies find no sex difference in the performance of men and women on spatial navigation tasks. For instance, Lawton, Charleston, and Zieles (1996) showed that, in a real environment route-reversal task, the speed and accuracy of men and women was not significantly different. Furthermore, in a study of brain activation during navigation in a virtual environment (VE), Sneider, Sava, Rogowska, and Yurgulen-Todd (2011) found no sex differences in task performance, even though there were sex differences in regional brain activation during learning.

A crucial point of focus in attempts to explain these sex differences in navigational performance (as well as the inconsistency in results of studies examining such sex differences) is that they may be attributable, at least in part, to the types of cues available during navigation tasks (Barkley & Gabriel, 2007; Picucci et al., 2011; Sandstrom,
Kaufmann, & Heuttel, 1998). Specifically, although research generally shows that men solve spatial problems more quickly than females do, it appears that the two sexes rely on different strategies, using different environmental cues, in their attempts to solve the same problem (Chamizo, Rodríguez, Torres, Torres & Mackintosh, 2014). For example, in a study that examined differences in wayfinding strategies and navigational support design, Chen et al. (2009) found that men reported a preference for utilizing an allocentric wayfinding strategy (i.e., they were more likely to rely on geometric information) whereas females reported that they were more likely to adopt an egocentric wayfinding strategy (i.e., they were more likely to rely on information based on landmarks; see also Lawton, 1994).

Thus regarding navigation-aiding cues, there are several features that appear to influence performance differences in males and females. Two of the main features that tend to be manipulated in spatial navigation research are (a) the distance from the cue to the organism (i.e., whether the cue is proximal to or distal from the organism), and (b) the physical features of the cue (i.e., whether it is a landmark cue or a gradient cue). A proximal cue involves “local [cues that] must spatially co-occur with the goal object,” whereas distal cues are not “directly associated with the goal object” (Carman & Mactutus, 2001, p. 333). A gradient cue “is too distant to provide accurate positional information but can nonetheless provide an accurate direction” (i.e., it contains geometrical information, like angles and shapes), whereas a landmark cue “is a local object that can be used to deduce position from the relative distances and positions of objects within an array” (L. Jacobs & Schenk, 2003, p. 288). Throughout the literature, these terms are often used interchangeably: Distal and gradient cues are regarded as a single type of cue, and so are proximal and landmark cues.

In most experimental navigation tasks, there is a preponderance of distal gradient cues (in fact, in some tasks, those are the only cues; Maguire, Burgess, & O’Keefe, 1999). In studies using those tasks, males tend to perform better than females (see, e.g., Astur et al.,
It appears, then, that 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 does not feature cues that females prefer to utilize in aid of their navigation, then skewed results in favor of males ought to be expected.

Evidence for this proposition is presented in many human and rodent studies. An elaboration of the finding by Astur et al. (1998), using a similar VE navigation task, demonstrated that although both men and women were capable of learning a target location, their efficacy was altered when the availability of distal cues was changed: Women performed better when landmark cues were made available compared to when they were not (Sandstrom et al., 1998). In a series of rodent studies, Rodríguez, Chamizo, and Mackintosh (2011) showed that, when learning spatial relationships to a target, the preferred sources of information in males were geometric cues, whereas females showed a greater preference for landmark cues.

Recently, a series of rodent studies has added a further nuance to the literature of sex-based cue preferences in spatial navigation. Those studies suggest that preference for landmark cues over geometric cues in females, but not in males, may be modulated by the nature or salience of the landmark. That is to say, when viewing the landmark from different perspectives, females only show a preference for it if its properties do not change when it is approached from different directions (Chamizo et al., 2014; Rodrigo, Gimeno, Ayguasanosa, & Chamizo, 2014; Tores, Rodríguez, Chamizo, & Mackintosh, 2014). In one of those studies, Tores et al. (2014) showed that female preference for a landmark cue over a geometric cue was evident when the cue was a plain white cone, but disappeared when it was a white pyramid. This effect was also observed when the comparison was made between a plain cone
and one divided into four different patterns: Females preferred the former, but not the latter, over geometric cues, indicating that this preference for landmark cues is only present when the cue looks the same regardless of the perspective from which it is approached.

Finally with regard to the literature on sex differences in navigation-aiding cues, there are studies suggesting that even when bias in distal versus proximal, or landmark versus gradient/geometric, cue availability is controlled, males still outperform females on spatial navigation tasks. For instance, Picucci et al. (2011) demonstrated, in a VE task that contained both geometric and landmark information, that women covered more distance and spent more time before correctly reaching the target.

Several other factors affect the results of studies investigating sex differences in spatial navigation. These factors include spatial anxiety/confidence and sex-steroid levels (Driscoll, Hamilton, Yeo, Brooks & Sunderland, 2005; Gabriel et al., 2011; Phillips & Silverman, 1997; Picucci et al., 2011). ‘Confidence’ might be defined as an individual’s reflection about the expectations she has about her abilities. With regard to spatial abilities, confidence is lower in women than in men (Moe & Pazzaglia, 2010). This lack of confidence seems to act as a de-motivator and contributes to under-performance on spatial tasks. For instance, in their study assessing various factors that contribute to sex differences in navigational ability, Picucci et al. (2011) found that women reported lower spatial confidence than men. They also demonstrated that women become more skeptical of their spatial skills when they are discouraged by lack of success, which is associated with a more confusing pattern of search for a target.

Furthermore, estrogen and testosterone appear to have an impact on spatial ability. Research has shown that phase of the menstrual cycle, as well as testosterone levels, may modulate sex differences in spatial performance (Andreano, Arjomandi, & Cahill, 2008; Hampson, 1995; Hausmann, Slabbekoorn, van Goozen, Cohen-Kettenis, & Güntürkün,
Specifically, females tend to show better performance when lower levels of estrogen are present, and males tend to show better performance when testosterone levels are higher. Although even low levels of estrogen are better than none at all, when high levels (such as those seen during the luteal phase of the menstrual cycle) are present women perform more poorly than during any other phase of the menstrual cycle (McCormick & Teillon, 2001).

**Effects of Stress on Cognitive Functioning**

The perception of negative (i.e., threatening) environments elicits a stress response in all living organisms. This response involves many different physiological changes within the organism, all in the service of improved adaptive functioning (Herman & Cullinan, 1997). During the adaptive response to a negative change in environment, attention is enhanced, allowing major brain processing to be directed toward a focus on the perceived threat (Tsigos & Chrousos, 2002).

In humans, the experience of a stressor\(^1\) results in the activation and coordination of physiological responses that involve autonomic, endocrine, metabolic, and immune systems (Kemeny, 2003; Lupien, McEwen, Gunnar & Heim, 2009). Two of the main biological systems involved in this response are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. Each can be activated by a variety of stressors. The ANS response involves the activation of the sympathetic nervous system (SNS), which is responsible for increasing, via the release of adrenaline into the bloodstream, heart rate and blood pressure when the organism is under stress. The HPA-axis response begins in the paraventricular nucleus of the hypothalamus. The experience of a stressor triggers the release of corticotrophin-releasing hormone (CRH), as well as arginine vasopressin (AVP), by the hypothalamus. Subsequently, the pituitary gland is stimulated to increase the amount of

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\(^1\)A stressor can be defined as a circumstance “that threaten[s] a major goal, including the maintenance of one’s physical integrity (physical stressors) or one’s psychological well-being (psychological stressors)” (Kemeny, 2003, p. 124).
circulating adrenocorticotropic hormones (ACTH), which is a key regulator of corticosteroid secretion by the adrenal cortex.

Corticosteroids contribute to physiological homeostasis by regulating and terminating the stress response (de Kloet, Oitzl, & Joëls, 1999; Kemeny, 2003; Lupien et al., 2009; Tsigos & Chrousos, 2002). The two primary receptors for corticosteroid hormones (corticosterone in rodents and cortisol in humans) are mineralocorticoid (MR) and glucocorticoid (GR) receptors. These are found in abundance in the hippocampus, amygdala, and prefrontal cortex, and thus increased cortisol levels affect functioning in these areas (de Kloet et al., 1999; Herman & Cullinan, 1997; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Putman & Roelofs, 2011). Some research suggests that increased levels of cortisol affect functioning in an inverted-U manner, such that small increases enhance functioning but large prolonged increases impair it (Lupien et al., 2009; Schilling et al., 2013).

The effects that the experience of stressors have on cortisol levels and, consequently, on cognitive functioning have been studied widely over the past half-century. It is now well known that in addition to triggering or exacerbating certain psychiatric disorders, stress dysregulates emotional states and can have negative effects on cognitive processes such as memory, executive functioning, and visual-spatial functioning (de Kloet et al., 1999; Dickerson & Kemeny, 2004; Lupien et al., 2009).

Within the literature of stress-induced effects on cognitive processes, many studies have investigated the effects of stress/elevated cortisol on the encoding, consolidation, and retrieval of neutral declarative memories. Elevated stress/cortisol appears to affect each of these processes slightly differently (Buchanan & Lovallo, 2001; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Smeets, Giesbrecht, Jelicic, & Merkelbach, 2007; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). An unpublished meta-analysis reviewed the effects of acute psychosocial stress on verbal declarative memory (Astor & de
Villiers, 2014). The authors noted that when cortisol levels are raised (after TSST exposure) during the encoding phase there is either a negative effect or no effect at all on memory performance; when raised during the consolidation phase, there is either a facilitating, impairing or no effect on memory performance; and, when raised during the retrieval phase, there are either no or negative effects on memory performance. Astor and de Villiers speculated that this inconsistency in the literature might be due, in part, to the lack of a consensus definition and operationalization of declarative memory. Such lack of consensus introduces multiple potential confounding variables that can influence the results obtained; for instance, their analyses suggested that using just delayed recall as a measure of declarative memory, as opposed to immediate recall/delayed recall/recognition, allows for less within-study confounds).

Another important area of interest in this literature is the effect that the experience or perception of a stressor has on executive functioning. Numerous studies have investigated the effects of elevated stress/cortisol on decision making and working memory, in particular. Empirical studies generally suggest that the experience of stress affects decision-making processes negatively, leading to more risk-taking behavior (Preston, Buchanan, Stansfield, & Bechara, 2007; Starcke & Brand, 2012; van den Bos, Harteveld, & Stoop, 2009). Similarly, previous research suggests that elevated cortisol levels generally have adverse effects on both accuracy and speed of performance on working memory tasks (Lupien et al., 2007; Schoofs, Preuß, & Wolf, 2008).

According to Dickerson and Kemeny (2004), only certain types of laboratory-based stressors lead to the typical physiological responses (and hence the cognitive consequences) outlined above. Specifically, they suggest that to increase the chances of eliciting substantial increases in cortisol levels, a laboratory-based psychosocial stressor must feature (a) a
motivated performance task, (b) relative uncontrollability of task outcome, and (c) the presence of social evaluation.

One psychosocial stressor that incorporates these three criteria is the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). The procedure of the TSST involves telling the participant that they are to prepare for a job interview. The participant is then allowed a short preparation period, which is followed by a free speech and mental arithmetic task lasting 5 minutes each (motivated performance tasks). The performance of these two tasks is conducted in front of an audience (whom the participants believe to be managers specially trained to monitor nonverbal behavior). They are under the impression that they are being recorded by a video camera and tape recorder to monitor their behavior. Should the participants complete their speech prior to the end of the time limit, they are then prompted with a series of standardized interview-like questions. Furthermore, if an error is made during the mental arithmetic task, the participant is asked to start from the beginning. These latter components incorporate both the relative uncontrollability of the task as well as the presence of social evaluation. Using all three of these criteria makes the TSST one of the most effective psychosocial stressors, and thus also one of the most commonly used (see, e.g., Dickerson & Kemeny, 2004; Giles, Mahoney, Brunyè, Taylor, & Kanarek, 2014; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Putman & Roelofs, 2011; Schoofs et al., 2008; Smeets et al., 2007).

There are, however, other factors that can influence whether, and by how much, a stressor increases cortisol levels. For example, under natural conditions cortisol has a diurnal cycle, with high levels evident in the early morning and a continued decline throughout the day. Therefore, pre-stress levels of cortisol will be higher if a stressor is experienced earlier in the day (i.e., in the morning), and will be lower if the stressor is experienced later in the day (i.e., afternoon or evening). Hence, the magnitude of the cortisol response to the stressor
will be smaller earlier in the day, as it would reach ceiling sooner (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004).

Additionally, there appears to be a sex difference in response to stressors. This difference arises, at least in part, because men and women respond differently by different environmental events. Men respond more to achievement (or challenge) stressors (i.e., those that involve needing to prove intellectual or physical superiority), whereas women respond more to social stressors (i.e. those that involve interpersonal concerns; Kudielka, Hellhammer, & Wust, 2009; Stroud, Salovey, & Epel, 2002). For example, because the TSST is more of an achievement than an interpersonal social stressor, it tends to produce a greater response in men than in women (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008; Kudielka, Buske-Kirschbaum et al., 2004).

Furthermore, previous studies investigating HPA-axis activity following the experience of stress have shown that the modulating role of the female menstrual cycle must also be taken into account (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). 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, the magnitude of their cortisol response is dampened significantly (Andreano et al., 2008; Conrad et al., 2004; Tsigos & Chrousos, 2002). Considering that female performance on navigation tasks seems to be worst during the luteal phase, this may indicate that females are often at a disadvantage when the effects of stress on navigation are tested during this phase. The reason for this is that their performance will be negatively affected by elevated levels of both cortisol and estrogen during testing.

Effects of Stress on Spatial Navigation

As mentioned previously, the hippocampal formation is critical for certain forms of spatial navigation (Astur et al., 2004; Burgess, Maguire, & O’Keefe, 2002; Roche et al.,
Specifically, it appears that a fully functional hippocampus provides a necessary component of the neural substrates underlying allocentric wayfinding strategies. When hippocampal function is disrupted, egocentric wayfinding strategies remain usable and become preferred (Sneider et al., 2011). Hence, one might predict that the experience of stress and a subsequent increase in cortisol levels would disrupt map-based navigation (i.e., navigation utilizing the hippocampus) but leave intact route-based navigation (i.e., navigation utilizing extra-hippocampal areas, such as the caudate nucleus).

Most animal studies focusing on this question have found results consistent with this prediction (see, e.g., Beiko, Lander, Hampson, Boon, & Cain, 2004; Cazakoff, Johnson, & Howland, 2010; Hölscher, 1999; Kim et al., 2007; Sandi et al., 2005). For instance, Snihur, Hampson, & Cain (2007) found that, when corticosterone levels were elevated, spatial ability in the Morris water task declined significantly in both male and female rats. Hence, it appears that the extreme sensitivity of the hippocampus to the experience of stress causes significant disruption in the ability to utilize cognitive map-based navigational strategies (i.e., to use hippocampal-dependent navigation; Sandi et al., 2005).

Organisms experiencing such elevations are not necessarily without navigational recourse, however. Rather, they appear to abandon map-based strategies and adopt route-based strategies (Elliot & Packard, 2008; Kim, Lee, Han, & Packard, 2001; Packard & Wingard, 2004). For example, Schwabe, Schachinger, de Kloet, and Oitzl (2010) demonstrated that stimulus-response (S-R) strategies (which are dependent on the caudate nucleus) are less susceptible to stress, and thus when rats experienced a stressor they showed a preference for such strategies over map-based ones. The task utilized in their study could be completed by the use of either navigation strategy, but when corticosterone levels were elevated, rats appeared to compensate for the dysfunction of the hippocampal-dependent system by reverting to S-R strategies.
Similarly, Schwabe et al. (2007) showed that, in humans, the use of map-based navigational strategies declines when cortisol levels are raised (see also Schwabe, Oitzl, Richter, & Schachinger, 2009). In their study Schwabe et al. (2007), using a 3-D spatial learning task, showed that exposure to the TSST before task completion was associated with increased use of an S-R strategy and consequent decreased use of more spatially-based strategies.

However, some studies have not confirmed the prediction that increased levels of cortisol will disrupt map-based navigation (see, e.g., Kitraki, Kremmyda, Youlatos, Alexis, & Kittas, 2004; Klopp, Garcia, Schulman, Ward, & Tartar, 2012; Meyer, Smeets, Giesbrecht, Quaedflieg, & Merkelbach, 2013; Richardson & Tomasulo, 2011). Some of these studies report no difference in performance following the experience of a stressor. For example, in a male-only rodent study, Faraji et al. (2013) found that restraint stress had no effect on spatial learning and memory in either the Morris water task or the ziggurat task (a dry-land task). Other studies report that the experience of acute stress has a positive impact on spatial navigation performance. For example, Duncko, Cornwell, Cui, Merikangas, and Grillon (2007) found that, in a men-only sample, performance on a virtual Morris water task improved after exposure to an acute psychosocial stressor.

Reasons for these differences in results are not postulated within the studies themselves. However, as Faraji et al. (2013) point out regarding inconsistent findings across animal studies, these inconsistencies 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. The Star Mirror Tracing Task (SMTT; Lafayette Instruments Corporation) and the cold-pressor test (CPT; Hines & Brown, 1932), as used by Richardson and Tomasulo (2011) 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 TSST (Kirschbaum et al., 1993), as used by Schwabe et al. (2007), 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. Hence, it appears that although stress hormones do affect map-based navigation, the effect the stress induction has on a subject is highly dependent on the level of increase in cortisol (Akirav et al., 2004; Kitraki et al., 2004).

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. However, even in those studies that have successfully increased cortisol/corticosterone (through pharmacological induction or via exposure to an HPA-axis activating stressor), there are still inconsistent results (see Cazakoff et al., 2010, for a review). Although most of these studies report that stress has a negative effect on spatial navigation (see, e.g., Sandi et al., 2005; Schwabe et al., 2007, 2009; Snihur et al., 2008), some report that there is no effect or that stress facilitates performance (Akirav et al., 2004; Klopp et al., 2012; Faraji et al., 2013;).

One possible reason for the existence of these inconsistencies emerges from a study conducted recently by Meyer and colleagues (2013). They reported that those participants who could be characterized as “low cortisol responders” (i.e., those whose cortisol levels were raised only a small amount following exposure to a stressor) showed negative effects on navigation performance, whereas those who could be characterized as “high cortisol responders” (i.e., those whose cortisol levels were raised to a much higher level following exposure to a stressor) showed improved performance. Similarly, Kitraki et al. (2004) found that, in rodents, a lower level of corticosterone was associated with impaired performance on a spatial learning task, whereas a higher level was associated with performance equivalent to
that of a control group. Hence, it might be that the studies discussed above, in which cortisol/corticosterone was successfully increased, differed in the level of cortisol responses to the stress inductions administered. For example, the cortisol levels of participants in the Klopp et al. (2012) study increased more, relative to baseline, than did the levels of participants in the Schwabe et al. (2007) study. Consistent with the argument being proposed here, participants in the Schwabe et al. study showed negative effects on navigational performance, whereas participants in the Klopp et al. study showed no effects of performance.

**Stress-Induced Sex Differences in Spatial Navigation**

Although 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, Conrad et al. (2004) found that acute stress (in their case, the product of a restraint paradigm) equally increased serum corticosterone levels in both sexes, but that subsequent spatial performance was impaired in male rats but enhanced in female rats. They further examined the effect of phase of estrous cycle in females and found that, despite females in the pro-estrous phase having higher levels of corticosterone than those in the estrous phase, there were no significant differences in spatial performance. Hence, stress affected females equally, independent of the phase of estrous cycle. This set of findings may imply that factors other than corticosterone increase facilitated female performance.

Richardson and Tomasulo (2011) 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 SMTT. They did note, however, that participants had slower reaction times after stress induction, and that men were more accurate than women. 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 (as mentioned previously, the SMTT only appears to affect ANS activity). However, Klopp et al. (2012) found similar results to this, utilizing the TSST to induce acute stress, and a virtual analog of the Morris water task as a measure of navigation performance.

Studies that utilized a stress induction method that reliably affects HPA-axis activity (and therefore hippocampal activity) are limited and have produced inconsistent results. K. Thomas, Laurence, Nadel, and Jacobs (2010) found that exposure to the TSST increased sex differences in spatial navigation. Specifically, women exposed to the stressor performed poorly, relative to no-stress controls, on map-based tasks; men 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). In contrast, Guenzel, Wolf, and Schwabe (2014) found that exposure to the socially evaluated cold pressor test (SECPT) did not differentially affect spatial learning and memory in a VE task in men and women, however women’s performance was adversely affected in a real-world navigation task.

The differences in results reported by K. Thomas et al. (2010), Gabriel et al. (2011), and Guenzel et al. (2014) 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 by administering a series of public speaking and mental arithmetic tasks, whereas the CPT involves placing the participant under physiological stress by requiring him to place his hand in a bucket of ice water. The SECPT, on the other hand, is an alternative version of the CPT in which the participant undergoes physiological stress while being observed by an experimenter and videotaped. Although all three methods produce reliable stress responses (von Dawans, Kirschbaum, & Heinrichs, 2011; Schwabe et al., 2008), only the TSST and SECPT raise cortisol levels reliably, and subsequently impair performance on
hippocampal-dependent tasks (Dickerson & Kemeny, 2004; Kirschbaum et al., 1993; Schoofs et al., 2007; Schwabe et al., 2008).

Furthermore, the tasks used to measure spatial performance differed across these studies. K. Thomas et al. (2010) used a VE task, based on the Morris water task, 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. The VE task used by Guenzel et al. (2014) was a variation of the radial arm maze normally utilized in assessing striatum-dependent stimulus-response based navigation. Those researchers altered the task so that successful navigation was achieved only via hippocampal-dependent spatial learning (thus ruling out any possibility of stimulus-response learning). During this task, participants were required to locate three different objects that were placed within one of eight radial arms. Again, the environment in this task only featured distal cues. 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. 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 in their original processing of the scene.

Although cue types and strategies play an important role in successful performance on the tasks featured in these three studies, it is difficult to compare their results accurately because two assess navigation directly while the other does not. Furthermore, the two navigational studies made available only distal cues, which were either landmark or gradient in nature. With the exception of these three studies, there is no published research on sex differences in the effects of stress on spatial navigation in humans.
Summary, Rationale, and Hypotheses

The review above 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 that are affected by increases in circulating cortisol. Another point is that there are notable methodological inconsistencies 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 affects hippocampal functioning (an area associated with map-based, but not landmark-based, spatial navigation strategies).

One primary aim of the current study was to improve upon the methodology of the studies conducted by K. 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 centers on the fact that neither of those studies took or reported physiological (e.g., cortisol and heart rate) measures. Hence, neither study provided psychophysiological or neuroendocrinological confirmation of a provoked physiological stress response. Although Guenzel et al. (2014) did report physiological measures, the stress induction paradigm used (i.e., the SCEPT) is less effective than, for example, the TSST, in raising cortisol levels for long enough to affect subsequent tasks (Giles et al., 2014; Quaedflieg, Meyer, & Smeets, 2013). If one is to study the effects of raised stress on hippocampal-dependent, map-based spatial navigation in humans, one has to show that an experimental manipulation is effective in raising cortisol levels, and in raising them for a sufficient length of time.
A second, related, area of methodological improvement is that, if one is to study the effects of raised stress on spatial navigation in an ecologically valid manner, 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 men and women appear to react differently to stressors. 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 men and women.

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 K. 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 cue type over another, or whether, in fact, both types of cues were present in that environment. Furthermore, it appears that Guenzel et al. (2014) utilized only distal gradient cues (i.e., mountains in the background), and thus differential cue preferences were not analyzed in their study.

Hence, the overall purpose of the current study is 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 minimize 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 allows the participant to use either in the service of efficient navigation.

Appendix A describes a pilot study that helped the development of such a spatial navigation task. Results from the pilot study suggested there might be significant sex differences in performance on that task, and also identified the potential for differential cue preferences in men and women. Those pilot data, then, provided the platform for further investigation into stress-induced sex differences utilizing the current (i.e., improved over previous work) methodology.

Hence, the study described in this thesis built on theoretical and empirical work published previously, and on the results of the pilot study described in Appendix A. I tested these specific hypotheses in a sample of young adult men and women:

1. In an unstressed condition, men will perform better on spatial navigational tasks than women, despite the availability of landmark cues.

2. Stress will impair spatial navigational performance in both men and women, but women will be more impaired by disruptions in landmark cues than men.

Furthermore, the current study adopted a mixed quasi-experimental pretest-postest design, rather than the between-subjects designs typical of previous research in this field. This design allowed for baseline measures and for the effects of stress on each individual’s ability to be observed. In this way, I was able to rule out the effects of potentially confounding individual difference factors (e.g., stressor reactions) that often complicate interpretation of data in this field (Hegarty, Montello, Richardson, Ishikawa & Lovelace, 2006; Hegarty, Smallman, & Stull, 2012).
Methods

Design and Setting

The current study adopted a 2 x 2 mixed quasi-experimental pretest-posttest 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., relaxed or stressed). The outcome variables were derived from the participant’s scores on two spatial cognitive tasks: the Computer-Generated (CG) Arena and a mental rotation task. Each participant was tested on two separate occasions, over 2 consecutive days. The first day’s testing served as the relaxed/control condition; the second served as the stressed/experimental condition.

The study took place in two venues at the Department of Psychology at the University of Cape Town (UCT). One venue was a computer laboratory where cognitive testing and questionnaire completion took place. The second venue was the room where participants underwent the experimental manipulation. Two female postgraduate researchers met the participants at the venues to conduct the data collection. There was no systematic attempt to match researchers to participants.

Participants

We recruited 62 undergraduate students (32 women and 30 men), between the ages of 18 and 25 years old ($M = 19.80$, $SD = 1.67$). They were recruited from undergraduate psychology classes at UCT, using the Student Research Participation Program (SRPP), and from the wider university community, using notice boards and print advertisements. Those participants recruited via SRPP ($n = 54$, 32 women and 22 men) received course credit. Those participants recruited from outside the Department of Psychology ($n = 8$, all men) were entered into a prize-giving draw. Potential participants were notified via the SRPP website or via advertisements of the study’s availability, of the relevant inclusion and exclusion criteria, and of the email address that would allow them to sign up (see Appendix B).
**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) age younger than 18 or older than 25, (b) smoking, (c) presence of depression or anxiety, (d) the use of any steroid-based medication, and (e) a body mass index (BMI) of more than 30 or less than 18. 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 consistent with criteria used in previous research (e.g., du Plooy, Thomas, Henry, Human, & Jacobs, 2014; Schoofs et al., 2008; Schwabe & Wolf, 2010; Human, Thomas, Dreyer, Amod, Wolf, & Jacobs, 2013).

In addition to these factors, there is also a modulating effect of the female menstrual cycle on stress responses (Kudielka et al., 2009). It appears that when women are in the luteal phase, they exhibit similar cortisol response patterns to men; however, when they are in the follicular phase or on oral contraceptives, their responses are significantly dampened (Kirschbaum et al., 1999). I therefore excluded women who were using oral contraceptives. I did not, however, only test women in the luteal phase as we wanted to gain an idea of the reaction stress induces in a more general population (women in the luteal phase only represent a proportion of the female population at any one time). I noted the phase of menstrual cycle each female participant was in so as to aid data interpretation. Recording of menstrual cycle phase was initiated by asking female participants to notify me at the start of their next period following the day of stress induction. Thereafter, I identified which phase of their cycle they were in during testing by calculating backwards (e.g., less than 10-12 days prior to onset is the luteal phase).

**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 construct validity (Beck, Steer, & Brown, 1996; Dozois, Dobson, & Ahnberg, 1998; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; Watson, Clark, & Tellegen, 1988). Furthermore, their usefulness in characterizing South African individuals has been demonstrated in previously published research (see, e.g., Rieckert & Möller, 2000; Ward, Flisher, Zissis, Muller, & Lombard, 2001).

On each day, the experimental procedures took place between 14h30 and 18h30 to control for cortisol’s diurnal cycle (Kudielka, Schommer et al., 2004; Maheu, Collicut, Kornik, Moszowski & Lupien, 2005).

![Timeline for Day 1 study procedures. MR = Mental Rotation; CG Arena = Computer-Generated Arena. Timeline provided in 5-min intervals, ranging from 0 minutes (start) to 30 minutes (end).](image)

**Day 1.** Figure 1 illustrates the timeline of the Day 1 procedures, all of which took place in the computer laboratory. Each participant was tested by one of two female postgraduate psychology students.

The researcher met the participant at the laboratory, and immediately provided him/her with a consent form (see Appendix C). The participant read and signed the document, and was given the opportunity to ask questions about the study procedures and related matters. Thereafter, the researcher measured the participant’s weight and height in order to calculate his/her BMI. The researcher then asked the participant to complete the Beck Depression Inventory-II (BDI-II; Beck et al., 1996) and the Trait form of the State-Trait...
Anxiety Inventory (STAI; Spielberger et al., 1983). These measures were used to ensure that, across groups, all participants were experiencing similar levels of depression and anxiety in their everyday lives, and to screen out individuals who reported 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 researcher scored the BDI-II while the 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 administered the mental rotation and CG Arena tasks.

**Measures of spatial ability.** The Card Rotations Test (CRT) from the Kit of Factor-Referenced Cognitive Tests battery (Ekstrom, French, Harman, & Derman, 1976) assesses 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. For each page, the participant is given 3 minutes to complete as many of the 80 individual items as s/he can. This test is an internally consistent measure of mental rotation (Spearman-Brown coefficient = .86; Hogan, 2012).

For the purposes of this study, the test was split so that the problems on one page were presented on Day 1 and those on the other were presented on Day 2. I counter-balanced the
presentation order to remove effects of between-page differences. Appendix D shows statistical analyses conducted to test the hypothesis that there were no between-page differences in difficulty. As can be seen, the analysis detected no significant differences.

The CG Arena (W. Jacobs, Laurance, & Thomas, 1997; W. Jacobs, Thomas, Laurance & Nadel, 1998; K. Thomas, Hsu, Laurance, Nadel, & Jacobs, 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 VEs to real-world spaces, and learning in a VE allows humans to make accurate judgments about metrics in real space (Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002; Boccia et al, 2014; Jheng & Pai, 2009; Loomis, Lippa, Klatzky, & Golledge, 2002).

The researcher read the participant a set of standardized instructions describing (a) the general characteristics of the two Arena rooms to which s/he would be exposed (a waiting room and an experimental room; see Figure 2), and (b) how to navigate within those rooms using the arrow keys on the keyboard. The CG Arena was presented on a desktop personal computer by custom-designed software. The Arena is viewed from a first-person perspective and consisted of a circular wall that formed the boundaries of the arena (i.e., the Arena wall) in a square room. The parameters characterizing each CG Arena room were set by user-defined variables (see Table 1).
Table 1

CG Arena Room, Target, and Motion Parameters

<table>
<thead>
<tr>
<th>Room Parameters</th>
<th>Experimental Rooms</th>
<th>Waiting Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions</td>
<td>200 x 200 x 242.24</td>
<td>200 x 200 x 45</td>
</tr>
<tr>
<td>Arena wall radius</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Arena wall height</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Participant eye height</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Target Parameters(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>15x15</td>
<td>-</td>
</tr>
<tr>
<td>Motion Parameters(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Move quantum</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Turn quantum</td>
<td>5</td>
<td>5.16</td>
</tr>
</tbody>
</table>

Note. All measurements are in CG Arena units. If one equates the length of a stride (10 units) to 1 metre, then the experimental room dimensions were 20 x 20 x 24.2m, the arena wall was 0.5m high and had a radius of 10m, and the target was 1.5 x 1.5m. \(^a\)On Day 1, the target was located in the Northeast quadrant, the center of which was approximately 150 units from the West and South walls. On Day 2, it was located in the Southeast quadrant, the center of which was approximately 150 units from the West wall and 75 units from the South wall. \(^b\)The move quantum approximates the shift in the participant’s view of the Arena with each forward or backward movement. The turn quantum approximates the shift in participant’s view with each right or left movement.

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 color). The Arena wall featured a marble-like texture. Participants were able to view the floor, up to three walls and ceiling of the waiting room when they navigated against the Arena wall.

The walls of the experimental room featured a panoramic picture that ran across the four walls. The Day 1 and Day 2 experimental rooms featured different pictures (see Figure 2). The Arena wall within the experimental rooms featured a brick-like texture. The ceiling of these rooms was not visible and the floor was colorless and texture-less. Participants were able to view the floor and up to two walls when they navigated against the Arena wall.
Figure 2. Experimental Room Design. Panel (a) shows the layout of the waiting room. Panel (b) shows a visible target trial in an experimental room (the target is a bright green square on the floor of the room). Panel (c) shows the layout of the Day 1 experimental room. Panel (d) shows the layout of the Day 2 experimental room. Proximal landmark cues are visible on, or close to the floor of, the Day 1 and Day 2 experimental rooms.

In the experimental room, 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 experimental rooms were divided into four quadrants (Northeast, Southeast, Northwest, and Southwest) by lines not visible to participants (see Figure 3).
Figure 3. Top-down schematic of CG Arena layouts. Note that room walls are represented by the large square bordering the circle. The top line of that square represents the North wall. The Arena wall is represented by the circle within the large square. The Arena itself is divided into the four invisible quadrants. Panel (a) shows the Day 1 location of the hidden target (the larger square in the Northeast quadrant) and of two proximal landmark cues (the smaller squares in the Northeast and Southeast quadrants). Panel (b) shows the Day 2 location of the hidden target and of two proximal landmark cues.

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 colored square on the floor of the experimental rooms; see panel (b) of Figure 2). This 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 (W. Jacobs et al., 1997).
On both Day 1 and Day 2, participants completed 34 separate trials in the experimental room (see Table 2). On each of these trials, the target (a large blue square) remained 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 the room differed from Day 1 to Day 2.
Table 2

**CG Arena Experimental Room: Trial Descriptions**

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

*Note. 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.*
The eight acquisition trials were similar to those contained in previous Arena designs (see W. Jacobs et al., 1997, 1998). These trials served as a learning set, allowing encoding of the hidden target’s location. Previous CG Arena studies (e.g., Guenzel et al., 2014; Skelton, Bukach, Laurance, Thomas, & Jacobs, 2000) have shown that learning should occur within the first 8 trials provided all aspects of the environment (dimensions, proximal cues, relations between distal cues) remain unaltered. Results from the pilot study demonstrated that orderly place learning occurred during the acquisition trials of the CG Arena used in the current study (see Appendix A). These data indicate, then, that similar map-based spatial navigation strategies are used to find the hidden target in this panoramic room as they were in the rooms described in previous studies (Jacobs et al., 1997, 1998; Jheng & Pai, 2009; Skelton et al., 2000; K. Thomas et al., 2001, 2010).

As Table 2 shows, 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 not 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 relating to a well-learned target, whereas changing the relations among stimuli will disrupt this performance (Fenton, Arolfo, Nerad, & Bures, 1994; W. Jacobs et al., 1998; Suzuki, Augerinos, & Black, 1980).

At the end of the Day 1 session, the researcher reminded the participant about his/her session for the next day. The researcher also asked the participant 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 the upcoming session.
Day 2. Figure 4 illustrates the timeline of the Day 2 procedures. The same researcher met the returning participants at the laboratory in which the Day 1 session had taken place. Prior to beginning procedures, she reminded the participants of their ethical right to withdraw from the study at any time during the session.

To measure heart rate, I 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 normalize to the participants’ heart rate. The researcher then took average heart rate measurements from each
of the following periods: (a) a 2 minute baseline immediately following the stabilization period (HRB), (b) a 5 minute period directly following the stress manipulation (HR1), and (c) a 5 minute period 40 minutes after the manipulation ended (HR2).

Participants rated their current level of general negative affect at three different times using the Negative Affect (NA) scale from the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988). Within the PANAS, the NA scale, but not the Positive Affect (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 correlate strongly with fluctuations in NA scores (Watson et al., 1988). The three PANAS reports were given as follows: the first (a baseline measurement) shortly after entering the laboratory (NA_B); the second 5 minutes after the end of the stress manipulation (NA_1); and the third 45 minutes after the manipulation ended (NA_2).

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 features 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 (STATE_B); the second 5 minutes after the end of the stress manipulation (STATE_1); and the third 45 minutes after the manipulation ended (STATE_2).

The researcher 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 after the end of the stress manipulation (CORT_1); and the third 45 minutes after the manipulation ended (CORT_2). These swabs are an easy, effective, and non-intrusive way to collect salivary cortisol samples, 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 analyzed.

The researcher administered the second page of the CRT after the stress induction procedure, 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 et al., 2014). The FFST combines procedures from the TSST (Kirschbaum et al., 1993) and the CPT (Hines & Brown, 1932). The room in which the stressor occurred featured bright lights, a video camera, and a two-person (one man, one woman) judging panel. Both judges were undergraduate research assistants from the UCT Department of Psychology.

The researcher read a set of standardized instructions introducing the FFST. The participant was asked to imagine auditioning for *Fear Factor*, and was told that s/he must convince a panel of judges that s/he is a suitable person to be on the show. The researcher told the participant that the judges were behavioral health experts who would analyze the participant’s verbal and nonverbal behavior with the aid of a video recording.

The participant was told the audition would comprise three tasks: 1) a 5 minute free motivational speech as to why s/he should be a *Fear Factor* contestant; 2) a 5 minute mental arithmetic task, demonstrating the ability to think under pressure; and 3) a 2 minute submersion of the dominant arm in cold water, demonstrating the ability 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 had 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 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 UCT Codes for Research. I 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 C) that outlined the study clearly, detailing what would be expected of them, and noting that their confidentiality would be 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 the Day 2 session. The researcher informed all participants that they had not been videotaped or evaluated in any way during the ‘interview’ section of the FFST. 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 (see Table 3 for list of individuals excluded), or if s/he showed any signs of distress at the end of the Day 2 session, s/he was given the contact details for the UCT Student Wellness Centre so that counselling could be initiated, if so desired.

Data Management and Statistical Analysis

I used the Statistical Package for Social Sciences (SPSS) version 20 to analyze the data.

Outcome variables. I scored the mental rotation task following standard procedures (i.e., by subtracting the number of incorrect responses from the number of correct responses). Hence, the maximum total score, for each page, was 80. The CG Arena software generates a number of outcome variables for each trial (e.g., time spent in the trial; time spent in each quadrant; time required to find the target; total path length, which includes distance moved onto the target). I used total path length to the target (which provides only the distance up to the point participants reached the target) as my primary Arena outcome variable. Hence, smaller values for this variable (i.e., shorter lengths to the target) indicate better performance.

Descriptive and inferential analyses. 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 analysis of variance (RM-ANOVA) 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. All post-hoc analyses conducted were planned in advance.

**Results**

**Final Sample Characteristics**

I excluded 16 participants (7 men and 9 women) because, after enrolling, (a) the research team discovered that they were not eligible for the study, or (b) procedural issues prevented them from completing both study sessions. Table 3 explains in detail why these individuals were excluded. The final sample size consisted of 46 participants (23 men, 23 women).

<table>
<thead>
<tr>
<th>N</th>
<th>Reason</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>Data lost</td>
<td>Computerised data lost due to hardware problems</td>
</tr>
<tr>
<td>2b</td>
<td>Did not complete</td>
<td>Procedural issues</td>
</tr>
<tr>
<td>3c</td>
<td>Did not complete</td>
<td>Cancellations on Day 2</td>
</tr>
<tr>
<td>1d</td>
<td>Did not complete</td>
<td>BDI-II score exceeded 20</td>
</tr>
<tr>
<td>1a</td>
<td>Did not complete</td>
<td>On steroid based medication</td>
</tr>
<tr>
<td>6d</td>
<td>BMI</td>
<td>BMIs exceeded 30</td>
</tr>
</tbody>
</table>

*Note. aOnly male participants. b1 man and 1 woman. c2 men and 1 woman. dOnly female participants.*

As Table 4 shows, independent sample *t*-tests detected, for the final sample of 46 participants, no significant between-sex differences regarding BMI, BDI-II scores, and STAI-Trait scores. The analysis did detect a significant between-sex difference with regard to age, however.
Table 4  
**Sample Characteristics: Descriptive statistics and between-group comparisons (N = 46)**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Male (n = 23)</th>
<th>Female (n = 23)</th>
<th>t</th>
<th>p</th>
<th>ESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>20.43 (1.92)</td>
<td>19.17 (1.07)</td>
<td>2.74</td>
<td>.005**</td>
<td>0.80</td>
</tr>
<tr>
<td>BMI</td>
<td>24.06 (2.42)</td>
<td>23.93 (2.76)</td>
<td>.17</td>
<td>.43</td>
<td>0.05</td>
</tr>
<tr>
<td>BDI-II</td>
<td>8.87 (6.05)</td>
<td>10.17 (7.01)</td>
<td>-0.68</td>
<td>.25</td>
<td>0.20</td>
</tr>
<tr>
<td>STAI -Trait</td>
<td>37.00 (9.47)</td>
<td>40.52 (11.32)</td>
<td>-1.149</td>
<td>.13</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*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. ESE = effect size estimate; in this case, Cohen’s $d$. Degrees of freedom for each between-group comparison were (2, 44).

* $p < .05$. ** $p < .01$. *** $p < .001$. All listed $p$-values are one-tailed.

Regarding *Age*, an exploration of outliers revealed no extreme outlying cases. Rather, it appeared to be the case that, on average, the age of women in the sample was significantly lower than that of men. I identified potential outliers by exploring the distribution of ages across the two groups. This exploration revealed that, in the group of men, there were two participants aged 24 and one aged 23, but that, in the group of women, there was no participant older than 22. However, even removing these three potential outliers did not lower the $p$-value to a level of non-significance. Although I did not expect age to have an influence on the outcome (particularly because all participants were between the ages of 18 and 25), due to the large effect size associated with the between-group comparison, I deemed it necessary to use the variable Age as a covariate throughout the subsequent analyses. To reduce the chances of an over-conservative test of within-subjects effects, I used mean centring of the covariate Age for all repeated-measures analyses (see, e.g., M. Thomas et al., 2009). M. Thomas and colleagues suggest this approach, and note that a repeated-measures analysis of covariance (RM ANCOVA) produces a weaker main effect than does an RM
ANOVA. Therefore, the RM-ANCOVA may not detect potentially significant effects that do exist in the data.

Regarding BMI, 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, the mean for each group fell within the range conventionally described as ‘minimally depressed’ (0-13). This variable is important to control for because of the confounding effects of pre-existing emotional states on stress responses (Kelly et al., 2008).

Regarding STAI-Trait scores, the sample appeared representative of the general population: When compared to the normative data for college students in the United States (men: $M = 38.30, SD = 9.18$; women: $M = 40.4, SD = 10.15$) supplied by the test manual (Spielberger et al., 1983), a single-sample $t$-test was not significant for men, $t(22) = -.66, p = .25$, or for women, $t(22) = 0.05, p = .48$.

**Effectiveness of the Stress Induction Method: Day 2 data**

The analyses described below tested the effectiveness of the FFST, and examined whether the level of stress induction on Day 2 was equivalent in men and women. For each variable listed in Table 5, I conducted a two-phase analysis using a 2x3 (Sex x Testing Stage) mixed-design ANOVA and then ANCOVA. The main effect of each of the repeated-measures factors are independent of the between-subject covariate of Age, and thus pure repeated-measures effects are reported for Testing Stage from an ANOVA (Annaz, Karmiloff-Smith, Johnson, & Thomas, 2009). Effects of the independent variable Sex and the interaction between Sex and Testing Stage are reported from an ANCOVA, thus controlling for any role age may play prior to any effect that may be detected. Degrees of freedom for the
within-subject effects therefore differ from those for the between-subject and interaction
effects. I ran planned comparisons to test pre-existing hypotheses about where within-group
differences existed.

Table 5
Self-Reported and Physiological Stress: Descriptive statistics (N = 46)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Men (n = 23)</th>
<th>Women (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STAI-State</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>37.04 (9.12)</td>
<td>39.17 (9.45)</td>
</tr>
<tr>
<td>Time 1</td>
<td>39.65 (9.42)</td>
<td>50.22 (14.22)</td>
</tr>
<tr>
<td>Time 2</td>
<td>35.83 (9.74)</td>
<td>42.96 (12.32)</td>
</tr>
<tr>
<td><strong>PANAS-NA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>14.65 (4.92)</td>
<td>14.55 (3.36)</td>
</tr>
<tr>
<td>Time 1</td>
<td>17.57 (6.26)</td>
<td>21.91 (8.79)</td>
</tr>
<tr>
<td>Time 2</td>
<td>14.13 (4.94)</td>
<td>15.68 (6.28)</td>
</tr>
<tr>
<td><strong>Heart ratec, d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>75.08 (15.04)</td>
<td>78.24 (11.59)</td>
</tr>
<tr>
<td>Time 1</td>
<td>98.30 (16.79)</td>
<td>102.80 (19.03)</td>
</tr>
<tr>
<td>Time 2</td>
<td>76.83 (11.35)</td>
<td>77.69 (9.51)</td>
</tr>
<tr>
<td><strong>Salivary cortisolg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.42 (3.94)</td>
<td>6.22 (3.19)</td>
</tr>
<tr>
<td>Time 1</td>
<td>12.58 (8.00)</td>
<td>7.77 (4.38)</td>
</tr>
<tr>
<td>Time 2</td>
<td>11.15 (6.32)</td>
<td>7.23 (3.08)</td>
</tr>
</tbody>
</table>

Note. Means are presented with standard deviations in parentheses. STAI = State-Trait Anxiety Inventory; PANAS = Positive and Negative Affect Scale. a,n = 22. bOne participant (a 19-year-old woman) did not complete this measure correctly. cMeasured in beats per minute (bpm). dDue to hardware malfunctions, complete sets of heart rate data were only available for 35 participants. e,n = 18; f,n = 17. gMeasured in nanomoles per litre (nmol/l).

**Participant self-report measures.**

**STAI-State.** Regarding the covariate Age, the analysis did not detect a significant
main effect, $F(1, 43) = 0.37, p = .55, \eta^2_p = .01$, nor did it detect a significant interaction effect
with Testing Stage, $F(1.75, 75.05) = 0.61, p = .53, \eta^2_p = .01$. These results suggest that the
effects on self-reported exposure to the psychosocial stressor did not vary substantially across the age range.

The analysis detected a significant main effect of Testing Stage, $F(1.75, 76.76) = 10.46, p < .001, \eta^2_p = .19$. After controlling for the effect of Age, the analysis detected a significant main effect of Sex, $F(1, 43) = 6.51, p = .01, \eta^2_p = .13$, but did not detect a significant Sex x Testing Stage interaction, $F(1.75, 75.05) = 2.12, p = .13, \eta^2_p = .05$. This pattern of data suggests that male and female scores on this instrument were significantly different overall, but that the change across time was no different in men and women. It also suggests, however, that, across the entire sample, there were significant changes in self-reported anxiety across the test session.

Planned pairwise comparisons revealed that, for the entire sample, there was a significant increase in STAI-State scores from Baseline to Time 1, $p = .001$, and that there was no significant difference between Baseline and Time 2 scores, $p = .35$. In addition, across the test session, female scores were significantly higher than those of males. Figure 5 illustrates this pattern of data.
Figure 5. Fluctuations in STAI-State responses during the stress induction procedure (N = 46). Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 1.36, 1.79, 1.63; Men = 2.00, 2.65, 2.40; Women = 2.00, 2.65, 2.40.

PANAS-NA. One participant (a 19-year-old woman) did not complete this measure correctly. Her data was therefore removed from the analysis. I conducted the analysis of PANAS-NA scores on the remaining 45 participants.

Regarding the covariate Age, the analysis did not detect a significant main effect, $F(1, 42) = 1.31, p = .26$, $\eta^2_p = .03$, nor did it detect a significant interaction effect with Testing Stage, $F(1.59, 66.84) = 0.84, p = .41$, $\eta^2_p = .02$. These results suggest that the effects on self-reported negative affect of exposure to the psychosocial stressor did not vary substantially across the age range.

The analysis detected a significant main effect of Testing Stage, $F(1.58, 67.90) = 21.09, p < .001$, $\eta^2_p = .33$. After controlling for the effect of Age, the analysis did not, however, detect a significant main effect of Sex, $F(1, 42) = 2.75, p = .11$, $\eta^2_p = .06$, and it did not detect a significant Sex x Testing Stage interaction, $F(1.59, 66.84) = 1.72, p = .19$, $\eta^2_p$
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 men and women. It also suggests that, across the entire sample, there were significant changes in self-reported negative affect across the test session.

Planned pairwise comparisons revealed that, for the entire sample, there was a significant increase in PANAS-NA scores from Baseline to Time 1, \( p < .001 \), and that there was no significant difference between Baseline and Time 2 scores, \( p = .68 \). Figure 6 illustrates this pattern of data.

Figure 6. Fluctuations in PANAS-NA responses during the stress-induction procedure (\( N = 45 \)). Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 0.60, 1.15, 0.84; Men = 0.88, 1.67, 1.22; Women = 0.90, 1.71, 1.25.

STAI-State and PANAS-NA scores were highly correlated at each measurement point: \( r = .53 \) at baseline, \( r = .70 \) at Time 1, and \( r = .66 \) at Time 2, \( p < .001 \) in each case. This
pattern of data confirms that participants’ self-reports of state anxiety and state negative affect were consistent across measures.

**Physiological measurements.**

**Heart rate.** Due to hardware malfunctions, complete sets of heart rate data were only available for 18 male and 17 female participants. Hence, the analyses described below pertain to those individuals only.

Regarding the covariate Age, the analysis did not detect a significant main effect, $F(1, 32) = 1.06, p = .31, \eta^2_p = .03$, nor did it detect a significant interaction with Testing Stage, $F(1.26, 40.43) = 0.29, p = .65, \eta^2_p = .01$. These results suggest that the effects on heart rate of exposure to the psychosocial stressor did not vary substantially across the age range.

The analysis detected a significant main effect of Testing Stage, $F(1.28, 42.33) = 127.28, p < .001, \eta^2_p = .80$. After controlling for the effect of Age, the analysis did not, however, detect a significant main effect of Sex, $F(1, 32) = 0.86, p = .36, \eta^2_p = .03$, and it did not detect a significant Sex x Testing Stage interaction , $F(1.26, 40.43) = 0.71, p = .44, \eta^2_p = .02$. Again, this pattern of data suggests that, on average, heart rate in men and women was not significantly different, and that the change across time was no different in men and women. It does suggest, however, that, across the entire sample, there were significant 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, $p < .001$, but that there was no significant difference between Baseline and Time 2 values, $p = .51$. Figure 7 illustrates this pattern of data.
Figure 7. Fluctuations in heart rate responses during the stress induction procedure ($N = 35$). Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 2.26, 3.05, 1.78; Men = 3.22, 4.36, 2.56; Women = 3.32, 4.49, 2.62.

**Salivary cortisol.** An assessment of the assumptions underlying the proposed inferential statistical tests indicated violations of normality and homogeneity of variances, and so I log transformed the data. This transformation resulted in the data no longer violating the above-mentioned assumptions, and so the transformed data were used in the analysis described below.

Regarding the covariate Age, the analysis detected a significant main effect, $F(1, 43) = 4.63, p = .04, \eta_p^2 = .10$. The analysis did not, however, detect a significant interaction with Testing Stage, $F(2, 86) = 1.38, p = .26, \eta_p^2 = .03$. These results suggest a significant positive association between cortisol levels and age.

The analysis detected a significant main effect of Testing Stage, $F(2, 88) = 24.82, p < .001, \eta_p^2 = .37$. After controlling for the effect of Age, the analysis did not detect a significant main effect of Sex, $F(1, 43) = .81, p = .37, \eta_p^2 = .02$, but did detect a significant
Sex x Testing Stage interaction, \( F(2, 86) = 7.58, p = .001, \eta^2_p = .15 \). Although this pattern of data suggests that, across the entire sample, there were significant changes in cortisol across the stress-induction procedure, it also suggests that the change across time was different in men and women (this even though there were no between-sex differences in overall cortisol levels).

Planned pairwise comparisons revealed that, for the entire sample, there was a significant increase in cortisol levels from Baseline to Time 1, \( p < .001 \), and that that increase over Baseline persisted at Time 2, \( p < .001 \). Figure 8 illustrates this pattern of data.

Further analysis of the interaction between Sex and Testing Stage, by means of tests of within-subject contrasts, revealed that there was a significant difference between male and female cortisol levels when examining the magnitude of the increase between Baseline and Time 1, \( p < .01 \), with men showing a greater response. However, when examining the magnitude of the difference between Time 1 and Time 2, there was no significant between-sex difference, \( p = .12 \).

I ran a 2 x 2 (Sex x Testing Stage) mixed-design ANCOVA using only Baseline and Time 1 measurements in order to determine if age plays a role in the sex difference found in the magnitude of response to the application of the psychosocial stressor. Regarding the covariate Age, the analysis did not detect a significant main effect, however there was a strong trend towards significance, \( F(1, 43) = 2.97, p = .09, \eta^2_p = .06 \), nor did it detect a significant interaction with Testing Stage, \( F(1, 43) = 1.21, p = .27, \eta^2_p = .03 \). These results suggest that the effects on cortisol levels of exposure to the psychosocial stressor did not vary substantially across the age range. After controlling for Age, the analysis detected a significant Sex x Testing Stage interaction, \( F(1, 43) = 12.19, p = .001, \eta^2_p = .22 \), with cortisol levels increasing more in men (Baseline Estimated Marginal Mean (EMM) = .70; Time 1 EMM = 1.00) than in women (Baseline EMM = .77; Time 1 EMM = .84).
Figure 8. Fluctuations in cortisol levels during stress induction procedure ($N = 46$). Standard error of means taken with a 95% confidence interval - (Baseline, Time 1, Time 2): Total = 0.03, 0.39, 0.31; Men = 0.05, 0.06, 0.05; Women = 0.05, 0.06, 0.05.

Qualities of the CG Arena: Day 1 data

The analyses described below assessed the general qualities of the CG Arena developed for the specific purposes of this study, and examined whether participants were able to utilize both proximal and distal cues within that Arena to navigate to a learned target location. One participant’s data for the Day 1 Arena protocol were lost due to software errors. Hence, data from this participant (a 21-year-old man) were excluded from all analyses of CG Arena data.

For all Day 1 and Day 2 variables, I calculated the optimal path length to target\(^2\) for each trial, and thereafter subtracted this value from each participant’s path length to target in

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\(^2\)I calculated optimal path length to target by running the Day1 and Day 2 Arenas 3 times each, and each time navigating to the target location in the most direct possible path. To ensure that I took that most direct path, I re-programmed the software to make the target visible on all trials. I then took the average across each set of 3 trials, and thus obtained, for both Day 1 and Day 2, the optimal length required to reach the target.
order to compute their deviation from the optimal path length to target. This strategy allowed me to remove any confounds that might have arisen from the varying distance of the starting point to the target across trials.

Unless otherwise stated, I ran all analyses on the log transformations of each variable (or computed variable) from this data, as assessment of assumptions underlying the proposed statistical analyses indicated violations of normality, of homogeneity of variances, or of homogeneity of regression slopes. These transformations resulted in the data no longer violating the above-mentioned assumptions.

**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 the same two-phase analysis described previously, using a 2 x 4 (Sex x Trials) mixed-design to ensure that there were no significant between-group differences in spatial performance.

Regarding the covariate Age, the analysis did not detect a significant main effect, $F(1, 43) = 0.02, p = .88, \eta^2_p < .001$, and it did not detect a significant interaction with Trials, $F(1.77, 76.15) = 0.40, p = .65, \eta^2_p = .01$. These results suggest that path length to target on the visible-target trials did not vary substantially across the age range.

The analysis detected a significant main effect of Trials, $F(1.80, 77.85) = 3.16, p = .03, \eta^2_p = .07$. After controlling for the effect of Age, the analysis did not, however, detect a significant main effect of Sex, $F(1, 43) = 0.21, p = .65, \eta^2_p = .005$, and it did not detect a significant Sex x Trials interaction, $F(1.80, 76.15) = 0.87, p = .41, \eta^2_p = .02$. This pattern of data suggests that all participants, regardless of sex, were equally capable of navigating and locating the visible target in the Arena. The curves presented in Figure 9 provide a measure of explanation for the main effect of Trials described above.
Figure 9. Average path length to target over visible target trials. Error bars indicate standard error of mean with 95% confidence interval.

**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. Again, I ran the same two-phase analysis described previously, this time using a 2 x 8 (Sex x Trials) mixed-design on the deviation from optimal path length data for the acquisition trials.

Regarding the covariate *Age*, the analysis did not detect a significant main effect, $F(1, 42) = 2.48, p = .12, \eta^2 = .06$, and it did not detect a significant interaction with Trials, $F(5.37, 225.43) = 1.38, p = .23, \eta^2 = .03$. These results suggest that path length to target on the acquisition trials did not vary substantially across the age range.
The analysis detected a significant main effect of Trials, $F(5.30, 227.95) = 27.51, p < .001, \eta_p^2 = .39$. After controlling for the effect of Age, the analysis detected a significant main effect of Sex, $F(1, 42) = 7.18, p = .01, \eta_p^2 = .15$, but did not detect a significant Sex x Trials interaction, $F(5.37, 225.43) = 1.38, p = .23, \eta_p^2 = .03$. A linear trend analysis was also statistically significant, indicating that there was an orderly learning curve from trials 1 through 8, $F(1, 43) = 111.43, p < .001, \eta_p^2 = .72$. Furthermore, planned pairwise comparisons revealed that men had a more efficient learning curve than women. Figure 10 illustrates these data.

![Figure 10](image-url)

*Figure 10.* Average path length to target over Day 1 acquisition trials. Error bars indicate standard error of mean with 95% confidence interval.

As Figure 10 illustrates, place learning appeared reasonably complete by trials 4-5. For this reason, I took the average deviation from optimal length on trials 6-8 as a baseline measurement against which to compare performance on the test trials (i.e., those trials during which walls or objects were removed or swapped). This step allowed me to identify the effect
each wall/cue removal or swap had on performance, which in turn would allow inferences about the effect that changing proximal and distal cues had on spatial performance.

To determine if the wall/cue changes made on each test trial had an impact on deviation from optimal path length to target, I ran a series of repeated-measures ANCOVAs, with each comparing performance on a particular test trial to baseline performance. Data from trials 17, 19, 21 and 29 were left untransformed despite non-normal distributions, as transformations resulted in the data violating either the assumption of homogeneity of variance (19) or homogeneity of regression slopes (17, 21, 29). Thus, for analysis involving those trials the untransformed baseline measurement was used.

Regarding the covariate Age, for all trials analysed the analyses did not detect a significant main effect, $F_s < 0.66, ps > .42$, nor did they detect a significant interaction with Trials, $F_s < 2.55, ps > .18$. These results suggest that, on the observed trials, path length to the target did not vary substantially across the age range.

As Table 6 shows, when changes were made to the experimental room (i.e., when walls or objects were eliminated from the Arena, or when walls or objects swapped positions), there were significant changes, relative to baseline, in participants’ deviation from optimal path length to target. Specifically, on three of the four swap trials (15, 19, and 31), and on one of the elimination trials (11), participants tended to take much longer path lengths, relative to baseline, to relocate the target. Of interest, however, is that on other elimination trials (13, 17, and 21), the opposite effect was present: Participants took a shorter path length, relative to baseline, to relocate the target. Figures 11 and 12 also illustrate this pattern of data.
Table 6
CG Arena Day 1: Comparison of test trial performance to baseline performance (N=45)

<table>
<thead>
<tr>
<th>Elimination trials</th>
<th>$F$</th>
<th>$p$</th>
<th>ESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 9 (N wall removal)</td>
<td>0.31</td>
<td>.29</td>
<td>0.08</td>
</tr>
<tr>
<td>Trial 17 (W wall removal)</td>
<td>3.28</td>
<td>.04*</td>
<td>0.39</td>
</tr>
<tr>
<td>Trial 13 (opposite/SW corner removal)</td>
<td>2.89</td>
<td>.05*</td>
<td>0.25</td>
</tr>
<tr>
<td>Trial 23 (critical/NE corner removal)</td>
<td>1.57</td>
<td>.11</td>
<td>0.22</td>
</tr>
<tr>
<td>Trial 29 (all walls removed)</td>
<td>0.02</td>
<td>.45</td>
<td>0.03</td>
</tr>
<tr>
<td>Trial 11 (object removal - NE)</td>
<td>27.35</td>
<td>&lt; .001***</td>
<td>0.95</td>
</tr>
<tr>
<td>Trial 21 (object removal - SE)</td>
<td>5.34</td>
<td>.01**</td>
<td>0.46</td>
</tr>
<tr>
<td>Trial 25 (all objects removed)</td>
<td>0.49</td>
<td>.24</td>
<td>0.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Swap trials</th>
<th>$F$</th>
<th>$p$</th>
<th>ESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 19 (Swap A - anticlockwise)</td>
<td>7.78</td>
<td>.004**</td>
<td>0.55</td>
</tr>
<tr>
<td>Trial 27 (Swap B - clockwise)</td>
<td>0.59</td>
<td>.22</td>
<td>0.15</td>
</tr>
<tr>
<td>Trial 15 (Swap objects - opposite side)</td>
<td>27.60</td>
<td>&lt; .001***</td>
<td>1.78</td>
</tr>
<tr>
<td>Trial 31 (Switch objects)</td>
<td>23.01</td>
<td>&lt; .001***</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Note. Degrees of freedom = (1, 43) for each comparison. ESE = effect size estimate; in this case, Cohen’s $d$.

*p < .05. **p < .01. ***p < .001. Bonferroni-corrected $p$-value = .05/45 = .001. All listed $p$-values are one-tailed.

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

The analyses described below served to test the hypothesis that, on Day 1 (i.e., without exposure to the TSST), men would perform better than women. I ran one-way ANCOVAs on the Day 1 data from the mental rotation and CG Arena tasks to identify whether there were any sex differences in performance under unstressed conditions.

**Mental rotation task.** Regarding the effect of the covariate Age, the analysis did not detect a significant main effect, $F(1, 43) = 0.13, p = .72, \eta^2_p = .003$. After controlling for the effect of Age, the analysis did not detect a significant between-group difference, $F(1,43) = 1.64, p = .10, \eta^2_p = .04$ (one-tailed $p$-value).

**CG Arena.** I grouped trials according to their general test conditions (distal: object removal, wall removal; proximal: object swap, wall swap). Regarding the covariate Age, for all trials analysed the analyses did not detect a significant main effect, $Fs < 2.26, ps > .14$. 

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**Figure 12.** Average path length to target for baseline trials and swap trials. Error bars indicate standard error of mean with 95% confidence interval. **Trial** Description – 19: Swap A – anticlockwise, 27: Swap B – clockwise, 15: Swap objects - opposite side, 31: Switch objects – with each other.
Table 7 shows the results of further analyses conducted on those data. After controlling for the effect of Age, the analysis detected only one significant sex difference: Men outperformed women on the set of object removal trials.

<table>
<thead>
<tr>
<th>Trial Group</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>F</td>
<td>p</td>
<td>ESE</td>
<td></td>
</tr>
<tr>
<td>Wall Removal</td>
<td>1.70 (0.33)</td>
<td>1.84 (0.44)</td>
<td>1.47</td>
<td>.12</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Object Removal</td>
<td>1.78 (0.49)</td>
<td>2.07 (0.46)</td>
<td>3.41</td>
<td>.04*</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Wall Swap</td>
<td>2.02 (0.84)</td>
<td>1.89 (1.11)</td>
<td>0.24</td>
<td>.31</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Object Swap</td>
<td>2.43 (0.61)</td>
<td>2.59 (3.50)</td>
<td>0.18</td>
<td>.34</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

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.

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

The analyses described below tested whether exposure to the TSST resulted in impaired navigation performance, and whether women’s navigation performance was impaired to greater extent than men’s.

Mental rotation task. Figure 13 illustrates male and female mental rotation performance across the two-day experimental protocol. I ran the same two-phase analysis described previously, this time using a 2 x 2 (Sex x Day) mixed-design repeated measures ANCOVA on the mental rotation scores from each testing day.

Regarding the effect of the covariate Age, the analysis did not detect a significant main effect, $F(1, 43) = 0.06, p = .80, \eta_p^2 = .001$, and it did not detect a significant interaction with Day, $F(1, 43) = 0.12, p = .73, \eta_p^2 = .003$. These results suggest that mental rotation performance did not vary substantially across the age range, and that all participants, regardless of age, were affected equally by exposure to the psychosocial stressor.
The analysis detected a significant main effect of Day, $F(1, 44) = 24.22, p < .001, \eta^2_p = .36$. After controlling for the effect of Age, the analysis detected a significant main effect of Sex, $F(1, 43) = 7.02, p = .005, \eta^2_p = .14$, as well as a significant Sex x Day interaction, $F(1, 43) = 11.83, p < .001, \eta^2_p = .22$. (All listed $p$-values here are one-tailed.)

Overall, planned pairwise comparisons revealed that participants performed better on Day 2 (i.e., after exposure to the psychosocial stressor), however this was largely driven by an improvement in the performance of men on Day 2. Men also performed better than women on both Day 1 and Day 2. Figure 13 illustrates the interaction between Sex and Day, showing that (a) on Day 1, men ($EMM = 48.53$) performed better than women ($EMM = 43.03$), and (b) on Day 2, while male performance ($EMM = 60.69$) improved, female performance ($EMM = 44.66$) was relatively unaffected by FFST exposure.

Figure 13. Effects of exposure to the Fear Factor Stress Test on mental rotation performance. Descriptive statistics for mental rotation task: Day 1: men $M = 48.83$ ($SD = 13.57$), women $M = 42.74$ ($SD = 13.10$); Day 2: men $M = 60.78$ ($SD = 12.83$), women $M = 44.57$ ($SD = 14.31$). Error bars indicate standard error of mean with 95% confidence interval.
**CG Arena.** To demonstrate that learning had occurred in the Day 2 CG Arena, I ran the same two-phase analysis described previously, this time using a 2 x 8 (Sex x Trials) mixed-design on the Day 2 acquisition trials data (with the specific outcome variable, being deviation from optimal path length on each of those trials).

Regarding the effect of the covariate Age, the analysis did not detect a significant main effect, \( F(1, 42) = 0.56, p = .46, \eta^2_p = .01 \), and it did not detect a significant interaction with Trials, \( F(5.55, 233.07) = 0.69, p = .65, \eta^2_p = .02 \). These results suggest that path length to target did not vary substantially across the age range, and that all participants, regardless of age, showed a similar learning curve across trials.

The analysis detected a significant main effect of Trials, \( F(5.52, 237.36) = 17.72, p < .001, \eta^2_p = .29 \). After controlling for the effect of Age, the analysis did not detect a significant main effect of Sex, \( F(1, 42) = 2.63, p = .11, \eta^2_p = .06 \), and it did not detect a significant Sex x Trials interaction, \( F(5.55, 233.07) = 1.55, p = .17, \eta^2_p = .04 \). Additionally, a linear trend analysis was statistically significant, indicating that there was an orderly learning curve from trials 1 through 8, \( F(1, 43) = 43.01, p < .001, \eta^2_p = .50 \) (see Figure 14).
Figure 14. Average deviation from optimal path length to target on Day 2 acquisition trials. Error bars indicate standard error of mean with 95% confidence interval.

I again ran the two-phase analysis described previously, this time using a 2 x 2 (Sex x Day) mixed-design to compare Day 1 performance to Day 2 performance. Each analysis compared performance on each Day 1 trial to performance on the corresponding Day 2 trial. Data from trials 17, 19, 21, and 29 were again left untransformed, for reasons explained above.

Regarding the covariate Age, for all acquisition trials analyzed, the analyses did not detect a significant main effect, $F_s < 1.44, ps > .23$, however there was a strong trend toward significance for Trial 3, $F(1, 42) = 3.76, p = .06, \eta^2_p = .08$. Furthermore, the analyses did not detect a significant interaction with Day, $F_s < 2.47, ps > .12$. These results suggest that, on all Day 1 and Day 2 acquisition trials, path length to target did not vary substantially across the age range, and that all participants, regardless of age, showed a similar pattern of performance from Day 1 to Day 2.
The analysis did not detect a significant main effect of Day for most of the acquisition trials, $F_s < 2.14, p_s > .15$, however there was a strong trend toward significance for Trial 7, $F(1, 43) = 3.07, p = .09, \eta^2_p = .07$. Furthermore, for some of the acquisition trials, after controlling for the effect of Age, the analysis did not detect a significant main effect of Sex, $F_s < 2.90, p_s > .10$, and it did not detect a significant Sex x Trials interaction, $F_s < 0.78, p_s > .38$.

However, the analysis did detect a significant main effect of Day for Trial 3, $F(1, 43) = 11.16, p = .002, \eta^2_p = .21$, suggesting that, on this trial, differences in performance across days. A pairwise comparison revealed that performance on Day 1 was worse than that on Day 2.

Furthermore, the analysis detected significant main effects of Sex for Trial 5, $F(1, 42) = 7.30, p = .01, \eta^2_p = .15$; for Trial 7, $F(1, 42) = 5.48, p = .02, \eta^2_p = .12$; and for Trial 8, $F(1, 42) = 4.03, p = .05, \eta^2_p = .09$. Pairwise comparisons revealed that, on these trials, men performed better than women.

The analysis also detected significant Sex x Trials interactions for Trial 3, $F(1, 42) = 5.95, p = .02, \eta^2_p = .12$, which showed that on Day 1, men ($EMM = 1.81$) performed better than women ($EMM = 2.33$), but that, on Day 2, women’s performance ($EMM = 1.30$) improved a great deal more than did men’s ($EMM = 1.54$). The analysis also detected significant Sex x Trials interactions for Trial 6 $F(1, 42) = 4.29, p = .04, \eta^2_p = .09$, which showed that on Day 1, men ($EMM = 1.00$) performed better than women ($EMM = 1.63$), and that, on Day 2, men’s performance ($EMM = 1.67$) declined relative to Day 1 whereas women’s performance ($EMM = 1.18$) improved over Day 1.

Regarding the covariate Age, for all test trials analyzed the analyses did not detect a significant main effect, $F_s < 1.88, p_s > .18$. For most test trials analyzed, the analyses did not detect a significant interaction with Day, $F_s < 2.48, p_s > .12$, however there was a strong
trend toward significance for Trial 17 (W wall removal), $F(1, 42) = 3.30, p = .08, \eta^2_p = .07$..

These results suggest that, on all Day 1 and Day 2 test trials, path length to target did not vary substantially across the age range, and that all participants, regardless of age, showed a similar pattern of performance from Day 1 to Day 2. The analyses did, however, detect a significant Age x Day interaction for Trial 31 (object switch), $F(1, 42) = 7.78, p = .01, \eta^2_p = .16$, suggesting that, on this trial, differences in performance across days is related to age differences.

**Main effect of testing day.** Table 8 shows the results for the main effect of Day. The analyses detected some significant effects when comparing Day 1 (relaxed) to Day 2 (FFST exposure) performance. Planned pairwise comparisons of data from these trials revealed that, across the entire sample, performance on Trials 13, 17, 21, and 27 was worse on Day 2, but that performance on Trial 11 was better on Day 2.
Table 8  
*CG Arena Day 1 vs. Day 2: Within-subject effects (N = 45)*

<table>
<thead>
<tr>
<th>Group</th>
<th>M (SD)</th>
<th>F</th>
<th>p</th>
<th>ESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 9 (N wall removal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>1.41 (0.74)</td>
<td>0.78</td>
<td>.19</td>
<td>0.14</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.52 (0.80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 13 (SW corner removal)</td>
<td></td>
<td>6.06</td>
<td>.01**</td>
<td>0.38</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.29 (0.70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>1.52 (0.49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 17 (W wall removal)</td>
<td></td>
<td>5.32</td>
<td>.01**</td>
<td>0.40</td>
</tr>
<tr>
<td>Day 1</td>
<td>44.48 (99.62)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>145.15 (337.93)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 23 (NE corner removal)</td>
<td></td>
<td>0.40</td>
<td>.26</td>
<td>0.12</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.31 (0.79)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>1.41 (0.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 29 (All walls removed)</td>
<td></td>
<td>0.34</td>
<td>0.28</td>
<td>0.12</td>
</tr>
<tr>
<td>Day 1</td>
<td>135.31 (78.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>163.23 (304.95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 11 (object removed - NE)</td>
<td></td>
<td>13.62</td>
<td>.001**</td>
<td>0.65</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.08 (0.59)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>1.71 (0.53)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 21 (object removed - SE)</td>
<td></td>
<td>13.21</td>
<td>.001**</td>
<td>0.76</td>
</tr>
<tr>
<td>Day 1</td>
<td>28.02 (62.54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>227.36 (363.80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 25 (All objects removed)</td>
<td></td>
<td>1.83</td>
<td>.09</td>
<td>0.28</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.57 (0.84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>1.80 (0.81)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 19 (Swap A - anticlockwise)</td>
<td></td>
<td>0.02</td>
<td>.45</td>
<td>0.01</td>
</tr>
<tr>
<td>Day 1</td>
<td>430.26 (644.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>420.79 (726.06)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 27 (Swap B - clockwise)</td>
<td></td>
<td>2.88</td>
<td>.05*</td>
<td>0.32</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.61 (1.12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>1.94 (0.88)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 15 (Swap objects)</td>
<td></td>
<td>0.01</td>
<td>.45</td>
<td>0.02</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.58 (0.54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>2.59 (0.61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 31 (Switch objects)</td>
<td></td>
<td>0.85</td>
<td>.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.15 (0.75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>2.03 (0.81)</td>
<td></td>
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</tr>
</tbody>
</table>

*Note.* Degrees of freedom = (1, 43) for each comparison. ESE = effect size estimate; in this case, Cohen’s $d$.  
* $p < .05$. ** $p < .01$. *** $p < .001$. All listed $p$-values are one-tailed.

**Main effect of sex.** The results discussed below pertain to the significant effects the analyses detected for Sex, after controlling for Age. All $p$-values reported below are one-tailed. The analysis detected significant main effects of Sex for Trial 9 (N wall removal), $F(1,
42) = 5.94, \( p = .01, \eta_p^2 = .13 \); for Trial 29 (all walls removed), \( F(1, 42) = 3.02, p = .05, \eta_p^2 = .07 \); for Trial 11 (object removed - NE), \( F(1, 42) = 6.71, p = .006, \eta_p^2 = .14 \); for Trial 17 (W wall removal), \( F(1, 42) = 2.74, p = .05, \eta_p^2 = .06 \); and for Trial 19 (Swap A - anticlockwise), \( F(1, 42) = 3.27, p = .04, \eta_p^2 = .07 \).

Planned pairwise comparisons revealed that, for each of those trials, men performed significantly better than women. Furthermore, for Trial 13 (SW corner removal), the analysis detected a strong trend toward statistical significance, \( F(1, 42) = 2.07, p = .08, d = 0.38 \). A pairwise comparison also revealed that, again, men performed better than women on that trial.

**Interaction effects: Sex and testing day.** The results discussed below pertain to the significant effects the analyses detected for the interaction between Sex and Day, after controlling for Age. All \( p \)-values reported below are one-tailed.

For Trial 9 (N wall removal), the analysis detected a significant interaction, \( F(1, 42) = 4.63, p = .02, \eta_p^2 = .10 \). Figure 15 illustrates this interaction and shows that, on Day 1, men (\( EMM = 1.31 \)) performed better than women (\( EMM = 1.52 \)), but that, on Day 2, male performance improved over Day 1 (\( EMM = 1.14 \)), whereas women’s performance was worse than on Day 1 (\( EMM = 1.90 \)).

For Trial 17 (W wall removal), the analysis detected a significant interaction, \( F(1, 42) = 3.20, p = .04, \eta_p^2 = .07 \). Figure 16 illustrates this interaction and shows that, on Day 1, men (\( EMM = 31.76 \)) performed better than women (\( EMM = 56.67 \)), but that, on Day 2, women’s performance (\( EMM = 236.53 \)) declined a great deal more than did men’s (\( EMM = 49.61 \)).
Figure 15. Interaction between testing stage and sex on CG Arena Trial 9. Error bars indicate standard error of mean with 95% confidence interval.

Figure 16. Interaction between testing stage and sex on CG Arena Trial 17. Error bars indicate standard error of mean with 95% confidence interval.
For Trial 23 (NE corner removal), the analysis detected a significant interaction, $F(1, 42) = 2.81, p = .05, \eta^2_p = .06$. Figure 17 illustrates this interaction and shows that, on Day 1, men ($EMM = 1.40$) performed more poorly than women ($EMM = 1.23$), and that, on Day 2, male performance improved over Day 1 ($EMM = 1.21$) whereas women’s performance was worse than on Day 1 ($EMM = 1.62$).

*Figure 17.* Interaction between testing stage and sex on CG Arena Trial 23. Error bars indicate standard error of mean with 95% confidence interval.
For Trial 19 (Swap A – anticlockwise), the analysis detected a significant interaction, $F(1, 42) = 3.23, p = .04, \eta_p^2 = .07$. Figure 18 illustrates this interaction and shows that, on Day 1, men ($EMM = 363.22$) performed better than women ($EMM = 494.39$), and that, on Day 2, women’s performance ($EMM = 684.05$) declined relative to Day 1 whereas men’s performance ($EMM = 145.57$) improved over Day 1.

*Figure 18.* Interaction between testing stage and sex on CG Arena Trial 19. Error bars indicate standard error of mean with 95% confidence interval.
For Trial 31 (Switch objects – with each other), the analysis detected a significant interaction, $F(1, 42) = 3.72, p = .03, \eta^2_p = .08$. Figure 19 illustrates this interaction and shows that, Day 1, there was little difference in performance between men ($EMM = 2.18$) and women ($EMM = 2.14$). On Day 2, however, women performed slightly more poorly than they did on Day 1 ($EMM = 2.27$), whereas men performed better than they had on Day 1 ($EMM = 1.78$).

*Figure 19.* Interaction between testing stage and sex on CG Arena Trial 31. Error bars indicate standard error of mean with 95% confidence interval.
Furthermore, for Trial 27 (Swap B – clockwise), the analysis detected a strong trend toward a statistically significant interaction, $F(1, 42) = 2.48, p = .06, \eta^2_p = .07$. Figure 20 illustrates this interaction and shows that, for men, there was little to no performance difference for men from Day 1 to Day 2 ($EMM = 1.75$), but that, for women, average performance was worse on Day 2 ($EMM = 2.12$) than on Day 1 ($EMM = 1.47$).

*Figure 20.* Interaction between testing stage and sex on CG Arena Trial 27. Error bars indicate standard error of mean with 95% confidence interval.
Similarly, for Trial 15 (Swap objects – to opposite side), the analysis detected a strong trend toward a statistically significant interaction, $F(1, 42) = 1.80, p = .09, \eta^2_p = .04$. Figure 21 illustrates this interaction and shows that, on Day 1, there was little difference in performance between men ($EMM = 2.59$) and women ($EMM = 2.57$). On Day 2, however, women ($EMM = 2.71$) performed more poorly than on Day 1, whereas men ($EMM = 2.47$) performed better than on Day 1.

![Figure 21. Interaction between testing stage and sex on CG Arena Trial 15. Error bars indicate standard error of mean with 95% confidence interval.](image)

**Discussion**

The purpose of this study was to investigate thoroughly the relations between acute psychosocial stress and spatial navigation performance. As part of the study, I aimed to develop and describe (a) a stress-induction method that would activate both ANS and HPA-axis responses in both men and women, and (b) a spatial navigation task that allowed
participants to use both landmark and gradient cues, both proximally and distally, and that could be used to observe potential sex differences under stress. That method and that task allowed me to test the hypotheses that (a) under normal, unstressed conditions, men will perform better on spatial navigational tasks than women, despite the availability of landmark cues, and (b) stress will impair spatial navigational performance in both men and women, but women will be more impaired than men when, after place learning has occurred, landmark cues are removed from the environment, or are moved to different places within the environment.

The Stress Induction Manipulation

Analysis of data from the Fear Factor Stress Test (FFST) indicated that it raised self-reported negative affect and anxiety, as well as objective physiological measures of heart rate and salivary cortisol, significantly in both men and women. Moreover, participants entered and left Day 2 of the study (i.e., the day on which they were exposed to the FFST) in the same state of relative calm, with a significant increase in subjectively-reported and physiologically-measured stress occurring in the middle phase of the procedure (i.e., immediately after FFST exposure).

However, the magnitude of cortisol increase from baseline to post-exposure was greater in men than in women. Although this pattern of data points towards a differential response to the FFST by men and women, one must consider the role played by the phase of the menstrual cycle in which female participants were during administration of the experimental procedures.

Unlike some previous studies in this field (e.g., Guenzel et al., 2014; Schwabe & Wolf, 2010), eligibility criteria for the current study did not specify that female participants had to be in the luteal phase of their cycle during administration of the experimental procedures. (Those previous studies included that eligibility criterion to enhance the
possibility of female cortisol responses being similar to those of males.) I made this methodological decision to increase the likelihood that I would observe the effects of stress induction in the general population: In everyday life, women are not always in the luteal phase when exposed to stressful situations.

I did, however, record the phase of menstrual cycle in which the female participants were during administration of the experimental procedures, so as to have some idea of the general distribution of menstrual cycle phase within the female sample. Although I sent post-study reminders to all female participants to notify me when their first period following their participation began, only 16 of the 23 responded with the required information. These responses indicated that, during the experimental procedures, 18% \( n = 3 \) of those female participants were in the luteal phase, whereas 82% \( n = 13 \) were in the follicular phase. This pattern of data might account for the observed sex difference in magnitude of cortisol responses: As Kirschbaum et al. (1999) postulate, when women are in the luteal phase, they exhibit similar cortisol response patterns to those of men, but when they are in the follicular phase, these response patterns are dampened significantly. An unplanned statistical analyses of the current data confirmed that postulation: A Wilcoxon signed-rank test analyzing immediate post-exposure cortisol levels detected a strong trend toward a significant difference between women in the follicular phase versus those in the luteal phase, \( Z = -1.60, p = .05, d = 0.34 \) (one-tailed).

Taken together, these data suggest that the observed sex difference in magnitude of cortisol response to the acute psychosocial stressor is not necessarily attributable to flaws inherent in the FFST. Hence, the results from this study, in combination with those from previous studies conducted in our laboratory (du Plooy et al., 2014; Human et al., 2013), suggest that the FFST induces a physiological stress response successfully, and can raise
cortisol levels consistently, in both men and women, and that it is therefore suitable for use in future stress-related research.

**Mental Rotation Performance**

Regarding the hypothesis that, under normal, unstressed conditions, men would outperform women on spatial tasks, analysis of the mental rotation data detected no sex differences. I assessed mental rotation ability in this navigation-focused study because that ability assists in holding a mental representation of the environment in which one is navigating, and subsequently assists with the appropriate execution of movement within that environment (Garden et al., 2002). Previously published research has shown, generally, a male advantage in mental rotation ability (see, e.g., Jansen-Osmann & Heil, 2007; Parsons et al., 2004; Peters et al., 1995). A separate line of research suggests, however, that these sex differences may be dependent on the type of mental rotation task utilized, particularly because the scoring of different tasks can emphasize different performance factors (Goldstein, Haldane, & Mitchell, 1990; Kaufman, 2007). For example, Goldstein et al. (1990) reported that sex differences on the Vandenberg-Kuse Mental Rotation Test (VK-MRT) disappear when the scoring procedure controls for the number of items attempted.

With regard to the CRT, the mental rotation task used in this study, its scoring involves subtracting the number of items answered incorrectly from the number of items answered correctly. That is to say, the CRT rewards accuracy over speed. Following from the findings of Goldstein et al. (1990), such control of a particular performance factor may have reduced the sex differences ordinarily found on mental rotation tasks. Furthermore, Jansen-Osmann and Heil (2007) note that the effect size associated with the advantage for men over women is smaller for the CRT ($d = 0.3$) than for the VK-MRT ($d = 0.9$; see also Peters et al., 1995). This piece of data may also help explain why, in the current study, there were no significant mental rotation performance differences between men and women.
Furthermore, previous research has shown that women in the follicular phase of their menstrual cycle fare better on mental rotation tasks than do those in the luteal phase, and that women’s performance is not significantly different from that of men when they are in the former phase (Jones, Braithwaite, & Healy, 2003; McCormick & Teillon, 2001; Moody, 1997). Given that more than half of the female sample was in the follicular phase at the time of test administration, the results of those previous studies may help explain why I did not observe any sex differences in mental rotation performance.

Regarding the hypothesis that stress will impair mental rotation performance in men and women (and more so in women), analysis of the Day 2 data compared to the Day 1 data indicated that, after FFST exposure, performance improved in men but was unaffected in women. This result disconfirms the stated hypothesis, and stands in contrast to previously published findings regarding the effects of stress on spatial ability (Gabriel et al., 2011; Sandi et al., 2005).

Possible explanations for the currently observed data relate to the role potential confounds can play in the mental rotation performance of men and women. For example, one might consider the role phase of menstrual cycle played in the level of cortisol increase for the female participants. As noted earlier, most women in this study were in the follicular phase of their menstrual cycle, and their cortisol levels were elevated only slightly following FFST exposure. Hence, the magnitude of post-FFST cortisol increase in women may not have been large enough to affect their performance, thereby leading to similar mental rotation scores under stressful and non-stressful conditions.

The Day 1 to Day 2 improvement in mental rotation performance seen in men is an interesting and unusual result, and one that is rather difficult to explain. Mental rotation performance is associated with neural activation in the parietal regions, primarily, although some studies suggest the prefrontal cortex (PFC) might also be involved (Jordon et al., 2002;
Weiss et al., 2002). Given that most of the literature on stress and cognition focuses on hippocampal-dependent tasks, studies examining the effects of stress on mental rotation performance are non-existent. Furthermore, those studies that do examine the effects of stress on cognition in PFC-dependent tasks (e.g., tasks assessing working memory; see, e.g., Elzinga & Roelofs, 2005; Schoofs et al., 2008) do not focus on mental rotation performance and underlying mechanisms that might be associated with stress-enhanced performance. Therefore, only can only speculate regarding reasons for the observed result.

First and foremost, one needs to consider the possibility of carry-over effects, given that no control was put in place to account for these. Having previously been administered the task the day before, the loss of novelty may have had a positive effect on the men’s ability to perform the task efficiently. Second, one could, again, consider the role cortisol levels might have played. Specifically, it may be possible to classify this male sample as being similar to the high cortisol responders described by Meyer and colleagues (2012; see also Kitraki et al., 2004), who noted that significant increases in cortisol levels were accompanied by an improvement in spatial performance. However, given that there is no specific level at which cortisol enhances (or impairs) cognitive functioning, one cannot be convinced entirely by this explanation. Third, exploration into other factors that might have contributed to enhanced mental rotation performance is needed before any firm conclusions can be made about the present result. For example, sex differences in performance factors on mental rotation tasks may play a role here. One such factor is the strategy utilized in mentally rotating an image. Previous research has shown that men use a holistic process to mental rotation, whereas women use a step-by-step strategic approach (Heil & Jansen-Osmann, 2008; Hugdhal, Thomsen, & Ersland, 2006). Therefore, one might hypothesize that exposure to stress facilitated the former strategy but not the latter.

Spatial Navigation Performance: Unstressed condition (Day 1)
Qualities of the CG Arena. Regarding development of the spatial navigation task, analysis of path length data from the visible-target CG Arena trials conducted on Day 1 of the experimental procedure detected no sex differences in performance. This pattern of data suggests that men and women were equally capable of navigating, using basic visual-perceptual and motor skills and stimulus-response strategies, in the CG Arena.

Furthermore, analysis of path length data (specifically, data regarding deviation from optimal path length) from the Day 1 acquisition trials showed that participants were able to place learn adequately. Additionally, the linear learning trend observed across the acquisition trials was similar to that found by W. Jacobs et al. (1998) in their development of a similar CG Arena (see also Skelton et al., 2000; K. Thomas et al., 2010). Taken together, these results indicate that once the location of the target was acquired, participants could relocate it easily and consistently in an environment whose crucial features and dimensions remained unchanged.

Analysis of the Day 1 CG Arena test-trial data suggested that participants relied primarily on proximal landmark cues, rather than on distal gradient cues (i.e., objects located within the Arena, rather than on the Arena walls), to locate and relocate the target. Task performance declined markedly when the position of the two landmark cues was changed, or when one of them (i.e., the object in the Northeast quadrant) was removed from the Arena.

Participants also displayed a marked decline in performance when the walls of the Arena were rotated in an anticlockwise direction. This result is consistent with previous studies showing that changing the relations among distal stimuli in an environment disrupts place-learning performance, even after the location of a target has been learned successfully (see, e.g., Fenton et al., 1994; W. Jacobs et al., 1998; Suzuki et al., 1980).

The Day 1 CG Arena test-trial data also delivered some unexpected results, however. For instance, task performance improved when one of the proximal landmark cues (i.e., the
object in the Southeast quadrant) was removed. One possible explanation for this unusual result revealed itself upon close examination of this specific object-removal trial: Optimal length (i.e., the shortest possible distance from start point to target) was shorter than the average path length participants took on the baseline trials (recall that baseline performance was calculated as the average performance across acquisition trials 6-8). Hence, because task performance on test trials was always measured against performance on the baseline trials, the current finding may simply be a product of poor environment design.

Other unexpected results were seen on trials that involved removal of distal gradient cues. Although previous studies (e.g., Fenton et al., 1994; W. Jacobs et al., 1998) suggest that removal of a subset of distal stimuli should not affect place-learning performance, in the current study the elimination of particular distal cues affected participants’ success in relocating the target. Specifically, task performance improved when the wall directly opposite the target (i.e., the West wall) was eliminated, as well as when the corner two walls opposite the target (i.e., the walls comprising the Southwest corner) were eliminated. It is unclear why participants’ navigational performance improved with the removal of these specific distal cues. One possibility may relate to the fact that the two distal cue elimination trials both involved the West wall, which contained both distal gradient cues and a distal landmark cue and was situated directly opposite the location of the target. It is possible then, that participants utilized the position of this distal landmark cue in relation to the proximal landmark cues in order to relocate the target. The removal of this cue, then, may have been more noticeable to participants, thus making it easier to relocate the target (as its absence would have been easily identified). However, further research is needed to shed light on what factors might have played a role in facilitating such improvement.

Taken together, these results suggest that, as in previous CG Arena studies (e.g., W. Jacobs et al., 1998; K. Thomas et al., 2010), other VE navigation studies (e.g., Picucci et al.,
participants utilized both landmark and distal gradient cues to navigate successfully toward (i.e., to locate and relocate) a target that was in a fixed position across trials.

**Sex differences under unstressed conditions.** Regarding the hypothesis that, under normal, unstressed conditions, men would outperform women on spatial navigation tasks, analysis of acquisition trial (hidden target) performance on the Day 1 CG Arena task found that men showed a significantly steeper learning curve than women. In other words, although all participants took shorter routes to the target the more they were exposed to the environment, the reduction in deviation from optimal path length to target as the block of acquisition trials proceeded was quicker in men than in women. One might therefore conclude that, in learning the location of the hidden target, men used the available cues more efficiently than women did.

This set of results is consistent with findings reported by Picucci and colleagues (2011), who demonstrated, in a VE task that contained both geometric and landmark information, that women covered more distance and spent more time before correctly reaching the target. Hence, it appears that, despite the inclusion of both landmark and geometric cues, men are more efficient than women at learning the location of a target in an environment that whose features remain constant.

Regarding performance on the Day 1 CG Arena test trials, there were no significant sex differences on trials that featured removal of distal cues or swaps of distal and proximal cues. Men performed better than women on a trial that featured the removal of a proximal landmark cue, however. This piece of data is generally consistent with findings from previously published studies (Chamizo et al., 2014; Gabriel et al., 2011; Picucci et al., 2011; Rodriguez et al., 2011; Sandstrom et al., 1998) which report that the conventionally observed
sex difference in spatial navigation performance (i.e., that men outperform women) may be attributed to the types of cues made available in the navigation tasks utilized. These studies showed that the incorporation of landmark cues in navigational tasks resulted in better navigational performance by women (with navigation performance improving to almost match that of males).

Similarly, the current study incorporated a variety of cue types (both distal and proximal landmark cues, as well as distal gradient cues), thus allowing men and women to have access to, and to utilize, the cues and strategies that work best for them. In doing so, the design of the CG Arena and of the study allowed me to clarify why there might be sex differences in navigational ability. Similar to findings reported by Sandstrom and colleagues (1998), it appears that men and women perform equally well when both landmark and geometric cues are available. However, when the landmark cues were removed, performance of the two sexes was markedly different: Women struggled to relocate a previously-learned target, but men did not. These results may therefore provide further evidence for the different navigational strategies preferred by men and women, as it appears that the availability of cue types plays a significant role in each sex’s ability to navigate toward a previously-learned target.

**Spatial Navigation Performance: Stress Condition (Day 2)**

Regarding the hypothesis that stress will impair spatial navigation performance, analysis of path length data from the Day 2 CG Arena acquisition trials showed that (a) all participants were able to place learn adequately, and (b) on most trials, participants performed similarly in the Day 2 Arena as on the analogous Day 1 trial. The sole exceptions here were (a) acquisition trial 3, on which the performance of all participants, on average, improved from Day 1 to Day 2, and (b) acquisition trial 8, on which male participants, but not female participants, performed more poorly on Day 2 than on Day 1. (Separate sections
below provide further discussion of sex differences in Day 2 performance, and in Day 2 versus Day 1 performance.) Hence, these data tend to disconfirm the a priori prediction.

Further regarding the abovementioned hypothesis, analyses of data from the Day 2 CG Arena hidden-target test trials (comparing those data to data from the analogous Day 1 trials) suggested that, on most of the trials, there was no correlation between increased stress levels and spatial navigation performance. On some trials, however, there appeared to be an negative correlation between stress and performance. Specifically, under stressful (Day 2) compared to non-stressful (Day 1) conditions, performance was significantly more impaired on elimination trials (e.g., when the proximal landmark cue in the Southeast quadrant, or when the distal cues directly opposite the target (i.e., West wall, Southwest corner), were removed from the arena) and on a swap trial (i.e., when the distal cues were rotated clockwise).

Taken together, these results suggest that, overall, stress does not appear to impair place learning, but that it might impair navigational performance when there is a change to the environment in which the place has been learned. The former result is inconsistent with those from numerous previous studies (e.g., showing that place learning is impaired following stress exposure (Beiko et al., 2004; Holscher, 1999; Sandi et al., 2005; Snihur et al., 2007). However, most of these studies involve rodents, and hence do not use virtual environment. Again one must also consider the result of not controlling for order effects. These disconfirmations of the a priori prediction may be due to the similarity of the temporal administration of tasks across the two Days and therefore loss of novelty to the overall experience. Of note here, though, is that, using a slightly different assessment of spatial ability (i.e., a 3-D spatial learning task), Schwabe et al. (2007) found that TSST exposure was associated with decreased use of spatially-based strategies (and increased use of S-R based strategies) in humans.
Furthermore, the Day 2 test trial results are consistent with the Day 1 results discussed earlier in suggesting that, to relocate the target, participants relied primarily on the proximal and distal cues mentioned above (i.e., the proximal landmark cue in the Southeast quadrant and the distal cues featured on the West wall and Southwest corner). Specifically, when these cues were removed, participants’ ability to relocate the target was significantly impaired under control conditions, and even more so under stressful conditions. One might suggest, therefore, that stress exposure breaks down the ability to relocate a learnt target when environmental changes occur (particularly when those changes affect cues that have been relied upon to learn, locate, and re-locate the target). This finding confirms, but only partially (i.e., only with regard to test trials, and not with regard to acquisition trials), the hypothesis that stress has an impairing effect on navigational performance.

However, the more important interpretations of the effects stress has on navigational performance are those that follow the analyses that included sex as a factor. This is because the effects of stress on navigation performance might differ significantly between men and women; analyses involving only pooled data ignore potential sex differences, and so do not provide a complete account of the observed patterns of performance.

**Sex differences under stress: Acquisition trials.** Regarding the hypothesis that stress will impair spatial navigational performance in both men and women (but more so in women), there were no sex differences in learning curves on the block of Day 2 acquisition trials. This result disconfirms the a priori prediction, and stands in contrast to the sex differences observed on the Day 1 acquisition trials (when, recall, men outperformed women).

Further analysis of data (i.e., RM-ANCOVA analyses of the Day 2 acquisition trials and the analogous Day 1 trials) did reveal some interesting sex differences, however. Specifically, the analysis detected a significant main effect of Sex (in favor of men) on trials
5, 7, and 8, indicating that, when data from the two days were taken together, men performed significantly better than women. Furthermore, although on all three of those trials men and women showed a general decline in performance from Day 1 to Day 2, their changes in performance across the two days was not significantly different (i.e., the main effect of Time was not significant).

More importantly, however, Sex (men vs. women) x Time (control [Day 1] vs. stress [Day 2]) interactions showed that women’s performance improved significantly more than did men’s on acquisition trial 3. Furthermore, on trial 6 men’s performance declined relative to Day 1, whereas women’s performance improved. Taken together, the findings from trials 3 and 6 may suggest that, in a virtual environment containing both landmark and gradient cues, there is an improvement (relative to performance under unstressed conditions) in female navigational performance after exposure to a psychosocial stressor. Although this result disconfirms the a priori prediction, it is consistent with data presented by Gabriel et al. (2011), who reported that, compared to unstressed controls, women exposed to a stressor showed better performance on a cue perception task, whereas men’s performance generally remained unaffected compared to unstressed controls.

**Sex differences under stress: Test trials.** Regarding the hypothesis that stress will impair spatial navigation performance in both men and women (but more so in women), analyses of data (i.e., RM-ANCOVA analyses of the Day 2 test trials and the analogous Day 1 trials) suggested that, on some trials, men’s performance was significantly better than women’s. Specifically, a significant main effect of Sex was found in favor of men on elimination trials (i.e., when the North, West, and Southwest distal cues were eliminated individually, when all the distal cues were removed, as well as when the proximal landmark cue in the Northeast quadrant was removed) and on a swap trial (i.e., when the distal cues were rotated in an anticlockwise direction).
Furthermore, analysis of the Sex (men vs. women) x Time (control [Day 1] vs. stress [Day 2]) effects detected some significant interactions. Specifically, it appears that stress enhanced men’s performance but impaired women’s performance on trials that featured (a) removal of the North and Northeast distal cues, (b) rotation of the distal cues in an anticlockwise direction, and (c) a change in location of the proximal landmark cues (i.e., when the objects were switched with each other, or moved to the other side of the Arena while remaining in the same relationship to one another). Finally, on the trial that featured clockwise rotation of the distal cues, women showed more impaired navigational performance on Day 2 than on Day 1, whereas men’s performance remained relatively constant.

The sex differences that were observed suggest that stress exposure appears to have negatively affected women’s ability to relocate the target under fluctuating environmental conditions. Interestingly, the opposite effect is seen with men: Their ability to adapt to the altered room was enhanced following exposure to the psychosocial stressor (e.g., on the elimination trials they used the remaining cues effectively). This pattern of data stands in contrast to previous research, which has generally shown that stress either impairs or does not affect navigation performance (Conrad et al., 2004; Guenzel et al., 2014; Klopp et al., 2012). For example, K. Thomas et al. (2010) demonstrated, using a similar CG Arena program, that women who were exposed to a psychosocial stressor performed more poorly than those who were not, whereas men who were and were not exposed to the stressor performed equally. However, that study, like most navigational tasks used in this research literature, did not focus on differences in performance when the environment in which place learning took place changes. The current results indicate that women’s navigational strategies are broken down when experiencing a stressor in a changing environment; however, it appears that
men’s performance is enhanced when an environment breaks down and is made simpler (in combination with experiencing a stressor).

**Overall findings and implications of results.** In summary, the current study set out to investigate (a) the effects of psychosocial stress on spatial navigation performance in an environment that featured both landmark and gradient cues, and (b) any sex differences in those effects. The main hypothesis of this study (i.e., that stress would impair the navigational performance of both men and women in the virtual environment, but would impair women’s performance to a greater degree) was not confirmed. However, the current analyses did detect some interesting sex differences in navigational performance in the presence of elevated cortisol. Specifically, the findings suggest that, generally, on test trials (i.e., trials that feature elimination of, or changes in relationships among, distal cues, or that feature changes in relations between proximal cues) in a previously learned environment, acute psychosocial stress enhances navigational performance in men, but impairs such performance in women. This improved performance by the men in this study mirrors their mental rotation performance discussed earlier, indicating that men’s spatial ability might, under particular conditions and on particular tasks, be enhanced following exposure to a stressor.

Furthermore, although females have a greater response to stress when they are in the luteal phase of their menstrual cycle, in the general population females differ in the phase of cycle they are in. Hence, the general impairment found in female participants under stressful conditions (in comparison to the general improvement found in male participants) can be considered indicative of a differential sex response to stressful conditions in the population.

The findings presented here may also provide some insight into why there is between-study inconsistency in the literature on sex differences in spatial performance following exposure to a stressor (e.g., K. Thomas et al. [2010] found impaired performance in women, but not men, exposed to a stressor, whereas Gabriel et al. [2011] found enhanced
performance in women, but not men, exposed to a stressor, and Guenzel et al. [2014] found no effects of stress on performance in either men or women. Specifically, by incorporating different types of navigation-aiding cues, the current study was able to better describe the environmental conditions under which sex differences are observed in a spatial navigation task following exposure to a stressor. In particular, sex differences in performance following exposure to the stressor were apparent only when certain aspects of the environment in which place learning had already taken place were altered. For example, when the Day 2 CG Arena environment featured alterations to distal cues nearby the target, analyses detected sex differences (men’s performance on these trials improved whereas women’s performance decreased); however, when that environment featured alterations in distal cues opposite the target, in a distant quadrant, both men’s and women’s performance was affected negatively by stress.

This pattern of results suggests that the types of navigation-aiding cues in an environment (as well as location of these cues relative to the target) do indeed play a significant role in eliciting sex differences in navigational performance following exposure to a psychosocial stressor. Specifically, it appears that sex differences only become apparent when there are changed relations between distal and proximal cues that have been used primarily to relocate a target on previous trials. Hence, studies that intend to assess sex differences in spatial navigation performance under stressful conditions must use navigational environments that contain cues (both proximal and distal cues, that contain both landmarks and gradients) providing the best opportunity for both sexes to learn the location of a target. Most tasks currently used to assess navigational ability are limited in that they do not incorporate one or more of these types of cues (e.g., a task may contain distal gradient cues, but no distal landmark cues or proximal landmark/gradient cues). Hence, one reason for the inconsistent results across previous studies may be the specific cues made available in the
tasks utilized (and, in K. Thomas et al. [2010] and Guenzel et al. [2014], the degree to which those cues were relied upon for navigation).

**Limitations and Directions for Future Research**

Several limitations of the current study should be addressed by future research seeking to explicate more clearly the role that navigation-aiding environmental cues play in stress-induced sex differences in spatial navigation.

**Homogenous sample.** One limitation of this study was the significant between-sex differences in age. Although all participants were between 18 and 25 years old, the average female age tended toward the lower end of the range, whereas the average male age tended toward the higher end. As such, I needed to use Age as a covariate in my primary statistical analyses, in order to rule out any possible influence this variable may have had on the outcomes. As noted previously, RM ANCOVAs produce weaker main effects than RM ANOVAs do (M. Thomas et al., 2009). Therefore, the analyses conducted here may not have detected potentially significant effects that do exist in the population. Future studies should age-match participants more closely, thereby avoiding the statistical need to control for age differences.

**Physiological data.** A second limitation of this study was the fact that, due to hardware malfunction, several sets of heart rate data were not available for use in the final statistical analyses. Furthermore, I did not take any other measures of ANS activity. Many studies in this field (e.g., Klopp et al., 2012; Schoofs et al., 2008) use salivary alpha amylase as a measure of such activity. Future studies should follow that lead so that multiple sources of data on the ANS stress response might be obtained.

Additionally, regarding the observed elevations in cortisol levels, results indicated that, although the FFST was successful in eliciting HPA-axis responses from women, on average the magnitude of response in women was not equivalent to that of men. As
mentioned previously, the female menstrual cycle modulates cortisol response to a stressor. Future research should aim to recruit equal numbers of women in the various phases of the menstrual cycle, thus allowing between-group comparisons of performance under stress in different phases of the cycle, while maintaining the ability to generalize to the population of women.

Furthermore, the level of changes in cortisol showed a main effect for sex. Specifically, it appears that the females of this sample were not affected by the stressor as much as men in terms of levels of cortisol increase. This is an important point to consider, given that a cortisol response underlies the effect of stress on spatial cognition; and has implications for the results found in this study. In particular, one needs to consider whether the differences found were due to navigation being affected by stress induction, or if the means of stress induction was not effective enough to produce stress in women.

**Mental rotation task.** The mental rotation task used in this study has a smaller effect size associated with the advantage for men over women than does a task such as the VK-MRT (Jansen-Osmann & Heil, 2007). The observed lack of sex differences normally apparent under unstressed conditions may therefore have been due to this reason. Hence, future research should consider using a mental rotation task such as the VK-MRT, in order to ensure that the effect is given a better chance of being detected by statistical analysis.

**Spatial navigation task.** The design of the environment in which participants navigated might need to be re-examined. It appears that, on some test trials, the optimal length to the target was shorter than the baseline length (recall that baseline performance was calculated as the average performance across acquisition trials 6-8). One way to control for this is to ensure that the start point is a sufficient distance away from the target on all trials (e.g., in a distant, rather than adjacent, quadrant). This way optimal length to the target for each test trial can be better compared to the optimal baseline length.
Furthermore, some would say that the environment utilized primarily involves egocentric wayfinding and does not adequately allow for an allocentric wayfinding strategy. Cue availability is one of the main variables that has contributed to the observed sex differences in previous research. The current CG Arena created for this study may have led to a confound between the constructs of egocentric and allocentric, given that the features of the “distal” environment were at more proximal to the target location than the “proximal” objects. In order to identify stress effects on spatial navigation, a virtual environment that better integrates the usage of both allocentric and egocentric wayfinding is needed. This could be done by diminishing the cue availability bias more adequately and therefore allow one to obtain more reliable sex differences.

**Summary and Conclusions**

Laboratory research into spatial navigation allows us to identify variables that affect everyday, real-world navigation. Such research therefore allows us to determine ways in which we might manipulate those variables so as to improve the efficiency of our navigation. Two variables that affect navigational performance are biological sex and physiological stress. However, few studies to date have investigated stress-induced sex differences in spatial navigation, and those few studies have delivered inconsistent conclusions and have left much room for exploration. The current study sought to resolve some of those inconsistencies, and to fill important knowledge gaps. Specifically, the purpose of the current study was to clarify the effects of acute psychosocial stress on sex differences in spatial navigation, using a novel stress-induction procedure and a novel spatial navigation task.

The study showed that the FFST is an adequate stress induction method that produces ANS and HPA-axis responses of the kind needed in this field (i.e., the FFST was demonstrated to induce elevated heart rate and cortisol levels in both men and women). The study also described the creation of a CG Arena that contains both landmark and gradient
cues, and that therefore can be used for thorough testing of spatial navigation in future research (with some of the modifications mentioned above). Furthermore, analyses of the data suggested that although stress does not appear to impair navigation performance in an unchanging environment, stress-induced sex differences in spatial navigation are apparent when the relations among various cue types are altered in an environment where place learning has already occurred. Hence, the major contribution of this research is that it demonstrated that features of the environment, and the way in which they are utilized in the service of navigation, are variables critical to understanding why there are sex differences in navigation following exposure to a psychosocial stressor.
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APPENDIX A

Pilot Study

The pilot study was conducted in 2012 at the UCT Department of Psychology. The primary aim of this study was to provide data supporting the proposed methodological improvements as a foundation for a research programme aimed at creating a clearer idea of whether stress-induced sex differences in spatial navigation do exist.

Methods

Design and Setting

The pilot study adopted the same design (2 x 2 repeated-measures factorial design) and methodology described in the current study. Thus the first predictor variable was the participant’s sex (i.e., male or female) and the second was the psychological state of the participant (i.e., stressed or relaxed). Outcome variables were derived from the participant’s scores on the same two spatial cognitive tasks: the CG Arena and a mental rotation 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.

Participants

Fourteen volunteers (9 males, 5 females) between the ages of 18 and 25 were enrolled. Because this study formed 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 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 the same exclusion criteria outlined in the current study.

Materials and Procedure

All materials utilised in the pilot study are described in detail in the Methods section of the current study.

Day 1. All procedures took place in the computer laboratory and the entire session lasted approximately 30 minutes. 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 C). Thereafter, the researcher asked the participant to complete the BDI-II and the Trait form of the STAI. Following completion of these questionnaires, the researcher measured the participant’s weight and height in order to calculate BMI. Thereafter, she administered the MR and CG Arena tasks.

Measures of spatial navigation. The CRT assessed mental rotation ability. For the purposes of the pilot study, the test was split so that the problems on one page were presented on Day 1 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 D).

The CG Arena was used to assess spatial navigation ability. The Arena was created using software previously used in our laboratory. As outlined in the Methods section of the current study, separate Arenas were created for Day 1 and Day 2 testing; however, the general layout and function of each trial remained the same.

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 and the entire session lasted approximately 1 hour.

Physiological and self-report measures were collected at three different times throughout this second session: the first (a baseline measurement) shortly after entering the laboratory, the second was taken 5 minutes following the end of the stress manipulation, and the third 45 minutes after the manipulation ended. The stress induction occurred after all baseline measures were collected.

To measure heart rate, we used the VU-AMS which 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. Participants rated their current level of general negative affect at these times using the NA scale from the PANAS. Participants also rated their current level of anxiety using the STAI-State form. We collected cortisol by means of saliva samples using SARSTEDT Salivette® Cortisol swabs. Once the samples were collected, we stored them 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. To induce stress, we exposed participants to the FFST.

The researcher administered the second page of the CRT after the stress induction. 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.

**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. 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.

**Effectiveness of the Stress Induction Method: Day 2 data**

Regarding the physiological and self-report measures, analysis of responses to the FFST 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. 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 female sample of the pilot study. Descriptive statistics for the physiological and self-report measures are presented in Table A1 below.
### Table A1

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

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

*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). 
*bn = 5; c* n = 6; 
*d* n = 3. 
*e*Measured in nanomoles per litre (nmol/l).

### Qualities of the CG Arena: Day 1 data

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.

Analyses of the Day 1 CG Arena data, comparing performance on each trial to a baseline performance suggested that participants relied primarily on proximal landmark cues (i.e., 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 distal cues (i.e., walls of the Arena) 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).

**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 A2 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.
Table A2

*CG Arena Day 1: Analysis of sex differences in path length across test trials (N = 12)*

<table>
<thead>
<tr>
<th>Trial Group</th>
<th>Males</th>
<th>Females</th>
<th>F</th>
<th>p</th>
<th>ESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall Removal</td>
<td>159.44 (34.38)</td>
<td>181.79 (69.81)</td>
<td>0.53</td>
<td>.49</td>
<td>0.39</td>
</tr>
<tr>
<td>Object Removal</td>
<td>215.88 (182.59)</td>
<td>177.36 (61.01)</td>
<td>0.16</td>
<td>.70</td>
<td>0.27</td>
</tr>
<tr>
<td>Wall Swap</td>
<td>462.30 (585.24)</td>
<td>319.34 (465.87)</td>
<td>0.17</td>
<td>.69</td>
<td>0.26</td>
</tr>
<tr>
<td>Object Swap</td>
<td>536.56 (387.97)</td>
<td>770.47 (750.22)</td>
<td>0.48</td>
<td>.50</td>
<td>0.38</td>
</tr>
</tbody>
</table>

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

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

**MR task.** 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 $\eta^2 = .36$, 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: 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 on Day 2 to analogous trials on Day 1. For elimination trials, all wall eliminations except trial 17 (removal of the west wall) were averaged together. Trials 11 and 25 (object elimination trials) were grouped together. One wall elimination trial (i.e., Trial 17) and one object elimination trial (i.e., Trial
were compared as individual trials because the analysis of Day 1 data indicated that the test manipulations on those trials had the largest impact on path length to the target relative to baseline. Furthermore, all swap trials were compared individually.

Results for the main effect of Testing Stage are displayed in Table A3 below. As Table A3 shows, there were no significant main effects for the relaxed versus stress conditions. The grouping of all the walls (except the West wall), as well as the clockwise swap, showed almost significant results with large effect sizes, $\rho$ (Cohen’s $d$) = .07 (0.74), and .06 (0.74), respectively. However, a significant interaction effect was found between sex and the object swap trial, $F(1, 10) = 3.90$, $p = .03$, $\eta^2 = .41$, which showed 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 the object switch trial; $\rho$ (Cohen’s $d$) = .18 (0.62), $F(1, 10) = 5.14$, $p = .05$, partial $\eta^2 = .34$. Further analysis revealed that females performed significantly better on the object switch trial than did males. No other significant results were obtained however power analyses did reveal that the sample size was too low to determine significant effects.
### Table A3

**CG Arena Day 1 vs. Day 2: Within-subject effects (N = 12)**

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

This pilot study showed 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 was about 40 participants (20 males and 20 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.
APPENDIX B

Example of Advertisement

UCT Department of Psychology SRPP

Dear Students,

This is an opportunity to earn your 3 SRPP points. The study takes place over two days. Day 1 is approximately 30 min and Day 2 approximately 1 hour. You must participate in both sessions to receive your 3 SRPP points. Sessions will be run in the afternoon (between 2.30 and 6.30pm).

To take part in the study you must meet the following criteria:

- Aged 18 - 25
- English first language speaker
- Not on any chronic medication (e.g., asthma, anxiety or depression medication)
- Females NOT on oral contraceptives
- A non-smoker

These are the available sessions*:

1. Friday # @ 2.30 and Saturday # @ 2.30
2. Friday # @ 3.30 and Saturday # @ 4.00
3. Friday # @ 4.30 and Saturday # @ 5.30

If you are interested in participating or have any questions, please email us at: stressandcognition2013@gmail.com.

Regards,

*These days are for example. The two day sessions provided were on a Monday and Tuesday, Wednesday and Thursday, or Friday and Saturday. The 3 time slots remained the same.
Wider University Community

The advertisement below was utilised as part of a larger study, therefore the inclusion criteria of race was not part of the inclusion criteria for the current study.

ARE YOU:
A White Male or Female
An English First Language Speakers
Aged 18 – 25 years
A Non-Smoker
Not on Chronic Medication
(e.g., asthma, anxiety, or depression medication)

IF YOU FIT THE ABOVE PROFILE THEN YOU ARE ELIGIBLE TO TAKE PART!

THIS STUDY IS INTERESTED IN LOOKING AT COGNITIVE PERFORMANCE UNDER DIFFERENT SITUATIONS.

If you are interested in participating, please email us:
APPENDIX C

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

Kevin G. F. Thomas, Ph.D.  Robyn Human, MA  Alyssa Amod
Department of Psychology  PhD Candidate  Honours student
University of Cape Town  Department of Psychology  University of Cape Town
021-650-4608  021-788-5536

Faculty of Health Sciences
Research Ethics Committee
Room E52-24, Groote Schuur Hospital, Old Main Building
Observatory 7925
Tel: 021-406-6338
Fax: 021-406-6411
Email: lamees.emjedi@uct.ac.za

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 D

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 based on data that was collected in a previous study in our laboratory. 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.