The effects of acute stress on spatial navigation in men and women.

by

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B.A., Vancouver Island University, 2010
M.Sc., University of Victoria, 2012

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Psychology

Supervisory Committee

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Abstract

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Stress is known to impair spatial navigation in rat models of declarative memory, and declarative memory in humans, but the effects on spatial navigation in humans are unclear. At least four models have been proposed to account for the cognitive effects of stress, based on the two different physiological stress response systems (the sympathetic-adrenal-medullary (SAM) and the hypothalamic-pituitary-adrenal (HPA) systems) and the effects of these responses on the hippocampus and (sometimes) other subcortical structures. In this dissertation, I examined the effects of an acute (experimental) stressor on human spatial navigation in three variations of virtual Morris water mazes designed to dissociate between hippocampus-dependent (allocentric) and hippocampus-independent (egocentric) forms of navigation. Results were considered in the light of all 4 models. Experiment 1 used a dual-strategy Morris water maze to test whether acute stress influences navigational strategy selection and whether this effect is mediated by the activation of the HPA or the SAM system. Surprisingly, stress increased hippocampus-based strategy selection, and did so in the presence of SAM but not HPA activation. Experiment 2 used new dual-strategy and place mazes to examine the effects of acute stress on both strategy selection and allocentric navigational performance. It also attempted to contrast the effects of stress at a short delay, which would favour mediation by the SAM system, and a longer, 30 minute delay (from stressor onset), which would favour mediation by the HPA system. Contrary to expectations, results revealed no effect of stress when tested immediately and sex-dependent impairments of performance (in females) and allocentric strategy selection (in males) at the delay. Experiment 3 used the same mazes as Experiment 2, plus a new cue maze to examine the effects of acute stress on strategy selection and both allocentric and egocentric navigational performance

after a 30 minute delay. Results confirmed that stress reduces allocentric strategy selection and impairs allocentric performance, but also has sex-dependent effects on egocentric performance: in females, stress enhanced navigation (as expected) but in males, stress impaired it. None of the 4 models provided a good explanation for these results, suggesting that current accounts of the cognitive effects of stress may be inadequate.

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List of Abbreviations

Theoretical Models

MYD	Modified Yerkes-Dodson
TDM	Temporal Dynamics Model

Stress Physiology

HPA	Hypothalamic-Pituitary-Adrenal
SAM	Sympathetic-Adrenal-Medullary
CRH	Corticotropin-Releasing Hormone
MR	Mineralocorticoid Receptor
GR	Glucocorticoid Receptor
NE	Norepinephrine

Stress Measurement

HR	Heart Rate
BP	Blood Pressure
SC	Skin Conductance
CORT	Cortisol
STAI	State-Trait Anxiety Inventory

Stress Induction

PASAT	Paced Auditory Serial Addition Task
TSST	Trier Social Stress Task

Navigation Testing

MWM	Morris Water Maze
uDS	updated Dual-Strategy maze
ITP	Inter-trial Probe
ITSP	Inter-trial Strategy Probe

Other Testing

FAPA	Farm Animals Paired Associates
IGT	Iowa Gambling Task

Acknowledgments

I wish to express my heartfelt thanks to a number of people who helped make this dissertation possible. Without a doubt, I would not have been able to complete this project without the dedicated and insightful support of Dr. Ron Skelton. I have come to learn that a supervisor who is willing to devote as much time and energy to a single graduate student as Dr. Skelton has is extremely rare. He has provided hey support throughout every phase of this project, and helped me overcome a number of difficult challenges. However, his influence on me has extended well beyond this particular piece of work – under his supervision, I have learned a great number of import skills that I will be able to employ in my career going forward. I am extremely grateful for everything he has done for me.

My dissertation committee members have been extraordinarily valuable to me throughout this work. I leaned heavily on the inspiration of Dr. Jacobs' empirical and theoretical work on the effects of acute stress and stress hormones on the brain throughout the development of this research. He has provided valuable advice on my methodology as well. Dr. Tony Robertson has been a huge support to me since I was an undergraduate student at Vancouver Island University. Indeed, without Dr. Robertson, I may not have pursued graduate studies in Neuro/Bio psychology at all. Finally, I owe much to Dr. Adam Krawitz, who graciously agreed to join my committee, despite the fact that my research topic is somewhat distant from his own. Despite this, he proved valuable advice on how to analyse my data.

I am also very grateful to Dr. Sonia Lupien. She is one of the world leaders in research on the effects of stress on the brain, and her work served as an important foundation for the present dissertation. I was humbled when she agreed to become my external examiner, and she has provided excellent feedback that has greatly improved the thesis at the revision stage.

I also owe thanks to a great number of others who helped me with this project in more indirect ways. Tom Ferguson, my labmate, was an excellent sounding board for ideas, and was always available to listen when I needed to vent frustrations (sorry about that). I also owe thanks to those who employed me throughout my graduate work, especially those at the Centre for Academic Communication – Nancy Ami and Laurie Waye – who not only provided funding, but also were extremely flexible and understanding when the pressure from my studies became difficult to manage.

Dedication

To my son (who kept me sane) and my wife (who is, miraculously, still with me).

Chapter 1: Introduction

Stress is important. All animals experience and respond to stress in various forms on a day-to-day basis. The human relationship with stress has wide-ranging implications, from athletics (e.g., Petruzzello, Landers, Hatfield, Kubitz, & Salazar, 1991) to academic performance (e.g., Stewart, Lam, Betson, Wong & Wong, 1999) to psychological (e.g., Horowitz, 1997) and medical (e.g., Cohen et al., 2012) health. Researchers have known for close to a century that acute stress impacts the way we think and what we remember (Yerkes & Dodson, 1908). A rapidly evolving field of research is currently investigating how, why and in what ways this occurs. Over the last 30 years, researchers have begun to understand the complex biopsychological interplay that underlies the impact of stress on how we think. The objective of the present dissertation is to contribute to this research by investigating the impact of stress on the neural systems that underlie human spatial navigation. Using several theoretical models as a guide, this work builds on research in both animals and humans which has demonstrated that the function of certain brain structures is particularly sensitive to the influence of stress hormones (e.g., Dias-Ferreira et al., 2009; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Schwabe, Schächinger, de Kloet, & Oitzl, 2009; Young, Sahakian, Robbins, & Cowen, 1999; see Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Kim & Diamond, 2002; Lupien & Lepage, 2001 for reviews).

Background

Stress.

What does "stress" mean?

Forty years ago, Hans Selye, one of the most influential researchers in the stress field, famously remarked, "Everybody knows what stress is and nobody knows what it is" (Selye,

1973). Despite the difficulties defining stress in a way that applies across all situations and species equally (Koolhaas et al., 2011; Kopin, 1995), certain characteristics of stress are largely accepted. First, environmental challenges perceived as aversive or threatening usually precede stress (i.e., "stressors"). Second, in humans, an unpleasant emotional experience (i.e., a feeling) usually accompanies stress (e.g., fear, anxiety, anger) (S.J. Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). Third, a physiological "stress response" helps the body prepare for and cope with perceived environmental challenges. An important part of the stress response includes the release of stress hormones such as cortisol and adrenaline (Joëls & Baram, 2009). Thus, the present work defines "stress" as an experience that occurs when an event (a stressor) is interpreted as aversive or threatening, which leads to unpleasant feelings and a state of physiological arousal (the stress response) that may lead to changes in cognition and behaviour.

Researchers often dichotomize stress (somewhat arbitrarily) based on its duration and intensity. *Chronic* stress is prolonged, low intensity stress that can be brought about by ongoing life circumstances, such as ill physical or mental health or an overwhelming workload. *Acute*, situational stress is episodic, high-intensity stress. Acute stress can arise from physical (e.g., pain) or psychological (e.g., public speaking) causes. Events perceived to be novel, unpredictable, or uncontrollable (Mason, 1968), or situations that involve socio-evaluative threat (e.g., public speaking; Dickerson & Kemeny, 2004) usually bring on psychologically based acute stress. In naturalistic settings, physical injury, unexpected emergency situations or crises, or any situation involving evaluation (e.g., public speaking, test-taking) often brings on acute stress.

Acute stress is modelled in laboratory settings using standardized tasks that involve physical discomfort or uncomfortable social situations. Perhaps the most popular experimental stressor is the Trier Social Stress Task (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). The

TSST reliably induces psychological and physiological stress by exposing participants to socio-evaluative threat. This is achieved by having participants give a 5-minute speech on an unfamiliar topic (without notes) in front of an audience and video cameras. After giving their speech, participants are given a challenging arithmetic task (e.g., count backward by 7's from 1089 as quickly as possible), during which their performance is checked by evaluators.

Stress physiology.

In order to better understand the effects of stress on brain function, it is important to understand the complex neurobiological mechanisms that may underlie them. When an environmental threat or challenge is perceived, the brain initiates a biological stress response to cope with the challenge. There are two independent systems, or "axes", which operate in concert and mobilize the body's resources in an aversive situation.

First, the rapid sympathetic-adrenal-medullary axis (SAM) system is engaged when sensory information about a potentially threatening environmental stimulus is sent to the hypothalamus, which then activates the sympathetic nervous system via the brain stem (Joëls, Fernandez, & Roozendaal, 2011). One consequence of this is the rapid release of epinephrine from the adrenal medulla. Although epinephrine cannot easily pass through the blood-brain barrier, it is able to influence the brain by activating beta-adrenoreceptors in the vagus nerve. Via the vagus nerve, epinephrine stimulates the release of norepinephrine (NE) from the nucleus of the solitary tract and the locus coeruleus. NE activates the basolateral part of the amygdala, which can activate (in the case of the hippocampus or caudate nucleus) or suppress (in the case of the frontal lobes) other brain structures via direct efferent connections (McGaugh & Roozendaal, 2002; Packard, Cahill, & McGaugh, 1994; Roozendaal, McReynolds, & McGaugh, 2004; Roozendaal & McGaugh, 1997) (see Roozendaal et al., 2009 for a review). In studies that

examine the effects of stress on the brain, changes in SAM axis activity are typically measured through measurement of cardiovascular activity (e.g., heart rate and blood pressure; Elzinga et al., 2005; Zoladz et al., 2011). Another, somewhat less common measure is skin conductance (e.g., Duncko et al., 2007). Increases in any of these measures are taken to reflect increased SAM axis activity as part of the stress response.

The second, slower hypothalamic-pituitary-adrenal (HPA) axis is engaged when threat-related information causes the hypothalamus to release corticotropin releasing hormone (CRH). This, in turn, signals the pituitary gland to release adrenocorticotropin into the bloodstream, which causes the cortex of the adrenal glands to release glucocorticoids (cortisol in humans, corticosterone in rats) into the bloodstream (Joëls et al., 2011, 2006; Lupien et al., 2007). Once released, glucocorticoids easily cross the blood-brain barrier and modulate brain activity in many structures via glucocorticoid (GR) and mineralocorticoid receptors (MR). GRs are widely distributed throughout the brain, while MRs are only found in the limbic system (Lupien & McEwen, 1997; Reul & de Kloet, 1985). Both are highly concentrated in the hippocampus (Lupien & McEwen, 1997; Reul & de Kloet, 1985). In studies that examine the effects of stress on the brain, changes in HPA axis activity are typically measured through measurement of salivary cortisol (e.g., Kirschbaum et al., 1996, Schwabe et al., 2007).

It is worth noting that there is presently no common physiological criterion for what constitutes a “significant” stress response. Some researchers define it as a significant increase in HPA activity (largely ignoring the SAM axis; e.g., Domes et al., 2002; Maheu et al., 2005), while others define it as significant increases in both HPA and SAM activity (e.g., Schwabe et al., 2007; Zoladz et al., 2011) , and many others simply use any significant change in physiology as an indicator of a meaningful physiological response (e.g.Thomas et al., 2010; Duncko et al.,

2007). Perhaps this is reasonable. It could be argued that the question of greatest interest is how stress changes behaviour and cognition, and then the next question is what the underlying physiological mechanism is. In other words, it could be argued that we are not yet sure that a particular increase in heart rate or cortisol is the "true" and complete stress response.

Effects of stress on cognition.

Stress has been shown to affect cognition in a variety of ways. For instance, research suggests that a number of executive functions are sensitive to stress. Studies investigating decision making have suggested that both acute (Porcelli & Delgado, 2009; van den Bos, Harteveld, & Stoop, 2009; Youssef et al., 2012) and chronic stress (Dias-Ferreira et al., 2009) can make individuals less sensitive to the outcomes of their decisions. Other research has linked acute stress to working memory (e.g., Young et al., 1999; see Lupien et al., 2007 for a discussion), where higher levels of stress are linked to poorer performance on working memory tasks. Still other research has shown that stress is inversely related to attentional control (Liston, McEwen, & Casey, 2009). However, because of the neurophysiological links between stress hormones and the hippocampus, much of the research over the past 3 decades has been focussed on the effects of stress on cognitive functions that are supported by the hippocampus.

Declarative memory.

Declarative memory is one important cognitive function that is supported by the hippocampus (Scoville & Milner, 1957) and that is known to be affected by stress and stress hormones. Declarative memory refers to memories of personal experiences or general facts that can be consciously accessed and communicated (Squire, 1992). The relationship between acute stress and human declarative memory has been intensely studied since Kirschbaum et al. (1996) showed that word recall was poorer when participants were exposed to an acute experimental

stressor prior to testing. In a sister study, Kirschbaum et al. (1996) found a similar effect on memory when participants were exposed to exogenously administered glucocorticoids, independent of stress. However, other research in this area has produced inconsistent results, with some studies finding that stress enhances (e.g.,Cahill, Gorski, & Le, 2003; Domes, Heinrichs, Reichwald, & Hautzinger, 2002; Hidalgo et al., 2012; Nater et al., 2007; Payne et al., 2007; Smeets, Giesbrecht, Jelicic, & Merckelbach, 2007; Zoladz et al., 2011), some finding that it impairs (e.g., de Quervain, Roozendaal, & McGaugh, 1998; Diamond et al., 2006; Elzinga, Bakker, & Bremner, 2005; Kirschbaum et al., 1996; Payne et al., 2007; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001; Zoladz et al., 2011), and still others (Hidalgo et al., 2012; Hoffman & al'Absi, 2004; Wolf et al., 2001) finding that it has no effect whatsoever on declarative memory performance.

Spatial navigation.

The present work is concerned with another important cognitive function that is supported by the hippocampus: spatial navigation. There are two known cognitive strategies that can be used to navigate large-scale space to reach a goal. One is *allocentric* navigation, which relies on a cognitive map -- a complex, flexible map-like representation that encodes absolute directionality (e.g., east, west) and configurations of spatial relationships amongst features of the environment (Tolman, 1948). The cognitive map is known to be mediated by the hippocampus (O'Keefe & Nadel, 1978) and both lesion (Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002; Morris, Garrud, Rawlins, & O'Keefe, 1982) and neuroimaging (e.g., Maguire et al., 1998) studies have shown that allocentric navigation relies on the hippocampus. The second type of navigational strategy is labeled *egocentric* navigation, which involves navigation based on simple stimulus-response associations between cues in the environment (usually proximal to the

goal) and/or sequences of body-turns (e.g., “turn left on Mckenzie”). Both lesion and neuroimaging studies have shown that egocentric navigation relies on the caudate nucleus (Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; Maguire et al., 1998; Whishaw, Mittleman, Bunch, & Dunnett, 1987).

Most studies using a virtual Morris water maze have used mazes set up to assess competence in allocentric navigation, though most include a “visible platform” condition to assess egocentric competence (For a review, see Livingstone-Lee, Zeman, Gillingham, & Skelton, 2014). Only a few have set up mazes specifically designed to test egocentric-only navigation (ibid, Livingstone and Skelton, 2007) A few have added an egocentric condition into a basically allocentric maze (Livingstone and Skelton, 2007). However, only the current lab has set up virtual MWM where participants are free to select between the two navigational strategies (Livinstone and Skelton 2007, (van Gerven, Schneider, Wuitchik, & Skelton, 2012).

Effects of stress on spatial navigation performance: Rodent evidence.

Experimental evidence in rat studies consistently shows that acute stress impairs hippocampus-based allocentric spatial navigation (see Cazakoff, Johnson, & Howland, 2010; Lupien & McEwen, 1997 for reviews). In a typical example, Park, Diamond, Conrad, Zoladz, & Fleshner (2008) exposed rats to 30 minutes of predator stress before testing them in a 6-arm radial arm water maze. They found that stress impaired rat performance both during maze acquisition (immediately after stress induction) and during retention testing, 24 hours later. Similarly, stress-level administration of exogenous glucocorticoids usually impairs hippocampus-dependent spatial navigation (e.g., Roozendaal, Griffith, Buranday, Dominique, & McGaugh, 2003; Vicedomini, Nonneman, DeKosky, & Scheff, 1986)(see Lupien & McEwen, 1997 for a review).

Although little research has explored whether stress can modulate caudate-based egocentric navigation, recent research using rodents raises the possibility. Schwabe, Schächinger, de Kloet, & Oitzl (2010) gave mice acute stress or corticosterone injections, and then tested them in either an egocentric or an allocentric spatial task. They found that stress or corticosterone injections impaired performance on the allocentric task, but left performance in the egocentric task unaffected. Quirarte et al. (2009) showed that corticosterone, infused directly into the caudate nuclei of male rats, improved performance on a cue-based egocentric MWM. In another study, Wingard and Packard (2008) activated the amygdala of male rats by infusing it with an anxiogenic drug (which mimics the actions of norepinephrine released during stress), then tested their performance on either an allocentric or an egocentric plus maze. They found that amygdala activation led to impaired performance on the allocentric maze, and enhanced performance on the egocentric maze. They attributed this to neural modulation by the amygdala of the hippocampus and caudate nucleus, respectively. Together, the evidence from these studies suggests that acute stress may enhance egocentric navigation, possibly via the release of stress hormones which act directly on the caudate or indirectly through the amygdala.

Effects of stress on spatial navigation performance: Human evidence.

There has been surprisingly little research into the effects of stress on human spatial navigation, largely because studies of the relationship between stress and hippocampal function have focussed on declarative memory (which cannot, by definition, be studied in rats). To date, there have only been four studies on the effects of acute stress on allocentric spatial navigation (Duncko, Cornwell, Cui, Merikangas, & Grillon, 2007; Guenzel, Wolf, & Schwabe, 2014; Klopp, Garcia, Schulman, Ward, & Tartar, 2012; Thomas, Laurance, Nadel, & Jacobs, 2010), one of

which also investigated its effects on egocentric navigation (Guenzel et al., 2014). All four studies used computerized virtual versions of traditional Morris water or radial arm mazes.

The results of investigations of the effects of stress on human spatial navigation have been less consistent than rat studies. The earliest study was conducted by Duncko et al., 2007. Experimenters induced stress in a group of male participants using a short-duration physical stressor (the Cold Pressor Task). They then tested navigational performance in an allocentric MWM 40 minutes later. Interestingly, they found that stress enhanced performance. It is worth note, however, that the stressor did not lead to significantly elevated cortisol levels. In a second study, Klopp et al. (2012) sought to replicate Duncko et al.'s (2007) findings using a similar procedure, but with a psychosocial stressor (the Trier Social Stress Test; TSST; Kirschbaum et al., 1993) and a sex-balanced sample. Although they were able to demonstrate that the stressor markedly elevated both SAM and HPA axis markers of acute stress, they found no effects of stress on navigational performance whatsoever. In another study, Thomas et al., (2010) also induced stress using the TSST and tested navigational performance in an allocentric MWM 30 minutes later. In contrast to Duncko et al. (2007), they found that stress impaired performance, but only for females, while male performance was unchanged. In this study, markers of SAM axis activation were elevated, but HPA axis activity was not measured. Most recently, Guenzel at al., (2014) gave male and female participants a hybrid stress task which incorporated both physical and psychosocial elements (the Socially Evaluated Cold Pressor Task; Schwabe, Haddad, & Schachinger, 2008). They then tested participants' navigational performance in separate egocentric and allocentric virtual radial arm mazes 25 minutes after stress induction. The authors found no impact of stress on performance in either task, despite significant increases in both SAM and HPA axis activity.

Effects of stress on spatial navigation strategy selection.

In the studies reviewed so far, the effects of stress on spatial navigation have been examined in situations where only one strategy (either a hippocampus-based allocentric or caudate-based egocentric) was available to the navigator at a time. Under naturalistic conditions, however, individuals can often solve navigational problems using either allocentric or egocentric strategies, or both. Accumulating evidence suggests that people are capable of acquiring and employing egocentric and allocentric strategies, and switching between them as the circumstances demand (Iaria et al., 2003; Igloi, Zaoui, Berthoz, & Rondi-Reig, 2009; Ferguson, van Gerven, & Skelton, 2015). Individuals can be predisposed to select one strategy over the other, although the factors that govern this bias are not well understood (van Gerven, Schneider, Wuitchik, & Skelton, 2012). It has been suggested that stress or stress hormones, through their actions on the amygdala, caudate and hippocampus, may be one factor that can bias navigational strategy selection (e.g., Schwabe et al., 2010a; Wingard & Packard, 2008). This idea is based largely on a) the neuroanatomical connections between the amygdala, hippocampus and caudate nucleus (Roozendaal et al., 2009), b) the distribution of glucocorticoid receptors in these areas (Lupien & McEwen, 1997), and c) observations about the effects of stress or stress hormones on navigational performance (i.e., in rats, stress or stress hormones usually impair hippocampus-based navigation, but enhance or have no effect on caudate-based navigation).

Two rodent studies have examined the possibility that stress or stress hormones can shift navigational strategy selection. Kim, Lee, Han, & Packard (2001) exposed rats to tail shocks, and then trained them in a modified MWM that could be learned egocentrically or allocentrically. A specially designed probe trial, which could discriminate between the two strategies, revealed an effect of stress on strategy selection. Specifically, the unstressed control animals exclusively

used an allocentric strategy, while half of the stressed animals used an egocentric strategy. In a more recent study, Schwabe et al. (2010) exposed mice to restraint stress or injected them with glucocorticoids, then trained them in a dual-solution navigation task that could be solved egocentrically or allocentrically. Similar to Kim et al.'s (2001) results, Schwabe et al. (2010) found that acute stress shifted mouse strategy selection from allocentrically-dominated to egocentrically-dominated. They extended Kim et al.'s (2001) findings by showing that administration of glucocorticoids produce the same effect.

To date, there have been no studies that examine whether acute stress can shift navigational strategy selection in humans. There are two studies, however, that raise the possibility. In one study, Schwabe et al. (2007) tested human participants in a task that required them to learn the correct card of 4 on a doll-sized table in a model room (1 cubic foot). By moving the one proximal cue on the table, they determined whether the participants had adopted a cue-based ("stimulus-response") or a configuration-based ("spatial") strategy. Consistent with rodent research, they found that stress increased the likelihood that participants would choose an egocentric strategy. In a related study, Schwabe, Oitzl, Richter, & Schächinger (2009) tested the effects of exogenous glucocorticoids on female strategy selection in the same model room task. Surprisingly and in contrast to their findings on the effects of stress on strategy selection, they found that glucocorticoids dose-dependently increased the likelihood that participants would choose an *allocentric* strategy. It should be noted that the task used in both of these experiments did not require navigation, and it is not clear whether it required the formation or use of a cognitive map. Indeed, acquisition and use of static spatial relations such as these have been more often attributed to the parietal lobe (Colby & Goldberg, 1999; Karnath, 1997).

Summary.

This review of the literature suggests that acute stress or stress hormones affect hippocampus-based forms of cognition, such as that required for spatial navigation. In rodents, stress or stress-levels of glucocorticoids generally impair performance in navigation tasks that require hippocampus-dependent cognition, and may enhance performance in tasks that require caudate-dependent egocentric navigation. The picture is less clear when it comes to studies of human navigational performance under stress. Acute stress can enhance, impair, or have no effect on allocentric (hippocampus-based) navigational performance. At the same time, acute stress may enhance or have no effect on egocentric navigational performance. Although few studies have examined the effects of acute stress on strategy selection in rodents, the evidence thus far suggests that acute stress leads to a bias towards egocentric navigation. As yet, there have been no studies examining the effects of acute stress on navigational strategy selection in humans. However, results from a study into the effects of acute stress on non-navigational spatial cognition paradigm were consistent with rodent research, suggesting that stress may also shift human navigational strategy selection from allocentric to egocentric.

Understanding the effects of stress on spatial navigation.

Much of the acute stress literature uses one of several cognitive-neuropsychological models to understand the effects of stress on cognition, especially hippocampus-based cognition. These models are often used to understand the relationship between stress and declarative memory (e.g., Kuhlmann, Piel, & Wolf, 2005), and sometimes to explain the often conflicting findings (e.g., Finsterwald & Alberini, 2013; Zoladz et al., 2011). However, they may also help to understand the confusing effects of stress and stress hormones on human spatial navigation. These models are based on neurophysiological mechanisms underlying the stress response, but

emphasize different elements of the stress response (e.g., HPA axis, SAM axis, or both) and different neuroanatomy. They all hinge upon direct or indirect actions of adrenal stress hormones on the hippocampus. Much of the evidence for such models comes from rodent studies, though efforts are now being made to apply them to human research.

Two "single-system" theories.

The Modified Yerkes-Dodson.

The Modified Yerkes-Dodson (MYD) model, based on the early work of Yerkes and Dodson (1908), seeks to explain the effects of stress on hippocampal function in terms of interactions between the adrenal stress hormone cortisol and hippocampal neurons (de Kloet, Oitzl, & Joëls, 1999; Metcalfe & Jacobs, 1998; cf. Finsterwald & Alberini, 2013; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). The central idea guiding the MYD model is that cognition peaks when there is an optimal level of cortisol present in the brain, but too high or too low levels of cortisol leads to cognitive impairment.

Metcalfe and Jacobs (1998) significantly advanced the MYD model by defining the mechanism by which stress-induced cortisol enhances or impairs hippocampus function[1]. Metcalfe and Jacobs proposed that the effects of stress on hippocampal function are tied to the occupation ratio of two different receptors for cortisol (cf. Lupien et al., 2007; Finsterwald & Alberini, 2013), both of which are densely concentrated in the hippocampus and have been linked to memory processes (Oitzl & De Kloet, 1992; Lupien et al., 1997; de Kloet et al., 1999; see Finsterwald and Alberini, 2013, for a review). The functions of these receptors are not entirely clear, but one function is thought to be memory modulation (de Kloet et al., 1999; Finsterwald & Alberini, 2013). Some authors have suggested that the MR influence on memory

[1] Note: Others (e.g., Lupien et al., 2007) have attributed this advancement to de Kloet et al. (1999). While de Kloet et al. (1999) refined the model, Metcalfe and Jacobs (1998) had already forwarded the essential elements.

derives from its role in acquisition and integration of sensory information (Lupien & McEwen, 1997) and behavioural reactivity to stimuli (de Kloet et al., 1999), while the GR influence derives from its role in promoting memory consolidation, both in the hippocampus and in other structures (e.g., frontal lobes)(Lupien & McEwen, 1997).

According to the MYD model, when the occupation ratio of MRs to GRs in the hippocampus reaches an optimal balance, memory is enhanced; when the occupation ratio is either too far below or above the optimal level, memory is impaired. MRs have a much higher affinity for cortisol than do GRs, (Joëls & Baram, 2009), and consequently, MRs are nearly saturated even at low levels of circulating cortisol. Occupation of GRs, in contrast, requires a moderate increase in circulating cortisol. Thus, without stress (i.e., when the HPA axis is not engaged), many MRs but only a few GRs are occupied. Increases in cortisol concentrations (commensurate with increases in stress-response intensity) leads to an increased ratio of GR to MR occupation in hippocampal neurons. As this ratio increases, neuronal efficiency is at first enhanced and then impaired, following a Yerkes-Dodsonesque inverted-U function. (Figure 1; Metcalfe and Jacobs, 1998; de Kloet et al., 1999; Lupien et al., 2007; Finsterwald & Alberini, 2013).

Much of the evidence for this model has been provided from both rat and human studies in which MR and GR activation has been pharmacologically manipulated. For example, in rats, pharmacological blockade of MRs or GRs, as well as the saturation of MRs and GRs, results in impairment in hippocampal function (Diamond, Bennett, Fleshner, & Rose, 1992; Oitzl & De Kloet, 1992). However, moderate levels of circulating glucocorticoids, with complete MR and partial GR saturation, results in enhancement (Diamond et al., 1992). Similar findings have been observed in humans. For example, Lupien et al. (2002) administered a glucocorticoid synthesis

inhibitor to participants (depleting MR occupation) and found significant impairments in declarative memory as a result. Similarly, studies that pharmacologically saturate MRs and GRs generally find memory impairment (see Het, Ramlow, & Wolf, 2005 for a review).

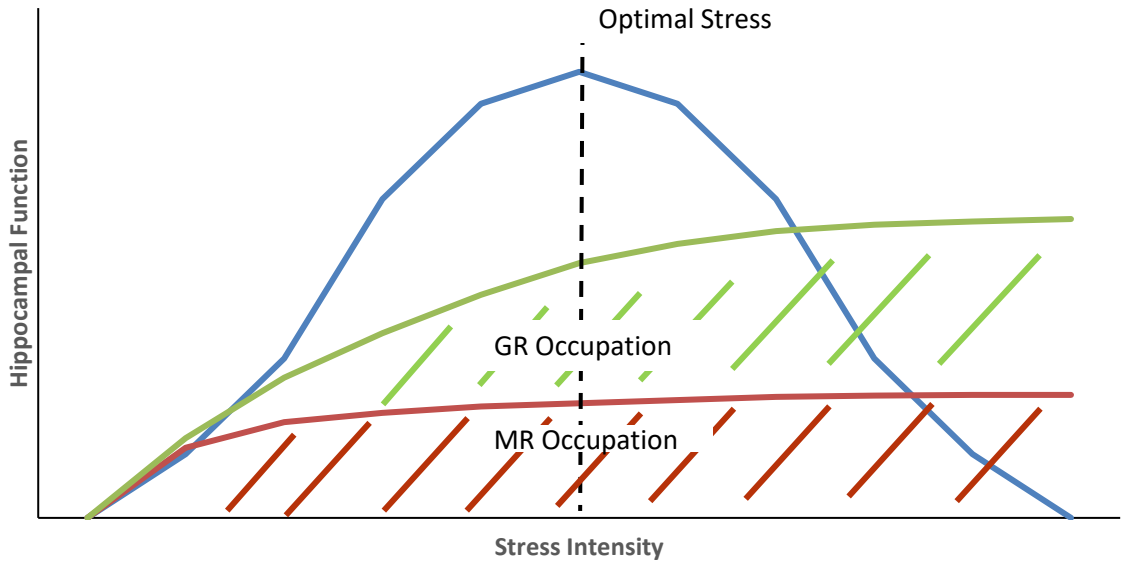


Figure 1. The Modified Yerkes-Dodson model.
Hippocampal function changes with increased stress intensity as a function of the changing occupation ratio of MRs and GRs. Modified from Finsterwald & Alberini, 2013.

The Temporal Dynamics model.

Another model that attempts to explain the effects of acute stress on hippocampal function is the Temporal Dynamics model (TDM; Diamond, Campbell, Park, Halonen, & Zoladz, 2007). This model postulates that the stress response initiates a pattern of time-dependent shifts in hippocampal efficiency (Figure 2), driven largely by changes in SAM axis activity. In the initial phase (phase 1), beginning shortly after the onset of acute stress and lasting for several minutes, the SAM axis activates the amygdala which, in conjunction with NE, CRH, and other neuromodulators, activates the hippocampus, enhancing its function for a brief period. Towards

the end of this period (on the order of seconds to minutes), and as the slower HPA axis becomes more influential, initial (synaptic) actions of glucocorticoids help to sustain hippocampal enhancement. The next phase (phase 2) begins as early as a few minutes after stress-onset and can last for several hours (even days). In this phase, the hippocampus enters a refractory state in which its efficiency is significantly reduced. According to the model, there are two reasons for this. First, the SAM-axis driven excitatory influence of the amygdala on the hippocampus is relatively short-lived (on the order of minutes). Second, stress-induced neuromodulators such as NE and CRH trigger a build-up of calcium in the hippocampus, leading to desensitization of NMDA receptors. This causes a decrease in the excitability of hippocampal neurons, and an increase in the threshold for the induction of long-term potentiation, thought to be a key element of hippocampus-based memory formation.

There is some evidence to support the TDM model. For example, Diamond and colleagues (2006) showed that if rats are trained in a MWM immediately after predator stress (i.e., during phase 1), but not 30 minutes after stress (i.e., during phase 2), performance in the maze is enhanced 24 hours later. Zolatz et al. (2011) replicated and extended this finding in humans. They asked participants to memorize a word list either immediately (i.e., during phase 1) or 30 minutes (i.e., during phase 2) after exposing them to a brief physical stressor (Cold Pressor Task). They found that when they tested participants' memory for the word items 24 hours later, it was enhanced when the list was memorized during phase 1, but impaired when the list was memorized during phase 2. However, other studies that test the effects of acute stress on hippocampal function in phase 1 or phase 2 have both confirmed (e.g., Schwabe, Bohringer, Chatterjee, & Schachinger, 2008; Smeets et al., 2007) and disconfirmed (e.g., Smeets et al., 2006; Takahashi et al., 2004) the predictions of the TDM model.

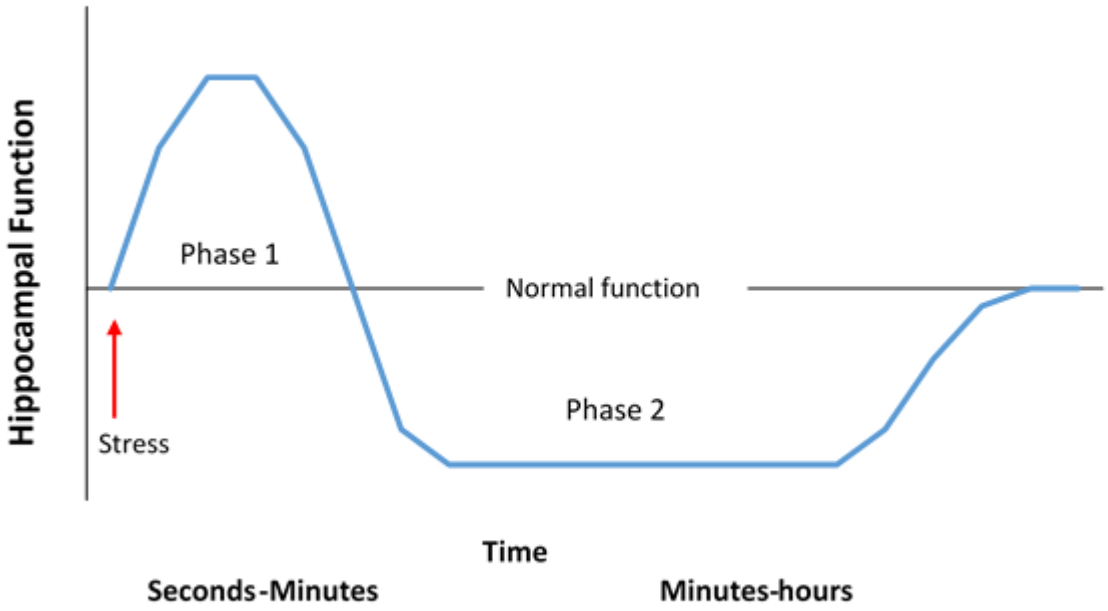


Figure 2. The Temporal Dynamics model.
Hippocampal activity is enhanced shortly after stress, and suppressed at longer delays.
Modified from Diamond et al., 2007.

Comparing single-system theories: Implications for navigation.

There are similarities and differences between the MYD and TDM in terms of their implications regarding the relationship between stress and spatial navigation. An important similarity is that both models focus on the influence of stress and stress hormones on the hippocampus. This means that, according to both models, the effects of stress on spatial navigation should be hippocampus-based, and should thus be manifested in allocentric navigational performance. An important difference between the models is that each focusses on different stress systems. According to the MYD model, effects of stress on hippocampal function (and thus allocentric navigation) should be related to the HPA axis activation. In contrast, according to the TDM model, the effects of stress on hippocampal function should be related to

SAM axis activation. Another key difference between the models is that for each, a different parameter governs the relationship between stress and hippocampal function. For the MYD model, stress *intensity* is key, with medium levels of stress intensity enhancing hippocampal function, and low or high levels impairing it. For the TDM model, stress *timing* is key, with stress enhancing hippocampal function immediately and for a short time after stress, and impairing it with longer delays.

It should be noted, also, that because both of these models are limited to the effects of stress on one neuroanatomical system, it might be argued that their implications for the effects of stress on spatial navigation are restricted to the performance domain. In other words, both make predictions about how stress might influence hippocampus-based allocentric performance, but neither consider how stress might shift behavioural dominance between the hippocampus and other systems, *per se*. Indeed, to interpret the effects of stress on strategy selection from a single-system theory point of view, it must be assumed that behavioural dominance resides with whichever system has greater activity. This assumption is made more explicitly in Dual-System theories.

Two "dual-system" theories.

Hot/Cool Systems model.

One theory that addresses the question of how stress might affect navigational strategy selection is the "Hot/Cool Systems" model, developed by Metcalfe and Jacobs (1998; cf., Metcalfe & Mischel, 1999).This theory dissociates between learning and memory systems that process emotionally neutral information and are associated with controlled, complex, reflective and flexible ("Cool" systems) cognition, versus learning and memory systems that process emotionally charged information and are associated with automatic, simple, reflexive, rigid, and

stimulus-response oriented ("Hot" systems) cognition. The model proposes that the hippocampus is central to Cool systems, while the amygdala is central to Hot systems. In line with the MYD model, the relationship between stress and hippocampus-based Cool system function is HPA driven and is U-shaped, such that low to moderate amounts of stress enhance its function, while too-low and too-high levels impair its function. However, the relationship between stress and the amygdala-based Hot system function is SAM driven and is linear, such that increasing levels of stress continues to enhance Hot system function up to very high levels (Figure 3). Thus, at low-to-moderate levels of stress, both systems are enhanced – the hippocampus-based Cool systems by the HPA and the amygdala-based Hot systems by the SAM—though Cool systems may dominate behaviour. At higher levels of stress, the hippocampus-based Cool systems becomes less efficient, and behavioural dominance shifts to the Amygdala-based Hot systems as they continue to become even more responsive.

To my knowledge, the effects of stress on cognition have not yet been explicitly tested from a Hot/Cool Systems perspective, but there is evidence that supports it. For example, one study (Heuer & Reisberg, 1990) compared memory for emotional and neutral elements of a slide show between participants whose arousal levels were mildly elevated versus participants with normal levels of arousal. After a two-week delay, participants who were mildly aroused had better memory for both emotional *and* neutral components, lending support to the idea that mild stress or arousal enhances both Hot and Cool system function. More recently, several studies have shown that higher, acute levels of stress enhance memory for emotional (i.e., Hot) content while simultaneously impairing memory for neutral (i.e., Cool) content (e.g., Payne et al., 2006, 2007). One interesting study (Schwabe, Bohringer, Chatterjee, & Schachinger, 2008) grouped participants according to how strongly they reacted to an acute stressor, based on salivary

cortisol measurement, and tested their recall for a list of words that contained both emotional and neutral words. The High Responder group remembered emotionally charged (Hot) words better than control, supporting the idea that Hot systems are enhanced at extremely high levels of stress. Furthermore, the Low Responder group remembered neutral (Cool) words better than control, consistent with the idea that moderate levels of stress enhance Cool system function. It should be noted that, in each of these cases, the hippocampus would still need to be functional (albeit at a somewhat reduced capacity), even at very high levels of stress, because it would be required for participants to recall *any* words (emotional or not; Buchanan, 2003).

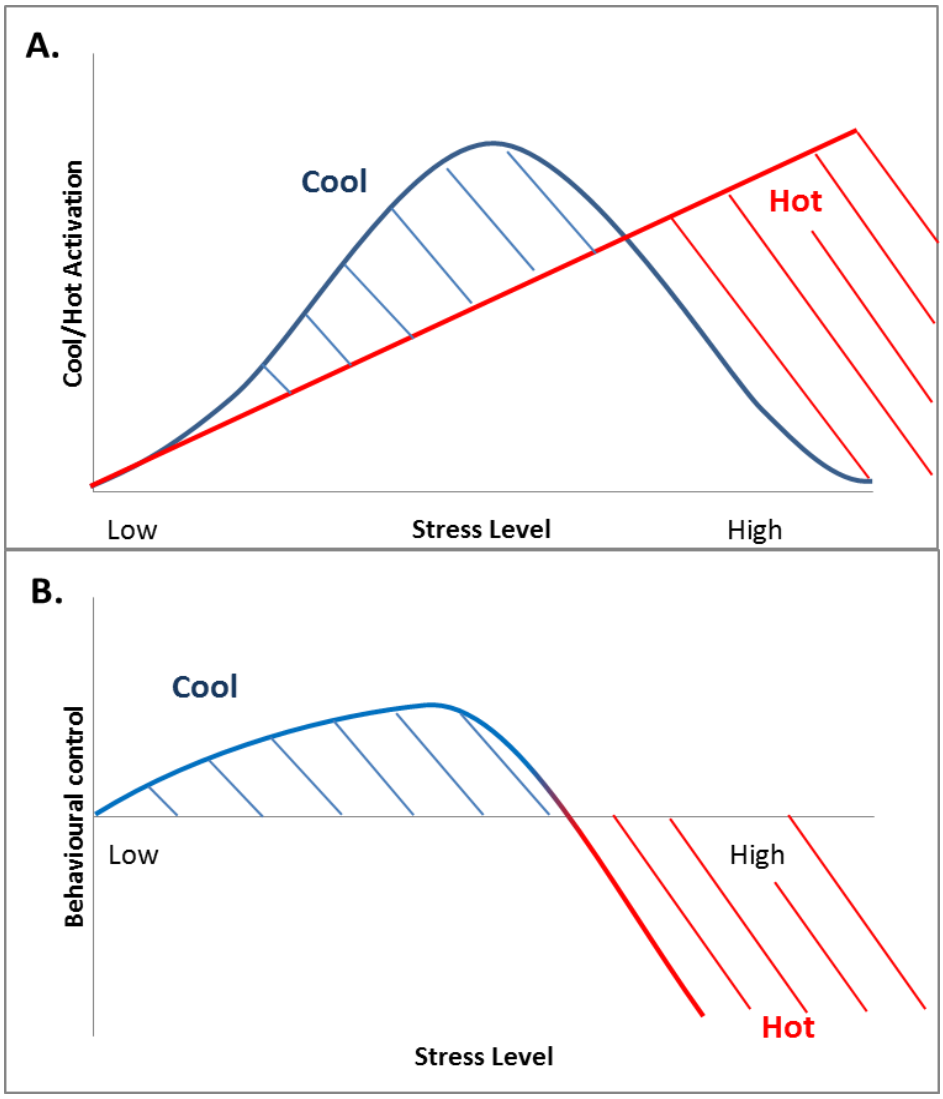


Figure 3. The Hot/Cool Systems model.
A) The relative activation of the Cool and Hot systems in response to increasing levels of stress, adapted from Metcalfe and Jacobs, 1998. B) Relative behavioural dominance of the Cool and Hot systems in response to increasing levels of stress.

The Uniform Shift model.

More recently, Schwabe (2013) developed a model of the effects of stress on brain function that has been dubbed the "Uniform Shift Theory" (Beck & Luine, 2010). In this model, Schwabe proposes that stress and stress hormones can orchestrate a shift in behavioural

dominance from largely hippocampus-centered systems to largely caudate-nucleus-centred systems (Schwabe, 2013; see Figure 4). Associated with this is a shift in learning strategies, from complex, flexible, "spatial" processing to simple, rigid, stimulus-response processing. According to this model, there are two mechanisms by which stress causes this shift. First, under normal conditions, relative activation of the hippocampus and caudate is kept in balance by mutual inhibition. Under stress, hormones released by both the HPA (glucocorticoids) and the SAM (norepinephrine) axes simultaneously enhance caudate and disrupt hippocampal activity, both directly and indirectly via the amygdala. This differential activation amplifies inhibition of the hippocampus by the caudate. Second, the shift in behavioural dominance is facilitated by the amygdala, which acts as a "conductor", biasing behavioural control to the caudate under stressful conditions.

Evidence for the Uniform Shift model comes from both animal and human studies. For example, animal studies using single-strategy navigation tasks have shown that acute stress or glucocorticoid administration impairs allocentric performance but leaves egocentric performance unaffected (e.g., Kim et al., 2001; Schwabe et al., 2010a; Xiong et al., 2003). Rodent research has also shown that stress, glucocorticoids, or intra-amygdalar injections of anxiogenic drugs can dramatically shift strategy selection bias in dual-solution navigation tasks from caudate-based egocentric strategies to hippocampus-based allocentric strategies (Kim et al., 2001; Packard & Wingard, 2004; Packard & Gabriele, 2009; Schwabe, Schächinger, et al., 2009). Moreover, animals that fail to switch saw impaired performance, while switching to egocentric navigation prevented deterioration of performance (Schwabe, Schächinger, de Kloet, & Oitzl, 2010a).

Human studies have also revealed evidence for the Uniform Shift model. For instance, in a behavioural study using a dual-solution spatial task (which did not involve navigation),

Schwabe and colleagues were able to show that acute stress increased the frequency of participants' egocentric strategy use, mirroring the effects they found in mice (Schwabe et al., 2007). Using fMRI, (Schwabe & Wolf, 2012) have been able to show that stress enhances caudate activity and disrupts hippocampal activity, and that these changes in activity are associated with changes in cognitive strategies associated with each. Furthermore, in a second experiment they were able to show strong functional connectivity between the amygdala and the caudate and hippocampus. Under stress, functional connectivity between the amygdala and the caudate increased, while connectivity between the amygdala and the hippocampus decreased (Schwabe, Tegenthoff, Höffken, & Wolf, 2012). This provides some support for the idea that the amygdala plays a prominent role in controlling the relative activation of caudate-based cognitive systems and hippocampus-based cognitive systems under stress.

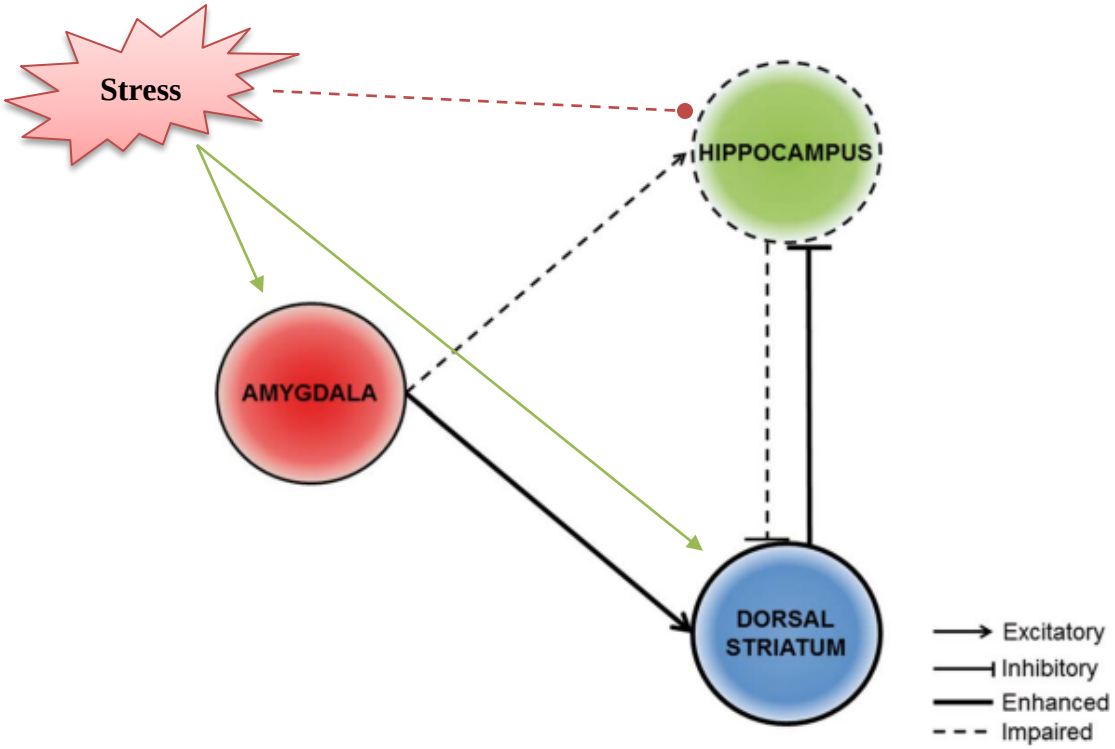


Figure 4. The Uniform Shift model.
Stress suppresses hippocampal function both directly and indirectly, via the amygdala. Adapted from Schwabe, 2013.

Comparing Dual-System theories: Implications for navigation.

There are similarities and differences between the Hot/Cool Systems and the Uniform Shift models in terms of their implications regarding the relationship between stress and spatial navigation. First, an important step in relating each model to spatial navigation is mapping the systems to spatial navigation strategies. This is straightforward for the Uniform Shift theory, which explicitly associates allocentric navigation with the hippocampus and egocentric navigation with the caudate, and posits that stress impairs the former, enhances the latter, and shifts behavioural dominance between the two. The Hot/Cool Systems model can also be related to spatial navigation. Importantly, Metcalfe and Jacobs explicitly associated Cool and Hot cognition with allocentric and egocentric navigation strategies, respectively. The link between

allocentric navigation and Cool cognition is straight forward, both conceptually (both are flexible, complex, reflective) and anatomically (both are hippocampus-mediated). Similarly, egocentric navigation is linked to Hot system cognition conceptually (both are simple, rigid, and stimulus-response oriented). In terms of anatomy, several studies have shown that that when the amygdala is activated by stress or stress hormones, it in turn activates the caudate nucleus (Packard & Wingard, 2004; Schwabe, Tegenthoff, Höffken, & Wolf, 2013; Schwabe & Wolf, 2012; Wingard & Packard, 2008). Furthermore, the amygdala has also been shown to contribute to egocentric navigation directly by attaching valence to particular stimuli (i.e., whether a stimulus should be approached or avoided (White & McDonald, 2002)).

Another important similarity between the dual-system theories is that both models consider the importance of the HPA *and* SAM axes in the relationship between stress and cognition. Thus, both models suggest that the effects of stress on the relative activation and engagement of neural systems involved in spatial navigation behaviour should be related to HPA *and* SAM axis activity.

Like the single-system models, the dual-system models differ in terms of the dimensions of the physiological stress response that they emphasize. Because the Hot/Cool Systems model is partly built upon the MYD model, the Hot/Cool Systems model also emphasizes the intensity of the stressor (and not the timing) as being the key factor in the relation between stress and cognition. In contrast, the Uniform Shift Model does not consider either stress intensity or timing as a major factor.

An important advantage for both of these models (over the Single-System models) is that both consider the effects of stress on navigation in both the performance and strategy selection domains. According to the Uniform Shift model, stress should generally enhance caudate

function and impair hippocampal function, leading to improved egocentric and impaired allocentric navigational performance. In the Hot/Cool systems model, stress should generally increase Hot system activity, which should lead to improvements in egocentric navigational performance. In contrast, stress can increase or decrease Cool system activity (and thus allocentric navigational performance) depending on the intensity of the stressor. (It is worth noting that stress intensity is not considered in the Uniform Shift model, but is an important modulating factor in the Hot/Cool systems model.) For both dual-system models, whichever system is more activated by stress gains behavioural dominance, and this should be reflected in navigational strategy selection bias.

Summary

The four models discussed above provide a means of understanding the effects of acute stress on hippocampus-based cognition. The two single-system theories (MYD and TDM) focus on different stress systems (HPA vs SAM), while the two dual-systems (Hot/Cool Systems and Uniform Shift) incorporate both stress systems. It is worth noting that none of the models incorporate both dimensions of the stress response—intensity and timing. The MYD and Hot/Cool Systems models focus on stress intensity, while the TDM model emphasizes stress timing. For the Uniform Shift Model, neither timing nor intensity are considered. These dimensions could be important in understanding how acute stress influences hippocampus-based cognition.

The role of Sex

A key factor in both stress and spatial navigation research is Sex. Sex is of particular interest because it is rarely considered in rodent research. In the human stress research, some studies suggest that males exhibit stronger physiological reactivity to acute psychological

stressors such as the TSST (both in terms of HPA and SAM axis activity; Kirschbaum et al., 1992; Kirschbaum et al., 1999). However, more recent research has shown this finding to be inconsistent (Guenzel et al., 2014, Wolf et al., 2001, see Kudielka and Kirschbaum, 2005 for a review). In contrast, females have been shown to exhibit stronger psychological reactions to psycho-social stressors (Kelly et al., 2008, Payne et al., 2007). Sex differences are more consistent in spatial navigation research, where a male advantage is one of the most reliable and robust findings. Males outperform females especially in tasks that are hippocampus-dependant (Astur, Ortiz, & Sutherland, 1998; see Coluccia & Louse, 2004, Lawton, 2010, and Voyer, Voyer, & Bryden, 1995, for reviews), including both real world navigation (Saucier et al., 2002), and 3D virtual tests of spatial navigation.

It is possible that sex differences in either dimension of stress reactivity (physiological or psychological) could interact with, or be the cause of, sex differences in spatial navigation ability. For example, Sindi, Fiocco, Juster, Pruessner, and Lupien (2013) showed that experimental testing situations themselves constitute stressors strong enough to elicit physiological stress responses (though they did not test for sex differences). It is easy to imagine a situation where females (having high spatial anxiety; Lawton, 2010) react more strongly than males to a test of spatial navigation ability, and the resulting increase in cortisol suppresses hippocampal function, leading to an impairment in performance. For this reason, sex differences in both stress reactivity and spatial navigation ability are closely watched in this dissertation.

The Present Research.

The primary purpose of this dissertation is to deepen our understanding of the relationship between acute stress and spatial cognition. To do so, the present research tested the

effects of an acute stressor on spatial navigation and spatial strategy selection using modified virtual Morris water mazes. These data were then examined in light of the 4 current models of the effects of stress on the hippocampus, amygdala and caudate.

The three experiments in the present dissertation are based on the central premise that acute stress, through either the HPA axis, the SAM axis, or both, should modulate hippocampal and possibly caudate function, and that this should be reflected in spatial navigation behaviour. The three experiments seek to understand the exact manner in which stress influences these anatomical structures and spatial navigation, which is predicted differently by the 4 different theories. The experiments tested the effects of acute stress on navigational strategy selection, navigational performance and the relationship between the two, and investigated potential modulating factors, including sex and time of day. All experiments used custom-made, computer-based virtual environments.

Chapter 2: Experiment 1

Introduction

Background.

An important methodological step in experimental stress research is to verify the efficacy of the experimental stressor. The present dissertation is especially interested in the physiological stress response, as this is considered to be the key driver of the effects of stress on cognition (de Kloet et al., 1999; Joëls et al., 2006; Lupien et al., 2007). To induce a stress response, I used a stressful version of the Paced Auditory Serial Addition task (PASAT, Gronwall, 1977). Comparable versions of the PASAT task have been used effectively in the past to activate both SAM (Lejuez, Kahler, & Brown, 2003) and HPA axis responses (McHugh, Behar, Gutner, Geem, & Otto, 2010). I decided to use the PASAT rather than other, more common experimental stressors largely for convenience. Our version of the PASAT maintains most of the elements that are thought to make the TSST stressful (e.g., psychosocial threat, lack of controllability, challenging cognitive task; Dickerson and Kemeny, 2004), but is cost effective and is easy to implement via PC. The TSST, in contrast, would require a large experiment space, video recording equipment, and a larger team of volunteers to act as an audience. The Cold Pressor Task (which involves the participant placing their hand in icy-cold water for extended periods) was also an attractive option. However, the Cold Pressor Task is known to be less effective than psychosocial stressors at activating both HPA and SAM stress responses (McRae et al., 2006).

In line with other acute stress research (e.g., Elzinga et al., 2005; Kirschbaum et al., 1996; Schwabe, Bohringer, et al., 2008), the present experiment used the measurement of HPA and SAM axes as a manipulation check to confirm the effectiveness of the experimental stressor. In Experiment 1, I expected that the PASAT would be effective at inducing a stress response, and

that this would be confirmed by 3 measures of SAM axis activity (Heart Rate, Blood Pressure, and Skin Conductance) and one measure of HPA axis activity (Salivary Cortisol).

The central issue in the first experiment in the present research program was whether an acute stressor, through the activation and influence of the HPA and SAM-stress axes, can influence spatial navigation strategy selection in a task where both strategies are available. This possibility is raised in part by evidence that stress can modulate navigational performance in situations where only one strategy is available. As discussed in Chapter 1, acute stress and stress hormones generally impair rodent performance in navigation tasks that require allocentric processing (only)(Cazakoff et al., 2010), and can enhance or have no effect on performance in navigation tasks that require egocentric processing (only) (Quirarte et al., 2009; Schwabe et al., 2010b). In humans, the effects of acute stress on performance in allocentric navigation tasks are mixed, and may be sex-dependent. For example, Duncko et al. (2007) observed an enhancing effect of acute stress on some performance variables (e.g., heading error) in their all-male study. In contrast, Thomas et al. (2010) found no effect of stress on male navigational performance, but observed a female impairment in navigational efficiency (Thomas et al., 2010). Two other recent studies found no effect whatsoever (Guenzel et al., 2014; Klopp et al., 2012). No effects were found in the one study that tested the influence of acute stress on performance in an egocentric task (Guenzel et al., 2014). More direct evidence for the idea that acute stress can shift navigational strategy selection comes from rodent studies in which both navigational strategies were available to choose from. These have shown that acute stress or stress hormones biased rodents to solve the tasks more egocentrically, and less allocentrically (Kim et al., 2001; Schwabe et al., 2010a). To date, no research has examined the impact of acute stress on navigational strategy selection in humans.

The possibility that acute stress can change navigational strategy selection is not only empirically, but also partly theoretically based. All 4 models of the effects of acute stress on cognition discussed in part 1 suggest that acute stress can shift navigational strategy selection between hippocampus-based allocentric navigation and caudate-based egocentric navigation. For three of these models (MYD, TDM, Hot/Cool Systems) the direction of this shift is variable, and depends on the intensity of the stressor and/or its temporal relationship with the navigational task. For one of the models (the Uniform Shift), stress should generally shift navigation from allocentric to egocentric.

In Experiment 1, I investigated the possibility that stress might change strategy selection. To do so, I exposed participants to an acute experimental stressor (the PASAT), and after a delay, tested their navigational strategy selection in a dual-strategy virtual Morris water maze. Based on both empirical evidence (e.g., Schwabe et al., 2007; 2010a) and the predictions of the theoretical models reviewed (see Part 1), I expected that the acute stressor would cause a stress response, and that this would suppress hippocampus-based allocentric navigation, leading to an increase in egocentric strategy selection. The MYD theory and Hot/Cool Systems model both predict that strong activation of the HPA axis will impair hippocampal function and thus allocentric navigation. The TDM theory predicts that given enough time after the stressor, hippocampal function (driven up by SAM activation) would go into a refractory period, resulting in impaired allocentric navigation. The Uniform Shift theory predicts that SAM and HPA activation together shift function to the caudate and away from the hippocampus, and would therefore impair allocentric navigation.

In line with the 4 theoretical models, SAM and HPA activity is often considered a mediating variable in the effects of stress on cognition. Accordingly, many studies have found

relationships between stress reactivity and performance on hippocampus-based declarative memory tasks (e.g., Domes et al., 2002; Elzinga et al., 2005; Zolatz et al., 2011). No study has examined the relationship between measures of stress reactivity (HPA or SAM) and navigational strategy selection. In the present study, I expected that changes in navigational strategy selection would be associated with changes in measures of stress reactivity**.**

Of secondary interest in Experiment 1 was the possible influence of natural circadian fluctuations in circulating cortisol. These variations are large enough to potentially modulate the impact of stress and stress hormones on hippocampal function (Lupien et al., 2007). There have already been experiments that have compared the effects of stress or stress hormones on hippocampal function during the circadian cortisol peak (morning) to its effects during the circadian cortisol trough (afternoon) and significant interactions have been found (Het et al., 2005; Maheu, Collicutt, Kornik, Moszkowski, & Lupien, 2005). To address the possibility that such an interaction might be reflected in navigation behaviour, the time of day of experimental runs was strictly controlled. Participants were run in the early (8:00-9:30) or late (9:30-11:00) morning only, and this was included as a factor in the analysis (Time of Day; TOD). Because of circadian variation, cortisol levels should be higher in the earlier time slot. Thus, I expected that the added cortisol released in response to acute stress should lead to a stronger effect on hippocampal function. This would be revealed as a Stress condition x TOD interaction, such that there would be a greater effect of stress in the early morning than in the late morning.

Another secondary interest in Experiment 1 was the possible influence of Sex. Sex is an important factor in both spatial navigation and stress research (Beck & Luine, 2010; Lawton, 2010). There are several ways in which sex might influence the effects of stress on navigation. For example, previous research has shown that men exhibit a higher cortisol response to acute

stress than women (Kajantie & Phillips, 2006; Kudielka & Kirschbaum, 2005; Sauro, Jorgensen, & Teal Pedlow, 2003). In addition, some authors have suggested that female sex hormones may protect against the influence of glucocorticoids on the hippocampus (Wolf et al., 2001). Given the possible sex differences in stress reactivity and sensitivity to stress hormones, I expected that the influence of acute stress on hippocampal function would be stronger in males than in females. This would be revealed as a Stress Condition x Sex interaction, such that there would be greater effects of stress on strategy selection in males than females.

A note on performance: Most authors consider performance to be an indicator of ability to navigate via a particular strategy. Supporting this idea are findings that stress impairs performance in allocentric, but not egocentric navigational tasks. However, in a dual-strategy maze, performance is not expected to be a particularly meaningful variable because if a participant is impaired at one strategy, they would be expected to simply switch to another, with no decrement in performance. This was seen in Schwabe et al. (2010a), in which stressed mice that switched navigational strategies from allocentric to egocentric were not impaired at navigation whereas that continued to navigate allocentrically were impaired.

Summary of hypotheses:

1. Manipulation check
    a. The effectiveness of the PASAT will be confirmed by 3 measures of SAM axis activity (Heart Rate, Blood Pressure, and Skin Conductance) and one measure of HPA axis activity (Salivary Cortisol).

2. Experimental hypotheses
    a. The acute stressor will cause a stress response, and this will suppress hippocampus-based allocentric navigation, leading to an increase in egocentric strategy selection.
    b. Changes in navigational strategy selection will be correlated with changes in measures of stress reactivity.
    c. There will be a greater effect of stress on strategy selection in the early morning than in the late morning**.**
    d. There will be greater effects of stress on strategy selection in males than females.

Method

Participants.

A sex-balanced sample of 127 healthy participants was recruited from the University of Victoria undergraduate pool. Participants were pre-screened for English as a first language and normal (or corrected to normal) vision. Using a demographics questionnaire, participants were screened for a history of psychological or neurological problems, with particular attention paid to the exclusion of participants who may be unusually sensitive to stress (e.g., those who suffer from generalized anxiety disorder). No participants were excluded on this basis. Participants were also screened for medications that might interfere with physiological reactivity to stress, such as glucocorticoid medication (e.g., for asthma). However, no participants reported using glucocorticoid medications. Eight participants who were on medication for depression (7) or ADHD (1) were not excluded[2]. Eleven participants were excluded from data analysis for one of four reasons: i) their prior experience with our mazes (2), ii) having English as a second language (2), iii) one felt nauseous during running, and iv) technical failures during or after the run (6). Ultimately, 116 participants were included in the data analysis. The average age of participants was 21.05 years (SEM = 1.62). A total of 8 participants reported that they used hormonal contraceptives, and 2 participants were smokers. These participants were not excluded. Participants were required to provide informed written consent. Ethics approval was obtained from the University of Victoria, Human Research Ethics Committee.

[2] Note: Given the possibility that medications for ADHD and depression may interfere with the physiological reactivity to stress (Gold, Licinio and Wong, 1995), I checked the physiological results with and without these 8 participants. Because the difference was minimal, and there were no changes to the significance (or lack of significance) for any of the physiological comparisons, I chose to include them in the analysis to maximize power.

Materials.

Stressor and manipulation checks.

PASAT.

To induce stress, I used a modified version of a well-known neuropsychological test, the Paced Auditory Serial Addition Task (PASAT; Gronwall, 1977). The PASAT requires participants to rapidly add single-digit numbers presented to them verbally (by the computer). This task requires participants to add each single digit number to the following number. For example, if the first numbers presented are "4", "1", "3", "7", the correct responses would be "5" (4 + 1), "4" (1 + 3) and "10" (3 + 7) and so on. My version of this task consisted of a practice block, with 14 digit presentations, and four test blocks, each with 60 digit presentations. Digits were presented at an easy interval of 2.7s in the practice. The presentation interval was shortened progressively from test blocks one to four to increase the difficulty. In my modified version, the intervals were shorter than in the original PASAT and comparable to those used in previous studies of stress (Gratz, Rosenthal, Tull, Lejuez, & Gunderson, 2010; Mathias, Stanford, & Houston, 2004). Specifically, I used 2.1 s, 1.7 s, 1.3 s, and 0.9 s, for each block respectively. Responses were made by clicking the appropriate digit in a boxed array. To enhance the element of socio-evaluative threat, auditory feedback (in the form of a loud, irritating buzz) announced each error (either a late or an incorrect response). For the control condition, I used an "*un*-Paced Serial Addition Task" (U-PASAT), recently developed in the UVic Spatial Lab. This task was identical to the PASAT in every way, except that the numbers to be summed were presented in pairs and subsequent pairs were not presented until a response was provided. In other words, there was no time pressure and no need to remember anything from the previous digits. In

addition, no error feedback was given. The total time for the PASAT or the U-PASAT (including practice) was approximately 10 minutes. Both were presented using Inquisit (Version 3) software.

HPA axis measurement.

Salivary cortisol was measured to assess the effectiveness of the PASAT to activate the HPA stress axis. Saliva samples were collected using standard oral swabs (Salimetrics). Samples were immediately frozen and stored on-site at -20°C. On the day of assay, samples were thawed and centrifuged at 3000 rpm for 15 minutes. Free cortisol in the samples was then analysed in duplicate using a commercially available competitive enzyme immunoassay kit (ELISA; Salimetrics, LLC). Mean cortisol concentrations were determined by interpolating the mean optical densities of the duplicates using 4 parameter logistic regression (elizaanalysis.com). Intra- and inter-assay coefficients of variation were 5.4% and 13.7%, respectively. The minimum detection limit of the assay was 0.08 nmol/l.

SAM axis measurement.

As is typical in the literature (Duncko et al., 2007; Elzinga et al., 2005, Zoladz et al., 2011), multiple measures were used to assess the effectiveness of the PASAT to activate the SAM axis. Measures included Heart rate (HR), skin conductance (SC) and blood pressure (BP) . HR (in beats per minute) was measured continuously (at 250hz) using a Zephyr^{tm} heart rate monitor (H x M BT Bluetooth) and SC (in microsiemens, µS) was measured continuously (at 10hz) using a Mindfield® eSense Skin Response system. HR and SC measurements were transmitted to an Android smartphone via Bluetooth. Commercially available smartphone applications (*Myfitnesscompanion* and *eSense Skin Response*) were used to collect, process and export HR and SC data in .CSV format to Microsoft Excel for further processing. Systolic and

diastolic BP (in millimetres of Mercury, mm Hg) were measured using an arm-cuff type digital blood pressure monitor (LifeSource).

Subjective stress measurement.

The State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970) was used to assess the effectiveness of the PASAT to induce anxiety, an indicator of the psychological experience of stress. I used the standard two, 20 item portions. In the STAI, responses to each item are assigned a score from 1-4, and these are aggregated into a final State or Trait anxiety score. Higher scores indicate higher levels of anxiety.

Spatial navigation.

Spatial navigation was investigated using the Dual-Strategy maze, a modified version of the Arena maze (Livingstone & Skelton, 2007; van Gerven et al., 2012), a virtual analogue of the MWM. The virtual environment was designed using the editor supplied by Unreal® (Epic Megagames). This environment was experienced in first-person, with a 90º field of view. Navigation was controlled using a game pad with only forward, left, or right functions activated, analogous to the movements available to a rat in the MWM. Location and movement data was recorded during navigation using Unreal® "demo" files and extracted using TRAM® software (Skelton, Ross, Nerad, & Livingstone, 2006).

Maze environment.

Navigational strategy selection and efficiency were assessed on a trial-by-trial basis using the Dual-Strategy maze (Figure 5). The maze environment consisted of a large, square room (visually 75 x 75 m square with 16.5 m high walls). The walls of the room were arbitrarily designated north, south, east and west. The north and south walls each had large single windows through which distinctive distal landscape features can be seen (mountains to the north, a body of

water to the south). The east and west walls each had three smaller windows through which hills that slope from the mountains to the water could be seen. Within the room is a circular arena, 42m in diameter, bound by a 1 m high wall. This prevented the participants from exiting the arena during trials without blocking their view of the windows and outdoor landscape. Proximal navigational cues were provided by an array of 8 distinctive objects perched on the arena walls within the room and aligned along cardinal and intercardinal axes. A small, circular green platform (approximately $1/6^{th}$ the diameter of the arena) represented the navigational goal.

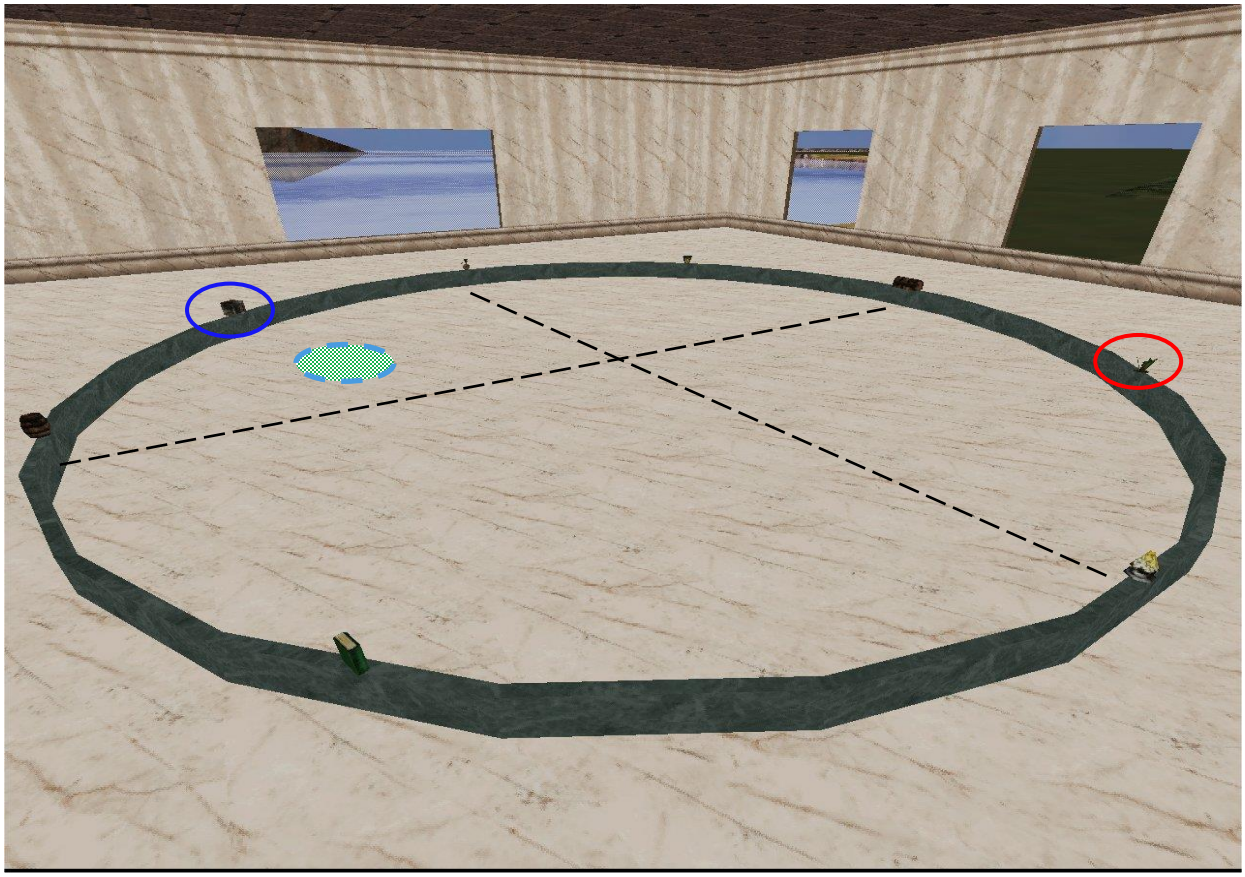


Figure 5. Experiment 1: A Standard trial in the Dual-Strategy maze.
The view is from the northeast from above normal eye-level to highlight the landscape outside the room (allocentric features) and the cue-object (box, blue circle) marking the position of the platform. Note that the platform was not visible until stepped on. In Inter-Trial Strategy Probes, the box switches places with the object opposite (fern, red circle). The trial was designated "allo" if the platform was reported to be located in the original quadrant, and "ego" if it is reported to be in the quadrant to which the box has moved. Dashed lines mark the boundaries of the quadrants.

Trial types: Practice.

Exploration trial. The purpose of the initial *Exploration* trial was to familiarize participants with the virtual environment and game pad controller. The Exploration trial allowed participants to freely explore the virtual environment. The start position was outside the arena, near the east wall, facing inward. Participants were encouraged to look at the landscape through the windows out all sides of the room. This trial ended when participants signal satisfaction with the controls and familiarity with the environment.

Visible Platform trials. The purpose of the four *Visible Platform* trials that immediately follow the Exploration trial was to ensure participants were capable of navigating to a specified target. These trials required participants to navigate to a visible platform as quickly and directly as possible from a start position just inside the arena. The platform was visible first in the center of the arena, then pseudo-randomly in the center of each of the 3 quadrants other than southeast (the location of the platform during testing trials). On these and all trials, a bell sounded when participants reached the platform. Once on the platform, participants were instructed to look around the room without stepping off of the platform and inform the experimenter when they were ready to move on to the next trial.

Pre-test Probe. The final trial in the practice phase was the *Pre-test Probe*. The purpose of the Pre-test Probe was a) demonstrate to participants that there were trials which have no platform and on which they were to go to a place in the room where they thought a platform was, and b) to determine whether participants were able to predict the platform location based on any information gathered to this point. This trial started from the east start position and ended when participants signalled that they had reached their best-guess spot.

Trial types: Testing.

Standard trials. The purpose of the ten standard, invisible platform trials (*Standard* trials*)* was to assess spatial navigation efficiency. These trials required participants to find and return to an invisible platform that was always located in the center of the SE quadrant. The platform remained invisible until stepped on, at which point it became visible and rose out of the floor with the now-familiar bell sound. Start positions varied pseudo-randomly from each of the cardinal points, just inside the arena wall, facing inward. Once the participants discovered the platform, they were encouraged to look around the room from that spot and try to remember

where they were, at least on the first 2-3 trials. The trial ended and the next one began when participants indicated that they were ready. Efficiency on standard trials was scored as per usual, using the distance (in platform radii) and latency (in seconds) required by participants to reach the platform.

Inter-Trial Strategy Probe trials. The purpose of the ten novel, explicit *Inter-Trial Strategy Probes* (ITSP) was to assess navigational strategy selection on a trial-by-trial basis. These trials were inserted between Standard trials, such that they alternated in pairs (i.e., Standard – ITSP, Standard – ITSP, etc.). ITSPs required participants to simply navigate to the place in the room where they thought the platform was located on the preceding standard trial. Participants were informed that on these trials, the platform would not appear and that no feedback would be provided. During these trials, the cue-object that always marked the quadrant containing the platform in the Standard trials was moved to the opposite side of the arena. Participants were not informed of this change. ITSP trials started at either NE or SW start positions near the arena wall, facing inward, such that the vectors to the two potential goal locations were equivalent. The trial ended when the participant said that they had navigated to the desired spot. If the participant identified the platform location as being in the original quadrant (i.e., in the southeast) the participant's strategy on that ITSP trial was considered to be allocentric. Conversely, if the participant identified the platform location as being in the quadrant that now contains the cue-object, their strategy was considered to be egocentric.

Probe trial. The final trial in the Dual-Strategy maze was a traditional (implicit) probe trial. This trial began as though it was a Standard trial. However, the cue was moved to the wall of the opposite quadrant and there was no platform. Participants were not informed of this change. The trial lasts for 50 s, at which time the usual bell sounded. As usual, the measure on

this trial was dwell time in the "correct" quadrant, but in this case, the correct quadrant was determined on the basis of the participant's predominant strategy on previous ITSPs.

Procedure.

A number of procedures were used to improve the accuracy of the cortisol measurements and ensure consistency across participants. To avoid contaminating saliva samples, participants were asked prior to the experimental session not to exercise strenuously, smoke, eat or drink anything (except water) in the hour before the session. To take advantage of the circadian elevation in circulating cortisol (Lupien et al., 2007), all experimental runs were conducted in the morning. Because cortisol concentrations decline rapidly in the morning (Edwards et al., 2001), only two experimental sessions were conducted per day, at either 8:00 am or 9:30 am. Time of day (TOD) was later included as a grouping variable.

Overall the procedure took about 85 minutes (see Figure 6). All testing was conducted in a quiet, distraction-free room, with one male and one female experimenter. Participants completed all computer-based tasks on a desktop computer with a 19" LCD monitor set to a resolution of 800 x 600. Upon arrival, participants were pseudorandomly assigned to the "stress" or "control" group such that approximately equal numbers of male and female participants were tested at each TOD (early or late morning).

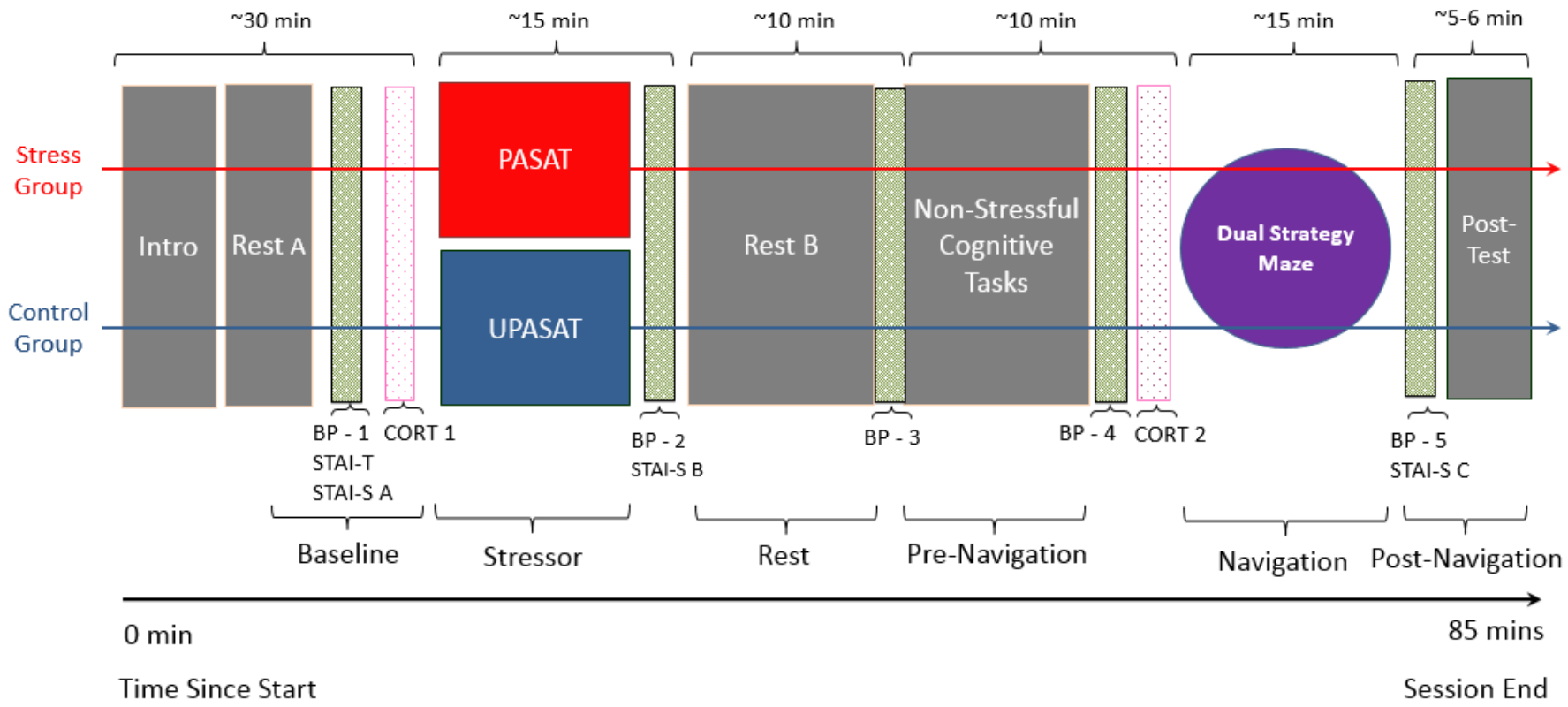


Figure 6. Experiment 1: Procedure.
Brackets at the top of the figure show estimations of task length (e.g. 30 min); brackets at the bottom specify the physiological and psychological measurement time points. Heart rate (HR) and skin conductance (SC) were measured continuously through the periods indicated (then averaged) whereas blood pressure (BP), salivary cortisol (CORT) and STAI-trait (STAI-T) and STAI-state (STAI-S) were measured as single points.

After consent was obtained, the session began with an initial 30 minute "pre-stress" resting period, intended to bring all participants to a similar state of physiological arousal. This phase included a short introduction, after which the HR monitor, BP cuff, and SC electrodes were fitted to the participants' chest, left arm and fingers, respectively. Participants then completed the short demographics questionnaire which asked about their age, sex, education, and history of neurological or psychological disorder. After completing the questionnaire (~1 min), participants were allowed to relax and read magazines. During this rest period, one experimenter stayed in the room but pretended to be busy with work on a separate laptop pc. The other experimenter left the room. When the 2nd experimenter returned after 10 minutes, participants completed the STAI (both "state" (STAI-S A) and "trait" portions). Immediately following completion of the STAI, and marking the end of the "pre-stress" phase, participants provided baseline saliva samples (CORT 1) and blood pressure measurements (BP1).

Next, participants in the stress group completed the PASAT. To heighten participants' experience of socio-evaluative threat (known to be particularly effective at increasing stress; Dickerson & Kemeny, 2004), stress group participants were told that it was a test of "thinking speed", but that most students "don't make too many mistakes". In addition, both experimenters observed the participants' performance intensely, and one recorded mistakes by making obvious notations in the participants' peripheral view whenever the error buzzer sounded. In contrast, participants in the control group were given the U-PASAT, were not given any socio-evaluative threat statements, and were simply told that the task tested their "ability to add single-digit numbers". While participants conducted the U-PASAT, one experimenter again pretended to work on a laptop, while the other experimenter left the room until the U-PASAT was complete.

To allow sufficient time for cortisol levels to peak (i.e., 20 – 40 minutes after stressor-onset; Dickerson & Kemeny, 2004), a second "post-stress" resting period followed, lasting approximately 20 minutes. This rest period was also intended to allow the sympathetic effects of stress on hippocampal function to subside (Elzinga et al., 2005). Immediately after completing the PASAT/U-PASAT, participants completed the "state" portion of the STAI a second time (STAI-S B) while BP measurements (BP2) were taken. They were then allowed to rest again by returning to their magazine reading. After 10 minutes, participants had blood pressure measurements taken a third time (BP3). After that, participants completed two non-stressful cognitive tasks for the remaining 10 minutes of the rest period. For this experiment, the main purpose of these tasks was simply to prevent drowsiness by keeping participants occupied. The first was the Iowa Gambling Task (IGT; Bechara, Damasio, Damasio, & Anderson, 1994), a decision-making task that requires the participant to repeatedly select cards from one of four "decks" to try to win imaginary money. The second was the Farm Animals Paired Associates task (FAPA), a memory task developed in-house that requires the participant to associate familiar farm animals with landscape backgrounds. The results for these tasks are only tangentially related to the present dissertation, and were not interesting (i.e., no main effects or interactions), and thus will not be discussed further. The "post-stress" resting period ended when, immediately following the second task and just prior to navigating in the virtual maze, participants gave a second saliva sample (CORT 2), and had blood pressure measurements taken a fourth time (BP4).

Participants were tested in the Dual-Strategy maze immediately after the second rest period. Maze trials were presented in the order described above, and participants were given

detailed instructions prior to starting each new trial type. The entire maze procedure, including all trials and instructions, required approximately 15 minutes.

After testing in the Dual-Strategy maze, participants completed a final STAI (state portion) and provided final BP measurements (BP5). To end the session, participants completed a short (3-4 minute) post-test questionnaire. The main purpose of the questionnaire was to gather information about possible factors that might have influenced navigational strategy selection, such as video-game experience, and to collect subjective assessments of the stressfulness of the experimental tasks.

Data analysis.

All statistical analysis was conducted using SPSS version 21.

To assess the effectiveness of the PASAT to induce stress I used a 2 x 2 x 2 ANOVAs, with Stress Condition, TOD and Sex[3] as independent variables, to examine group differences for each of the physiological measures, as well as STAI score. Measures of SAM axis activity (HR, SC, systolic and diastolic BP) and the psychological experience of stress (STAI score) were calculated as percentage of baseline. For HR and SC, percentage of baseline was based on the average of all measurements taken during the stressor period (PASAT/UPASAT) divided by the average of all measurements taken over the 5-minute baseline period immediately prior to the stressor. For BP and STAI, percentage of baseline was based on the measures taken immediately after the stressor divided by the measures taken immediately before the stressor. I also calculated a SAM composite score, which combined the 4 SAM measures. This score was calculated by converting each of the SAM activity measures into z-scores, then multiplying them by weights to balance their contribution to the score such that HR, SC and BP (including both Systolic and

[3] It should be noted that I used a quasi-experimental design and that Sex was a predictor variable. However, throughout the rest of the chapter and in the general discussion I will be referring to sex as a variable in a manner consistent with the literature (e.g., du Plooy, Thomas, Henry, Human, and Jacobs, 2014; Wolf et al., 2001).

Diastolic) each contributed 33%. Due to the skew in the distribution, the measure of HPA axis activity (salivary cortisol concentration) was converted to a log10 value and calculated as change from the pre-stressor baseline to pre-navigation levels (delta log(CORT)).

To assess navigational strategy selection, I conducted a 2 x 2 x 2 (Stress Condition x Sex x TOD) ANOVA[4] on average Allo %. Allo % is calculated by taking the number of ITSP trials that were designated as "allo" and dividing that by the total number of ITSP trials in which the participant chose a strategy.

$$\text{Allo\%} = \text{Allo trials}/(\text{Allo trials} + \text{Ego Trials})$$

To assess navigational efficiency, average latency and distance to the platform on standard trials were analysed using a 2 x 2 x 2 (Stress Condition x Sex x TOD) ANOVA. To assess participants' navigational knowledge, I conducted a 2 x 2 x 2 (Stress Condition x Sex x TOD) ANOVA on "correct" quadrant dwell time. The "correct" quadrant was determined based on a classification criterion (based on participants' preferred strategy on ITSPs). Finally, relationships between physiological measures of stress and navigational strategy selection were assessed using Pearson correlations.

Results

Manipulation checks.

Both physiological and psychological measures indicated that the PASAT was effective at inducing stress (Figures 7-9). Condition x Sex x TOD ANOVAs revealed a main effect of

[4] Note: ANOVA relies on the assumption that the data are normally distributed, although it is robust to violations of this assumption (Driscoll, 1996; Schmider, Ziegler, Danay, Beyer, & Bühner, 2010). Nevertheless, because the allo% data were non-normally distributed (bimodal), I used a distribution-free test (Kruskal-Wallis) to verify the ANOVA results when appropriate. The results of these checks did not change interpretations.

Condition on each of the 4 measures of SAM activity. Stress elevated both systolic ($F(1,116) = 9.49$, $p<.01$, $\eta^2 = .11$), and diastolic ($F(1,116) = 20.54$, $p<.001$, $\eta^2 = .16$) BP, as well as HR ($F(1,116) = 13.59$, $p<.001$, $\eta^2 = .12$) and SC ($F(1,116) = 10.01$, $p<.01$, $\eta^2 = .09$) (Figure 8). However, neither Sex nor TOD had any main or interaction effects on any of the SAM measures (all p's > .12). This pattern was also reflected in the SAM Composite score, with a main effect of Condition ($F(1,116) = 37.94$, $p = .000$, $\eta^2 = .26$), but no main or interaction effects for Sex or TOD (all p's > .06). I did observe the expected morning decline in cortisol concentration ($F(1,116) = 4.87$, $p = .03$, $\eta^2 = .05$). The effect of the PASAT was to partly sustain salivary cortisol concentrations as reflected by the declining levels in the Control group (Figure 9), though the resulting difference between stress and control groups was not significant ($F(1,116) = 3.01$, $p = .09$, $\eta^2 = .03$, Figure 9). Regarding the subjective experience of stress, a 2 x 2 x 2 ANOVA revealed that the PASAT caused participants in the Stress group to report significantly higher levels of anxiety on the STAI than those in the Control group (Control $M = 5.03\%$, Stress $M = 60.34\%$, ($F(1,116) = 92.78$, $p < .000$, $\eta^2 = .46$). Again, there were no main or interaction effects with Sex or TOD (all p's > .07).

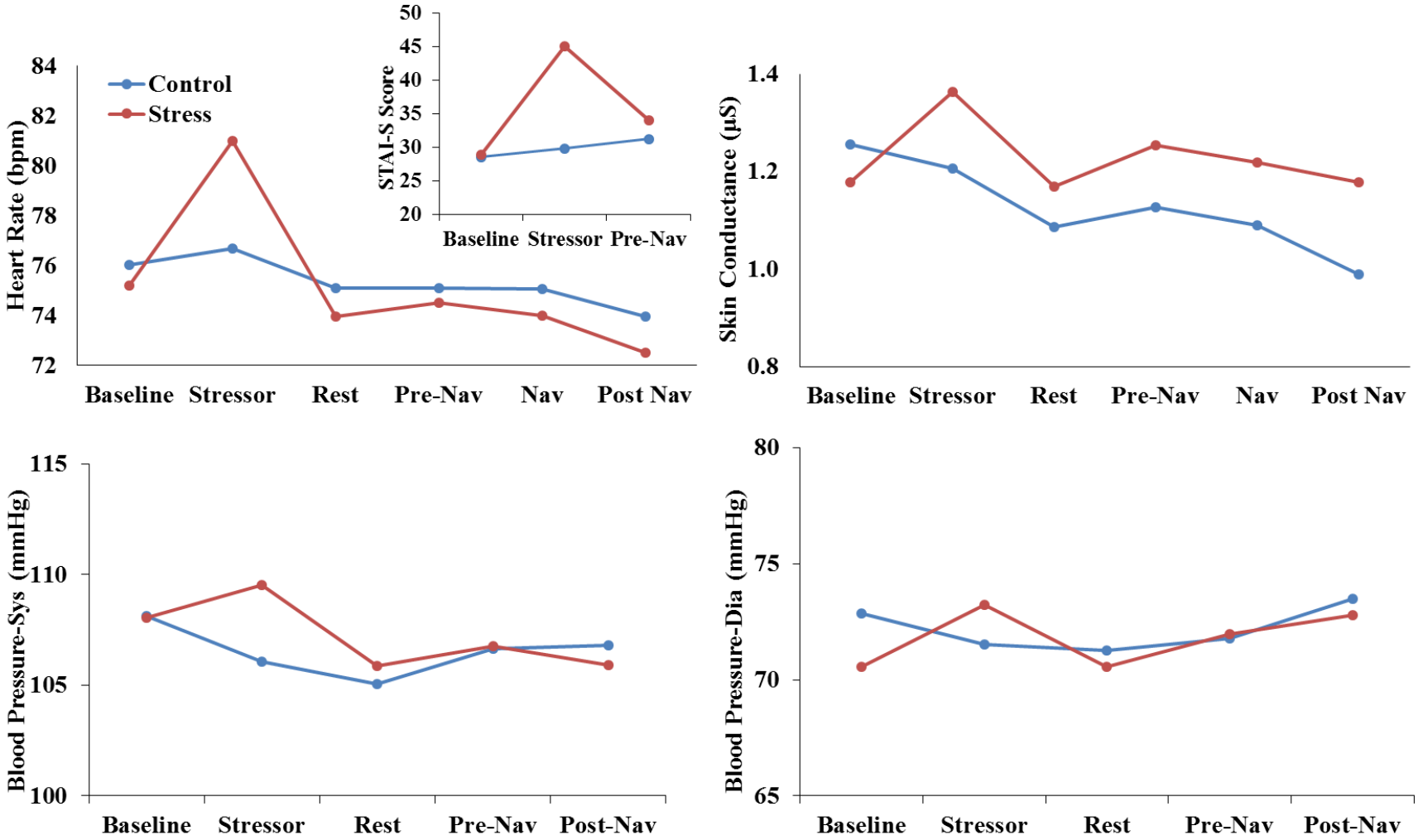


Figure 7. Experiment 1: Time course of the PASAT effect on physiological measurements.
Y-axes denote: Heart Rate (HR), Skin Conductance (SC), Systolic Blood Pressure (BP-Sys), Diastolic Blood Pressure (BP-Dia), and State-Trait Anxiety Inventory-State Portion (STAI-S; inset).

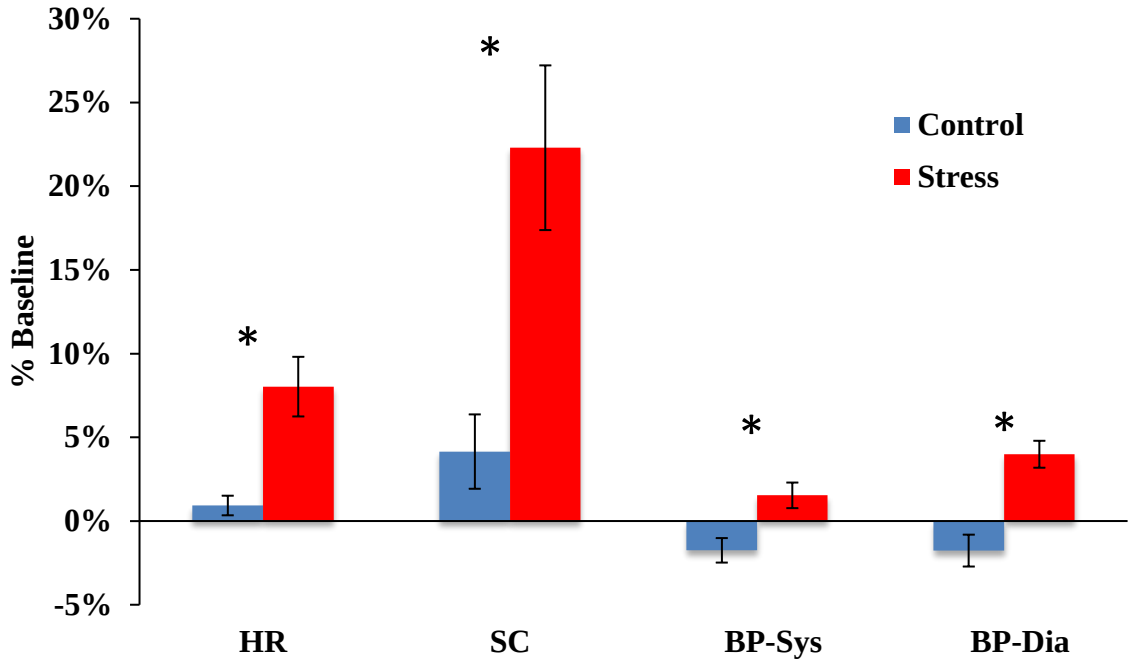


Figure 8. Experiment 1: The effect of the PASAT on SAM measures.
The effect of the PASAT on heart rate (HR), skin conductance (SC), systolic (Sys) and diastolic (Dia) blood pressure, expressed as mean % Baseline. Error bars are SEM. *p < .01.

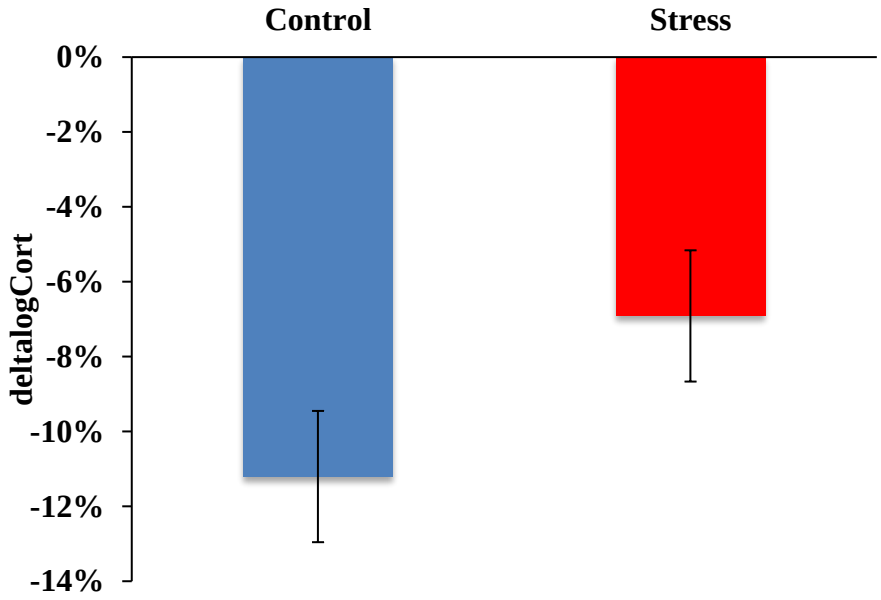


Figure 9. Experiment 1. The effect of the PASAT on salivary cortisol.
Values are expressed as mean delta Log(CORT). Error bars are SEM. *p* = .08. Note: decreasing cortisol concentrations in both the stress and control groups reflects the normal circadian drop in endogenous cortisol in the morning.

Stress and navigational performance.

Analysis of navigational efficiency revealed only the commonly observed sex differences in the virtual MWM. Separate Condition x Sex x TOD ANOVA's revealed that male participants required slightly less time than female participants to reach the platform on visible platform trials (Male M = 3.02, Female M = 3.82 s, $F(1,115) = 22.38$, $p<.001$, $\eta^2 = .17$) and on standard trials (Male M = 6.87, Female M = 9.71, $F(1,115) = 16.7$, $p<.001$, $\eta^2 = .13$). However, condition and TOD had no effect on visible platform trial or standard trial latency, and there were no interactions (all p's > .28). Separate factorial ANOVAs revealed that neither Condition, Sex, nor TOD had any effect on distance or correct quadrant dwell time, nor were there any interactions (all p's > .30).

Stress and navigational strategy selection.

PASAT stress caused a shift in strategy selection in the virtual maze. A Condition x Sex x TOD ANOVA revealed a main effect of condition, such that stressed participants had a higher allocentric bias than un-stressed participants (Stress M = 66.70%, Control M = 41.10%, $F(1,115) = 10.31$, $p<.01$, $\eta^2 = .09$) (Figure 10). Although control female participants tended to navigate egocentrically more often than control male participants, and stress seemed to make the female participants navigate as allocentrically as male participants, there were no main effects of sex or TOD, and no 2- or 3-way interactions (all p's > .16).

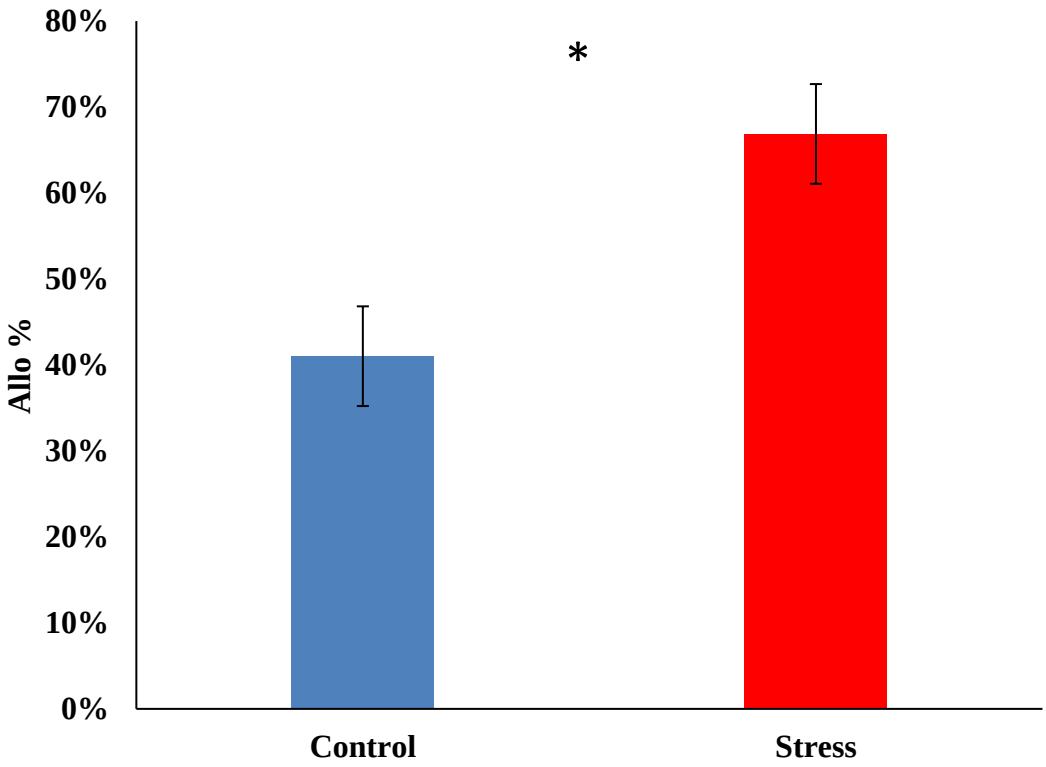


Figure 10. Experiment 1: The effect of the PASAT on navigational strategy selection. The effect is expressed as average Allo%. Error bars are SEM. *p < .01.

Navigation, HPA, and SAM axis activation.

Correlations between navigational strategy selection and physiological measures of the stress response indicated that strategy selection was weakly related to some measures of SAM axis activity, but not the one measure of HPA axis activity. Allo% was positively related to diastolic (r =.19, p<.05) and systolic (r =.18, p=.06) BP. In other words, greater positive changes in BP were associated with more allocentric strategy selection. Allo% was also positively related to the SAM Composite score (r =.20, p<.05). However, no relationships were observed between Allo% and HR (r = .14, p = .15) or SC (r = .08, p = .42), and no relationship was observed between Allo% and salivary cortisol (r =.03, p = .80).

Discussion

Experiment 1 has revealed several interesting results. First, importantly, the results from the physiological measurements (HR, BP, SC, CORT) suggest that the PASAT caused a biological stress response (i.e., acute stress), as expected. This was more strongly represented by measures of SAM axis activity, however. Although the PASAT appeared to mitigate the morning drop in salivary cortisol levels to some degree, the difference between those who were and those who were not exposed to the PASAT was not significant. Second, the results from the behavioural measurements revealed that acute stress influenced spatial navigation strategy selection in the Dual-Strategy maze. Surprisingly, the hypothesis that acute stress would lead to a shift in navigational strategy selection towards egocentric navigation was disconfirmed – that is, stress strongly biased strategy selection *away* from egocentric and *towards* allocentric navigation. Consistent with my expectation, however, the results revealed relationships between physiological stress reactivity and strategy selection. Although the relationships were weak, increased blood pressure (a measure of SAM axis activity) was associated with more allocentric strategy selection. This makes sense, given that the stress group selected allocentric strategies more often. However, Experiment 1 was not able to reveal evidence that either TOD or Sex are important modulating factors in the relationship between stress and navigational strategy selection or performance, as no interactions between Stress condition and Sex or TOD were found.

The behavioural results in Experiment 1 were surprising. Contrary to my predictions, stress led to more allocentric and less egocentric strategy usage. This finding is contrary to the existing animal research on the effects of acute stress on strategy selection. In rodents, stress causes a bias towards more egocentric, rather than allocentric strategy use (Kim et al., 2001;

Schwabe et al., 2010a). The present finding is also contrary to one human study that tested the effects of acute stress on strategy selection in a small-scale model room (Schwabe et al., 2007). They found that stress shifted strategy selection from a putatively hippocampus-based "spatial" strategy to a putatively caudate-based stimulus-response strategy. Importantly, however, this study did not test spatial navigation. Indeed, to my knowledge, Experiment 1 in the present research program is the first to test the effects of stress on human navigational strategy selection.

The surprising effect of stress on strategy selection was not predicted by any of the 4 neurobiological theories of the effects of acute stress on hippocampal function, all of which agreed on the prediction that acute stress should lead to less allocentric navigation. However, the results can be accommodated by the TDM, MYD and Hot/Cool Systems models. According to the TDM model, the bias toward allocentric strategy selection may have resulted from a stress-to-test interval that was too short. The TDM model (see Chapter 1) posits that the effects of stress on hippocampal function have a specific temporal profile. Primarily through the rapid actions of the SAM axis, stress first enhances hippocampal function, which leads to a refractory period in which its function is impaired. Thus, according to this model, it is possible that navigation was tested too soon—before the refractory period, while hippocampal function was still enhanced by SAM activity and still maintained behavioural dominance. One problem with this interpretation, however, is that all physiological measures of SAM axis activity had long returned to baseline before navigation was tested. Still, these measures were indirect, and do not necessarily mean that SAM axis excitatory influence on the hippocampus had abated by the time navigation was tested.

According to the MYD model and the Hot/Cool Systems model, the bias toward allocentric strategy selection may have resulted from stress intensity that was too low. These

models posit that low-to-moderate levels of stress intensity, and the commensurate elevations in cortisol, enhance hippocampal function, while extremely low or extremely high levels impair it. Thus, it is possible that the PASAT was sufficiently stressful to enhance hippocampal activation, and thus behavioural dominance, but not to impair it. A problem with this explanation is that there was no significant difference in cortisol levels between stressed and unstressed participants in Experiment 1. Thus, it is difficult to argue that the effects of stress were mediated by cortisol, as these models would suggest.

The strategy selection data from Experiment 1 do not fit well with the predictions of the Uniform Shift model. This model posits that stress leads to hippocampal impairment, caudate enhancement, and a shift in behavioural control from the hippocampus to the caudate. By this model, there is no situation where acute stress can bias behavioral control *towards* hippocampus-based processes.

As discussed above, one possible explanation for the surprising effects of stress on strategy selection is that stress enhanced hippocampal function via the SAM axis. Another, somewhat distressing explanation for the surprising findings is that the virtual maze may not have accurately dissociated between egocentric and allocentric strategies and that stress might have acted via impulsivity to produce the observed results. That is, it is possible that acute stress did bias participants towards more egocentric navigation, as theory predicts, but the design of the maze led to egocentric navigation that was miscategorised as allocentric navigation. Navigational responses on ITSP trials were characterized as allocentric if the participant went to the quadrant that had previously held the platform (as defined by distal cues) and that was opposite the one marked by the cue object perched on the arena wall. Given that the choice of quadrant to go to may have been made right at the start of the trial (cf. Mueller, Jackson, & Skelton, 2008), there

are three ways in which egocentric responses might have been characterized as allocentric navigation. One possibility is based on the previous observation that participants learned about the objects flanking the cue object in a Dual-strategy maze (Livingstone and Skelton, 2007). In the present experiment, when the egocentric cue object was moved from its location in the Standard trial to its location on the opposite side of the arena in the ITSP trial, the flanker objects on either side of it were remained where they had been, in the "allocentric" quadrant. At the start of the ITSP trial, the target egocentric cue object was just off-screen to the left or right and the participant needed to swivel one way or the other in order to see it. However, the flanker objects were still visible (see Figure 11). Because these flanker objects were not moved between Standard and ITSP trials, they were still in the "allocentric" quadrant, and the egocentric response of travelling towards them would have been characterized as allocentric. Further, it is possible that the effect of stress was to increase the likelihood of participants impulsively selecting a travel direction based on immediately visible cues.

The second way in which an egocentric response might have been mis-characterized as an allocentric response is if participants had come to use the view out a window as a single cue by which to navigate (i.e. a "snapshot"; Eichenbaum, Stewart, & Morris, 1990), so the window becomes a guidance cue (Trullier, Wiener, Berthoz, & Meyer, 1997). If stress is enhancing impulsivity, then the window closest to the platform location might overshadow the much smaller "egocentric" cue objects as a navigational guidance cue (especially if the cue object was not immediately visible). This would lead to the mischaracterization of a participant navigating egocentrically to the window as navigating allocentrically.

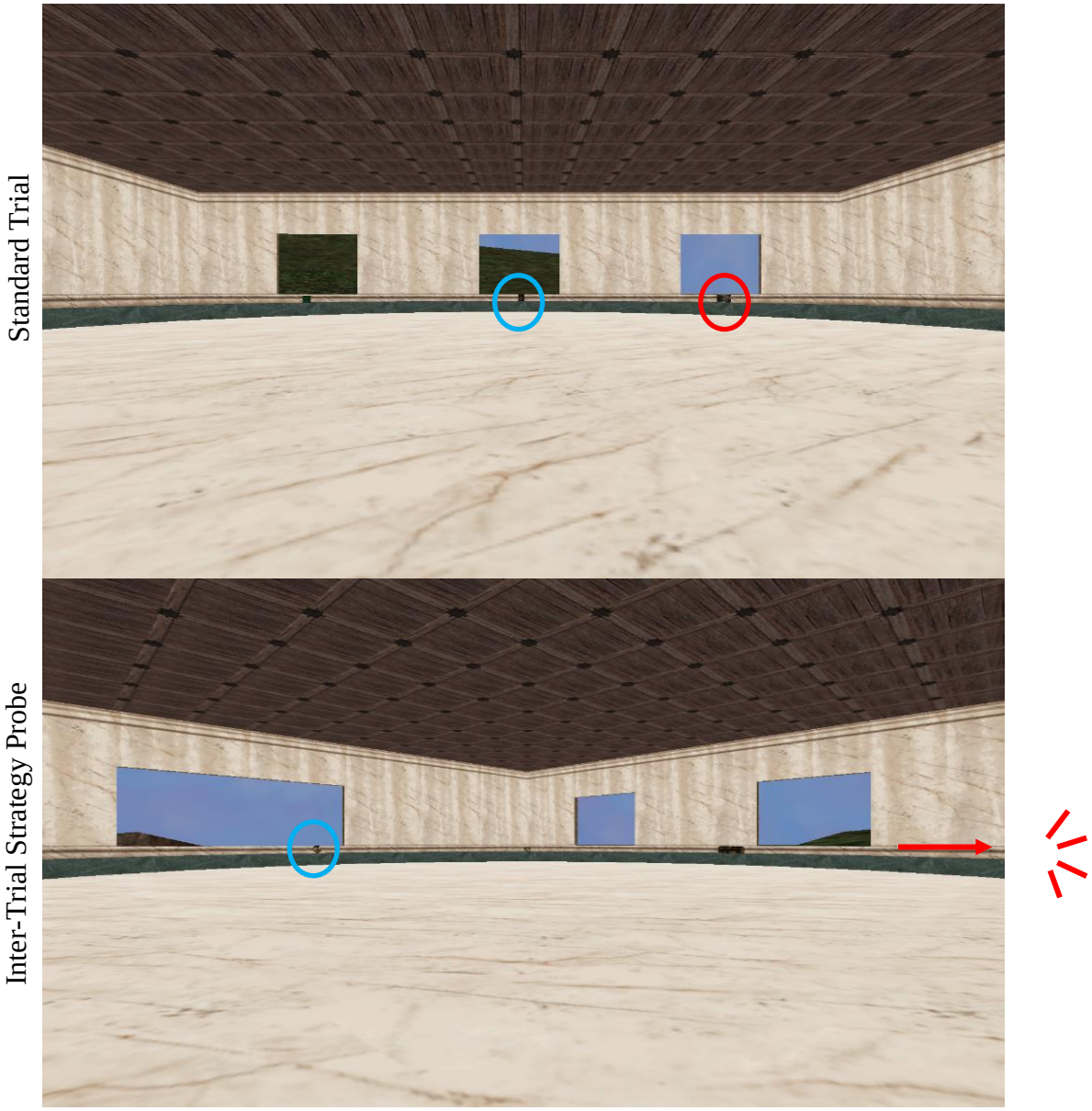


Figure 11. Sample starting views in the Dual-Strategy maze.
Standard trial (top): the red oval highlights the target egocentric cue, and the blue oval highlights one of the two flanker cues. Corresponding Inter-trial Strategy Probe (bottom): the red arrow and lines indicate the new location of the target cue (not visible at the start), and the blue oval highlights one of the flankers (unmoved from the Standard trial).

The third way is the most subtle. In this experiment, the platform, located in the centre of the quadrant, was some distance from the cue object on the arena wall. This means that anyone navigating predominantly via the cue object would either have to compute the location of the platform incorporating the distance and direction of the displacement, or would have to move to the centre of the arena before heading in a straight line to the cue object and bumping into the platform, thereby making the distance to the wall irrelevant. (Note: this pattern of travel was never noticed despite the manual review of every trial by every participant). This means that because the cue object was not right at the location of the goal, the additional spatial cognition required to find the platform might well have been allocentric and/or reliant on hippocampal function. In other words the maze might not have been distinguishing allocentric and egocentric cognition, but rather distinguishing two forms of spatial cognition, one of which was more allocentric than the other. Given the degree of speculation involved in this line of thought, it seems risky to speculate even further as to how stress or impulsivity might have caused a shift from allocentric navigation to "allocentric-ish" navigation. Nevertheless, it seems prudent to remove this complicating factor from the design of the dual-strategy maze.

A final point of discussion relates to the lack of gender differences in Experiment 1. As noted in Chapter 1 (see pgs. 26-27), sex is considered to be an important variable in both stress and spatial navigation research, and research in both areas has revealed sex differences before (see Lawton, 2010, and Kudielka and Kirschbaum 2005, for reviews). However, in the present experiment, there were no sex differences in stress reactivity or in navigational strategy selection. On the face of it, this is somewhat surprising, given the general sense in the literature that a) males are superior to females at allocentric navigation (and therefore select this strategy more often; e.g., Saucier et al., 2002), and b) males react more strongly to psychological stressors

(Kirschbaum et al., 1999). However, the data supporting these conclusions are not consistent. Numerous recent studies have found no sex-based differences in physiological reactivity to psychological stress (e.g., Guenzel et al., 2014, Wolf et al., 2001, see Kudielka and Kirschbaum, 2005 for a review). Furthermore, the present results are consistent with a recent study from our lab that showed that, when tested directly, males and females did not show a differential preference for one navigational strategy over another (van Gerven et al., 2012). Taken together, this suggests that sex may be a less important factor in both spatial navigation and stress research than previously thought.

In conclusion, the results of Experiment 1 suggest that acute stress can bias the way people navigate from one strategy to another. Surprisingly, the direction of this bias was opposite than expected – that is, stress led to more, not less, hippocampus-based allocentric navigation. Although the stressor appeared to be effective in eliciting stress, its intensity was sufficient to elicit SAM-axis activity but not HPA activity. Because there is no evidence of mediation by (HPA-driven) cortisol, and because of the direction of the effect (more allocentric, not more egocentric strategy choice), the findings of Experiment 1 do not fit well with the MYD, Hot/Cool Systems, or Uniform Shift theories. Even though the direction of the stress effect was opposite than expected, the findings might still be accommodated by the TDM model, which is SAM based. Taken together, the surprising results suggest two possibilities: either a) there was a methodological problem with Experiment 1 (e.g., the protocol was not timed well or the maze did not accurately measure strategy), or b) the effects of stress operate differently on the mechanism(s) that underlies navigational strategy selection than they do on the mechanism(s) that underlies navigational performance. These possibilities were explored in Experiment 2.

Bridge to Experiment 2

Experiment 1 revealed a surprising effect of stress on navigational strategy selection: stress caused more, not less allocentric navigation. Although the effect of stress on human navigational strategy selection has never been tested prior to Experiment 1, the effect was surprising because empirical evidence (from rat navigation studies and human spatial cognition studies) and theoretical models predicted that acute stress should lead to more egocentric and less allocentric navigation. Thus, Experiment 2 investigated this result more deeply, in an effort to determine whether it was a novel discovery, or simply the result of methodological factors. Experiment 2 also investigated whether the effect of stress on navigational strategy selection generalizes to allocentric navigational performance.

Chapter 3: Experiment 2

Introduction

Background.

The results of Experiment 1 showed that acute stress can shift navigational strategy selection in humans. Interestingly, however, the direction of the effect was different than that which would be predicted by theory or by previous empirical evidence (e.g., in rodent navigation), with acute stress biasing participants towards more (not less) hippocampus-based allocentric navigation and less (not more) caudate-based egocentric navigation. One goal of Experiment 2 was to see if this surprising finding was the result of methodological factors or if, indeed, it was a new discovery.

The results of Experiment 1 did not fit well with the four theoretical models reviewed in Chapter 1. The Uniform Shift model, in particular, cannot account for the data. By this model, stress always biases behavioural control away from the hippocampus and towards the caudate through the combined effects of the HPA and SAM axes. From this point of view, then, strategy selection should be biased towards caudate-based *egocentric* solutions, not hippocampus-based allocentric solutions – precisely the opposite of what was observed in Experiment 1. In contrast, while the MYD and Hot/Cool Systems models can accommodate the behavioural results of Experiment 1, they do not square well with the physiological results. The MYD and Hot/Cool Systems models posit that mild-to-moderate stress leads to the release of a small amount of cortisol through the HPA axis. This may bring about an optimal balance of receptor occupation in the hippocampus, enhancing its function and giving it behavioural dominance (measured as more allocentric strategy selection). However, there were no differences in cortisol levels between groups in Experiment 1, nor did cortisol concentrations correlate with strategy selection.

In other words, there was little evidence of HPA-based mediation of the effect of stress on strategy selection in Experiment 1, and thus little support for an MYD- or Hot/Cool Systems-based explanation.

The TDM model can accommodate both the physiological and behavioural results of Experiment 1. The TDM model holds that the hippocampus is first enhanced, then impaired by stress, and that this effect is largely driven by the SAM axis. The SAM axis was strongly activated in Experiment 1 (according to HR, SC, and BP measures), and some SAM measures were correlated with strategy selection (BP). By the TDM theory, it is possible that the observed effect of stress on navigational strategy selection was the result of the lingering excitatory influence of the SAM axis on the hippocampus. If the hippocampus was still highly activated by the SAM axis by the time navigation was tested, it makes sense that the hippocampus still dominated behaviour, and biased strategy selection towards allocentric. This potential explanation warrants further exploration in Experiment 2, which attempted to achieve better separation of the influences of the SAM and HPA axes by taking advantage of their known time courses.

As discussed in the previous chapter (see Chapter 2: Discussion), another explanation for the surprising findings of Experiment 1 is that the virtual Dual-Strategy maze, for several reasons, may not have accurately dissociated between egocentric and allocentric strategies. First, participants may have navigated egocentrically via the "flanker" cue which remained in the "allocentric" quadrant. Second, the participants may have used a window as a snapshot-guidance cue and navigated egocentrically to the allocentric platform. Third, the spatial displacement of the cue object from the platform may have required participants to navigate somewhat allocentrically rather than purely egocentrically or allocentrically. The first two could

have been enhanced by stress via an increase in impulsivity and the third could have been affected in a number of ways.

These potential methodological confounds were addressed in Experiment 2, which used an improved virtual maze to attempt to achieve better separation of egocentric and allocentric navigation (see Figure 12). In the new, "updated Dual-Strategy" (uDS) maze, the key egocentric cue objects were larger and contained within the arena, and the target egocentric cue always floated directly above the goal and was always visible from the start of trials. This is more in line with traditional animal versions of the egocentric MWMs (cf. Packard & McGaugh, 1992; Whishaw et al., 1987; Williams et al., 2003; see Vorhees & Williams, 2006, for a review). Moreover, all distractor cues were identical. These differences improved the separation of egocentric and allocentric navigation by a) eliminating the (potentially allocentric) computation required to estimate the distance between the cue and the platform location, and b) reducing the likelihood that participants could use distracter cues to locate the goal egocentrically.

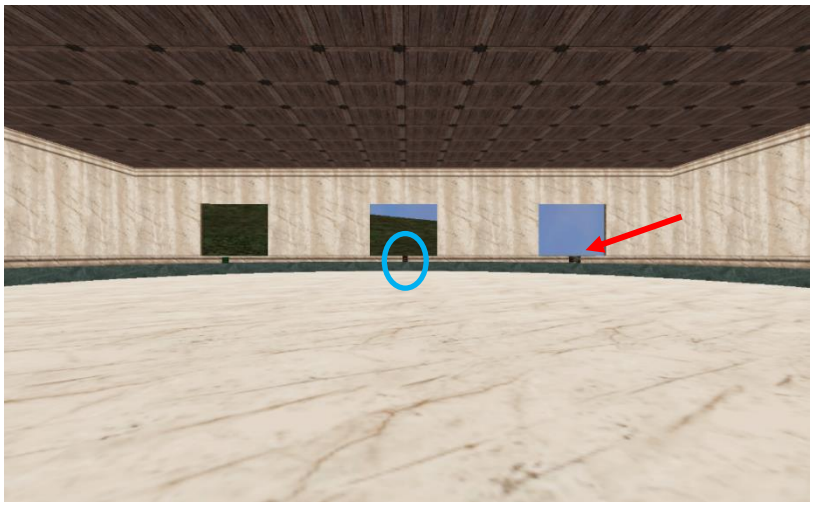
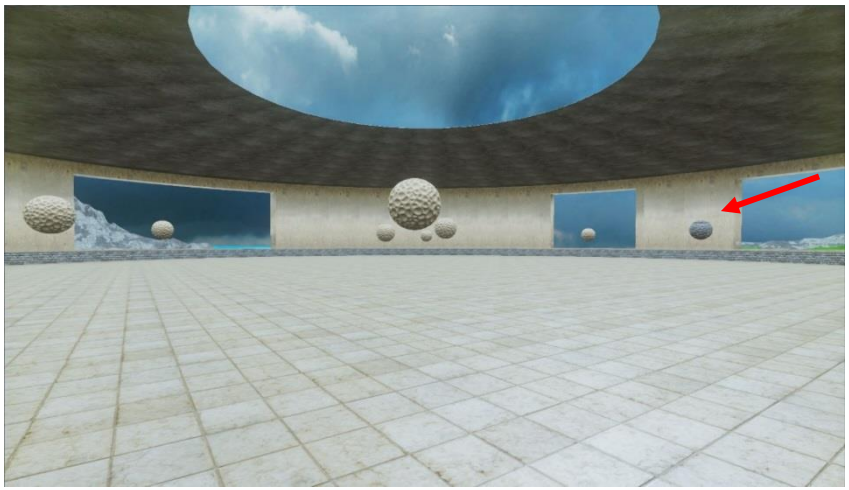
	Original Dual-Strategy maze	Updated Dual-Strategy maze
Standard Learning Trial (West start)		
Strategy Probe (North east start)		

Figure 12. Comparison of original and updated Dual-Strategy maze starting views.
Views from the start of the first trial pairs in the original Dual-Strategy maze (left) and the updated Dual-Strategy maze (right). The top panels are starting views from the first Standard learning trial, the bottom panels are starting views from the corresponding strategy probes. The red arrow indicates the target cue object. Note that the cue object is not visible at the start of the strategy probe in the original Dual-Strategy maze (bottom left), but one of the flankers is (blue ovals).

While the effects of acute stress on navigational strategy selection in Experiment 1 were surprising, not surprising was the lack of effects of stress on navigational performance. This is because, as mentioned in Chapter 2, navigational performance is confounded with strategy selection – that is, when the use of one navigational strategy is impaired (e.g., by stress), participants can simply switch to an alternate strategy when one is available to compensate. Indeed, in Schwabe et al.'s (2010) study, stressed rodents did just that in a dual-solution maze.

Nonetheless, the effect of stress on human hippocampus-based (allocentric) navigational performance remains of key interest. In rodents, acute stress and stress hormones appear to generally impair allocentric navigational performance (e.g., Park et al., 2008; Schwabe et al., 2010a; Xiong et al., 2003) though not always (Warren, Castro, Rudy, & Maier, 1991; see Cazakoff et al., 2010, for review). In humans, existing evidence for effects of acute stress on allocentric navigational performance is mixed. In one study that used an all-male sample (Duncko et al., 2007), stress enhanced allocentric navigational performance. In contrast, Thomas et al. (2010) found that acute stress impaired only female performance, leaving male performance unaffected. Two other studies (Klopp et al., 2012; Guenzel et al., 2014) found no impact of stress on allocentric navigational performance whatsoever, regardless of sex (although in the Guenzel et al., 2014 study, this may have been because navigation was tested in a virtual radial arm maze). Importantly, each of these studies tested the effect of stress on navigation at approximately the same delay after stress onset (~30 min), timed to coincide with stress-induced peak HPA activity (i.e., peak cortisol). To date, no study has examined the impact of acute stress on allocentric spatial navigation immediately after stress, when the SAM axis is still highly active.

Key Issues.

After establishing an effect of stress on navigation in Experiment 1, Experiment 2 sought to explore the effect in more detail. Specifically, Experiment 2 sought to examine 3 key issues. The first was whether the surprising effect of stress on strategy selection in Experiment 1 would replicate using improved methods that were designed to better separate egocentric and allocentric navigation and to achieve cleaner separation between control and stressed groups. The second was whether (and how) acute stress influences allocentric navigational performance. The third was whether the effects of stress on navigational strategy selection and performance are time dependent, reflecting the influence of either the SAM axis (early effects) or HPA axis (delayed effects).

Approach and hypotheses.

Based on the TDM model and the findings of Experiment 1, the premise of Experiment 2 is that the effects of stress on navigation are primarily mediated by the SAM axis. This suggests that, if the stress effect on strategy selection was measured accurately in Experiment 1, this effect should replicate in Experiment 2, which used the same timing and a similar, but improved maze (see Method). Furthermore, the effects of stress on both navigational strategy selection and performance should be parallel both in direction and time-course.

In order to re-test the effects of stress on strategy selection, test its effect on allocentric navigational performance, and examine the time dependency of the stress effect on navigation, Experiment 2 exposed half of the participants to acute stress (using the PASAT) and then tested their navigational strategy selection and performance. Strategy selection and performance were both tested immediately after the stressor, and, in different participants, at the same 30 minute delay (from stressor onset) as in Experiment 1. Strategy selection was tested in the new, updated

Dual-Strategy maze (uDS maze). As already noted, this maze should achieve better separation of egocentric and allocentric navigation by making the key egocentric cue visible from the start and reducing the likelihood that distractors could be used egocentrically. Navigational performance was tested using a traditional allocentric virtual Morris water maze (the Place maze) that was similar to the uDS maze in most respects except that there were no proximal cues that could be used egocentrically (see Methods for details).

A key consideration in Experiment 2 was what participants should be given to do during the delay period. In Experiment 1, this period was occupied (in part) by a declarative memory task (the FAPA) and a decision making task (IGT). While these tasks were intended to prevent boredom during the delay period, they appeared to elevate physiological arousal on some measures (SC and BP, data not shown). Furthermore, 72% of participants reported at least one of these tasks to be somewhat stressful in the post-test questionnaire. Given that neither test yielded particularly useful data, it seemed reasonable to use a more pleasant and less stressful task to occupy participants during the delay period. In a previous study from our lab (Steinberg & Skelton, 2013) 81% of participants reported that navigating in a similar allocentric virtual MWM was “pleasant”. The added benefit of this navigational task was that it could serve as the test of the immediate effects of the stressor.

Thus, Experiment 2 used a counter-balanced design, such that stressed and control groups were sub-divided based on maze order. That is, half of the participants navigated in the Place maze first (testing the effect of stress on immediate allocentric performance) and then navigated in the uDS maze (re-testing the effect of stress on delayed strategy selection). The other half of the participants navigated in the uDS maze first (testing the effect of stress on immediate strategy selection) and then navigated in the Place maze (testing the effect of stress on delayed allocentric

performance). SAM axis activity (heart rate, blood pressure, and skin conductance) was measured at each phase (Stress, immediate, and delayed navigation) and compared to pre-stress baseline. Although there were no sex differences in strategy selection in Experiment 1, previous research has found sex related performance differences in allocentric navigational tasks (e.g., Thomas et al., 2010). Thus, Experiment 2 continued to watch for sex differences in the effects of stress.

The premise that the effects of stress on navigation are mediated by the SAM axis led to several hypotheses in Experiment 2. First, it was expected that the effect of stress on strategy selection that was observed in Experiment 1 would replicate in Experiment 2, despite the repositioning of cues in the redesigned maze, and despite the change in activities during the delay period. Specifically, it was expected that acute stress would lead to an increase in allocentric strategy selection. Second, given that the effects of SAM axis activation of hippocampal function should occur rapidly (according the TDM model), then acute stress should increase allocentric strategy selection when tested immediately just as it did at a delay. Third, acute stress should enhance allocentric *performance* when tested immediately as well as at a delay, presumably also via rapid SAM-based enhancement of hippocampal function. Fourth, it was expected that the effects of stress on navigational strategy should have the same time course as the effects on performance. Finally, it was expected that the magnitude of the effects of stress on navigational strategy selection and performance would be related to the magnitude of its effect on SAM axis activation.

Summary of hypotheses:

1) The acute stress effects on strategy selection in Experiment 2 will replicate Experiment 1.
    a. That is, stress will lead to increased allocentric strategy selection at a 30 min delay after stress onset.

2) Acute stress will increase allocentric strategy selection when tested immediately after stress.
3) Acute stress will enhance allocentric navigational performance when tested immediately after stress and at a 30 min delay.
4) The timing of effects of stress on strategy and performance will be congruent.
5) The effects of stress will be related to the level of stress-induced SAM activation.

Method

Participants.

One hundred and twenty-two healthy participants (57 male) were recruited from the University of Victoria undergraduate pool. As in Experiment 1, participants were pre-screened for English as a first language and normal (or corrected to normal) vision. Using a demographics questionnaire, participants were screened for a history of psychological or neurological problems, with particular attention paid to the exclusion of participants who may be unusually sensitive to stress (e.g., those who suffer from generalized anxiety disorder). Three participants were excluded on this basis. One participant reported having a bad migraine headache during the session, and was excluded on this basis. Ultimately, 118 participants were included in the data analysis. The average age of participants was 21.05 years (SD = 3.35 years). Participants were required to provide informed written consent. Ethics approval was obtained from the University of Victoria, Human Research Ethics Committee.

Materials.

Stressor.

Experiment 2 used the same acute stressor task as Experiment 1: the PASAT (see Chapter 2: Method for task details). As a reminder, the PASAT requires participants to add single-digit numbers serially, as quickly as possible, within a very limited period of time. The control version (the U-PASAT) consists of pairs of single-digit numbers that have to be added together, with no time constraint or audience.

Manipulation checks.

As with Experiment 1, Experiment 2 measured heart rate (HR), skin conductance (SC) and blood pressure (BP) to assess the effectiveness of the PASAT to induce a physiological stress response. Heart rate (in beats per minute, BPM) was measured continuously (at 250 Hz) using a Zephyr^{tm} heart rate monitor (H x M BT Bluetooth) and Skin Conductance (in microsiemens, μS) was measured continuously (at 10 Hz) using a Mindfield eSense Skin Response system. Systolic (SB) and diastolic (DB) blood pressure (in millimetres of Mercury, mm Hg) were measured using an arm-cuff type digital blood pressure monitor (LifeSource). Also like Experiment 1, Experiment 2 used the State-Trait Anxiety Inventory (STAI) to measure the psychological experience of stress.

Unlike Experiment 1, however, Experiment 2 did not measure salivary cortisol. This was because a) salivary cortisol measurement and analysis is expensive, and b) the cortisol data from Experiment 1 was not particularly revealing about the relationship between acute stress and spatial navigation.

Spatial Navigation.

Updated Dual-Strategy maze.

Experiment 2 assessed spatial navigation strategy selection using the "updated Dual-Strategy" (uDS) maze (Figure 13). The uDS maze was similar to the original Dual-Strategy maze but used newer software (Unreal Development Kit (UDK); Epic Games), which portrayed a more realistic environment and allowed for faster, easier data extraction using the R software package. The visual layout of the uDS maze was based on the original Dual-Strategy maze and was similar in most respects. Thus, there was a circular arena inside a larger room with windows that looked out onto the landscape. A circular platform within the arena (invisible until stepped

on) represented the navigational goal. As with the Dual-Strategy maze, the uDS maze had mountains visible to the “north”, and hills visible in the “east” and “west” that sloped towards a body of water and an island visible in the “south”. To increase the difficulty and to achieve better separation between egocentric and allocentric navigation, there were three key visual differences between the Updated and the original Dual-Strategy mazes. First, the arena and room in the uDS maze was comparatively larger than in the original Dual-Strategy maze, with a smaller platform (Updated: 10-to-1 arena-to-platform-diameter ratio vs Original: 6-to-1 ratio). Second, the room that houses the arena was circular, rather than square, eliminating geometric features of the room (e.g., the corners) as potential cues. Third, rather than using an array of distinct cue-objects perched on the arena wall to support egocentric navigation, the uDS maze contained an array of spheres, floating at head level, one of which was always directly above the invisible platform. This sphere was distinguished from distractors only by its slightly darker shade of gray (see Figure 13).

The method for assessing navigational strategy selection in the uDS maze was the same as in the original Dual-Strategy maze (see Chapter 2: Method). The trial procedure included alternating sets of 10 Standard and 10 ITSP trials. Once again, on each Standard learning trial, participants were started from randomly varying start positions near the arena wall, and asked to find the platform as quickly and directly as possible. The platform was invisible until stepped on, but remained in a fixed location, and was always marked by the dark-gray cue-sphere floating above it. On each ITSP, they were started from a different position near the arena wall and asked to navigate to the place where they thought the platform was, based on the preceding Standard trial. However, on ITSPs, the dark-gray cue-sphere was moved from its usual place in the centre of the SE quadrant on Standard trials to the centre of the NW quadrant. Thus, if the participant

indicated the platform was located in the SE quadrant, they were assumed to have been navigating allocentrically and that trial was designated “allo”. If the participant indicated the platform was in the NW quadrant (where the cue-sphere now was), they were assumed to have been navigating egocentrically and that trial was designated “ego”. As in Experiment 1, an Allo% measure was calculated based on the number of trials designated as “allo” out of 10.

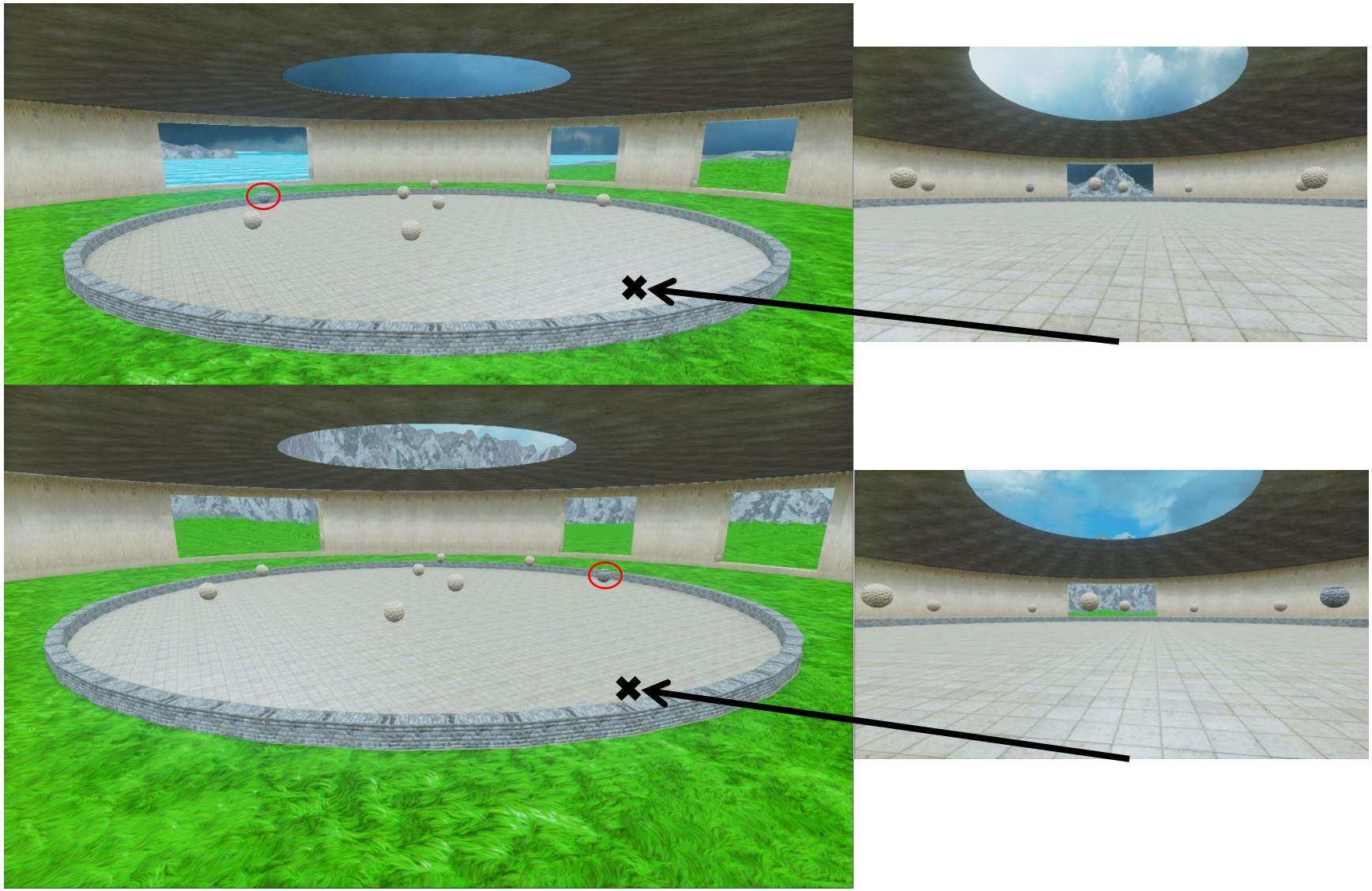


Figure 13. Experiment 2: Standard trial views of the uDS maze.
Left: Views from the northeast (top) and southwest (bottom), from above normal eye-level to highlight the landscape outside the room (allocentric features) and the cue-sphere (circled in red in the larger views) marking the position of the platform. Black X's show the starting location for the corresponding starting views on the right. Right: North (top) and south (bottom) starting views.

Place maze.

Experiment 2 assessed allocentric navigational performance using the "Place maze" (Figure 14). The design of the Place maze was based on that of the Arena maze (Livingstone-Lee et al., 2011), a virtual MWM analogue built using Unreal® software (Epic Megagames). Like the uDS maze, the Place maze was also built using the newer Unreal Development Kit (UDK; Epic Games) software. It was identical to the uDS maze in most respects, including the visual layout, start positions and the fixed nature of the platform (although it was fixed in the NE quadrant, not the SE, as with the uDS maze). However, there were no intra-maze cues (i.e., floating spheres) in the Place maze, making allocentric navigation (e.g., using a configuration of distal landscape cues) the only efficient strategy. Navigational performance was assessed on Standard trials in terms of the mean latency and distance required for participants to find the platform (averaged over trials). Whereas in the uDS maze Inter-Trial Strategy Probes (ITSPs) provide a measure of strategy selection, in the Place maze *Inter-Trial Probes* (ITPs) provide an indicator of the accuracy of participants' knowledge of the platform location. This "Place Error" variable is calculated as the Euclidean distance between the actual platform location (centre) and the participants' estimate, averaged over trials.

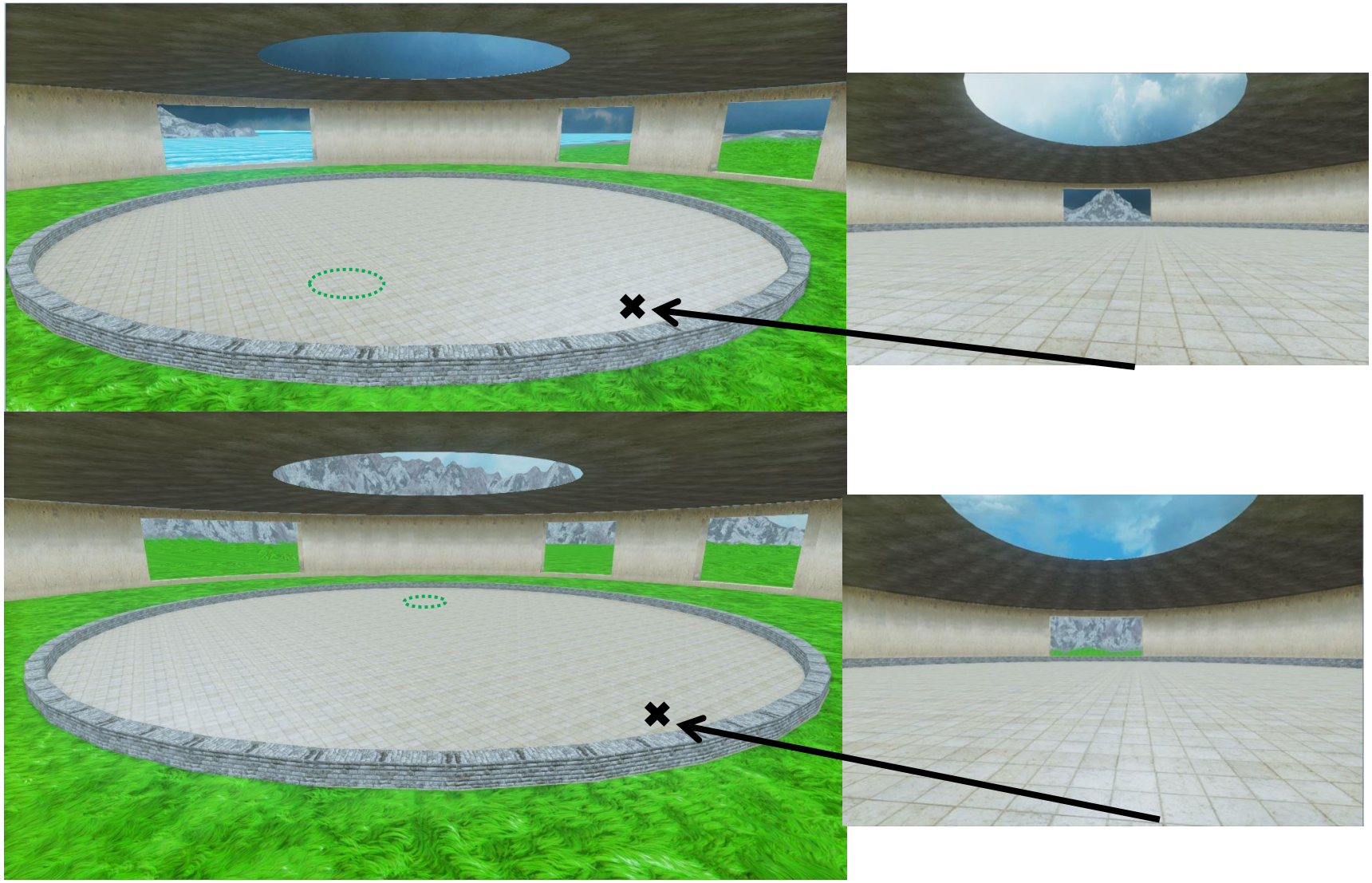


Figure 14. Experiment 2: Views of the Place maze.
Left: Views from the northeast (top) and southwest (bottom), from above normal eye-level to highlight the landscape outside the room (allocentric features). Dotted green ovals show the approximate location of the hidden platform. Black X's show the starting location for the corresponding starting views on the right. Right: North (top) and south (bottom) starting views.

Procedure.

The procedure for Experiment 2 was similar to Experiment 1 (see Figure 15). Testing was conducted in a quiet, distraction-free room, with one male and one female experimenter. Participants completed all computer-based tasks on a desktop computer with a 19" LCD monitor set to a resolution of 800 x 600. Experimental sessions were run between 9:00am – 5:00 pm. Overall the procedure took about 85 minutes.

After consent was obtained, the session began with an initial 25-30 minute "pre-stress" resting period. This period included a short introduction, after which participants were equipped with the HR monitor, BP cuff, and SC electrodes, then completed a short demographics questionnaire, in the same manner as Experiment 1. After participants had completed the questionnaire, the experimenters left the room and participants were allowed to relax and read magazines. When the experimenters returned after 10 minutes, participants completed the STAI (both "state" and "trait" portions) and then gave baseline blood pressure measurements (BP1). This marked the end of the "pre-stress" phase.

Immediately following the "pre-stress" phase, participants completed either the PASAT or the UPASAT, using a procedure identical to Experiment 1 (see Chapter 2: Method). Immediately after completing the PASAT/U-PASAT, participants completed the "state" portion of the STAI a second time (STAI-S B) while BP measurements (BP2) were taken.

In the "immediate testing" phase that followed, half of the participants had their navigational strategy selection tested in the uDS maze, and half had their performance tested in the Place maze. The trial procedure for both mazes was the same, and trials were presented in the same order described in Experiment 1 (see Chapter 2: Method). Briefly, participants were first given a set of practice trials, which included a) an exploration trial, in which participants could

look out the windows and explore the room outside the arena for as long as they wished, b) 4 visible platform trials, in which participants had to navigate to a visible platform from different start positions, and c) a Pre-test Probe trial, in which participants had to guess where the platform would be hidden on subsequent trials by moving to that location. After practice, participants completed 10 pairs of Standard and Probe trials (ITSPs for the uDS maze, or ITPs for the Place maze). Participants were given detailed instructions prior to starting each new trial type. The immediate testing maze procedure, including all trials and instructions, required approximately 15-20 minutes. As soon as participants had finished navigating in all trials, they completed a third STAI (STAI-S C) while providing BP measurements (BP3). This marked the end of the immediate testing phase and the beginning of the "delayed testing" phase.

In the delayed testing phase, the participants who were tested in the uDS maze first (i.e., in the immediate testing phase) now had their performance tested in the Place maze, while those who were tested in the Place maze first now had their strategy selection tested in the uDS maze. In both mazes, delayed testing started with a Pre-test Probe trial, after which participants were given the usual 10 pairs of Standard and Probe trials. The delayed testing maze procedure required approximately 10-15 minutes.

After delayed testing, participants completed a final STAI (STAI-S D) and provided final BP measurements (BP4). To end the session, participants completed a short (3-4 minute) post-test questionnaire. The main purpose of the questionnaire was to gather information about possible factors that might have influenced navigational strategy selection, such as video-game experience.

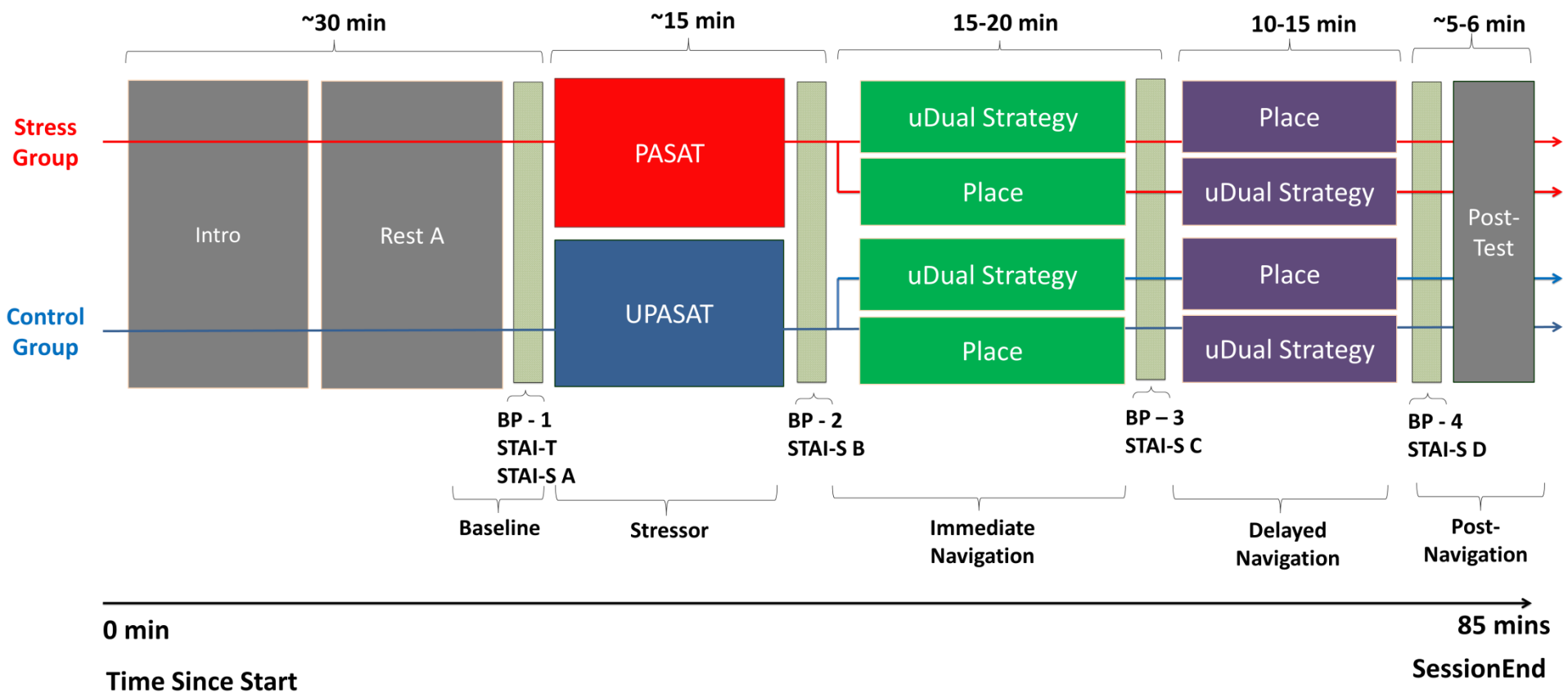


Figure 15. Experiment 2: Procedure.
Brackets at the top of the figure show estimations of task length (e.g. 30 min); brackets at the bottom specify the physiological and psychological measurement time points. Heart rate (HR) and skin conductance (SC) were measured continuously through the periods indicated (then averaged) whereas blood pressure (BP) and STAI-trait (STAI-T) and STAI-state (STAI-S) were measured as single points.

Data analysis.

All statistical analysis was conducted using Microsoft Excel and SPSS version 21.

To assess the effectiveness of the PASAT to induce stress, I entered Stress Condition and Sex[5] into 2 x 2 ANOVAs to examine group differences for each of the individual physiological measures, the composite SAM score (see Experiment 1), as well as STAI score. Measures of SAM axis activity (HR, SC, systolic and diastolic BP) in response to stress and the psychological experience of stress (STAI score) were calculated as percentage of baseline, in the same manner as Experiment 1. For HR and SC, percentage of baseline was based on the average of all measurements taken during the stressor period (PASAT/UPASAT) divided by the average of all measurements taken over the 5-minute baseline period immediately prior to the stressor. For BP and STAI, percentage of baseline was based on the measures taken immediately after the stressor divided by the measures taken immediately before the stressor (See Figure 15: BP 2/BP 1). Similarly, measures of SAM axis activity during navigation were also calculated as a percentage of baseline using measurement periods comparable to those of SAM activity during the stressor. However, BP at navigation was not measured separately for the immediate maze because the measurements would be the same as that used for SAM axis activity during stress (i.e., BP 2/BP 1).

I assessed navigational strategy selection by conducting 2 x 2 x 2 (Stress Condition x Delay x Sex) analyses of variance (ANOVAs) on average Allo % in the uDS maze. Allo % is calculated in the same way as in Experiment 1, by taking the number of ITSP trials that were designated as "allo" and dividing that by the total number of ITSP trials in which the participant chose a strategy. I assessed navigational performance by conducting 2 x 2 x 2 (Stress Condition

[5] It should be noted that like Experiment 1, Experiment 2 used a quasi-experimental design and that Sex was a predictor variable. However, throughout the rest of the chapter and in the general discussion I will be referring to sex as a variable in a manner consistent with the literature (e.g., du Plooy et al., 2014; Wolf et al., 2001).

x Delay x Sex) ANOVAs on average latency and distance to the platform on standard trials, and on place error on ITPs. Finally, relationships between physiological measures of stress and navigational strategy selection and performance were assessed using Pearson correlations.

Results

Manipulation checks.

Both physiological and psychological measures indicated that the PASAT was effective at inducing stress (Figures 16, 17). Condition x Sex ANOVAs revealed a main effect of Condition on each of the 4 measures of SAM activity. Stress elevated both systolic ($F(1,110) = 23.29$, $p<.000$, $\eta^2 = .18$), and diastolic ($F(1,110) = 21.21$, $p<.000$, $\eta^2 = .17$) BP, as well as HR ($F(1,110) = 33.76$, $p<.000$, $\eta^2 = .24$) and SC ($F(1,112) = 17.24$, $p<.000$, $\eta^2 = .14$) (Figure 17). However, Sex did not have any main or interaction effect on any of the SAM measures (all p's > .09). This pattern was also reflected in the SAM Composite score, with a main effect of Condition ($F(1,119) = 68.47$, $p = .000$, $\eta^2 = .37$), but no main or interaction effects for Sex (all p's > .23). Regarding the subjective experience of stress, a 2 x 2 ANOVA revealed that the PASAT caused participants in the Stress group to report higher levels of anxiety on the STAI than those in the Control group ($F(1,112) = 112.68$, $p<.000$, $\eta^2 = .50$), but again, Sex had no main or interaction effects (all p's > .12). .

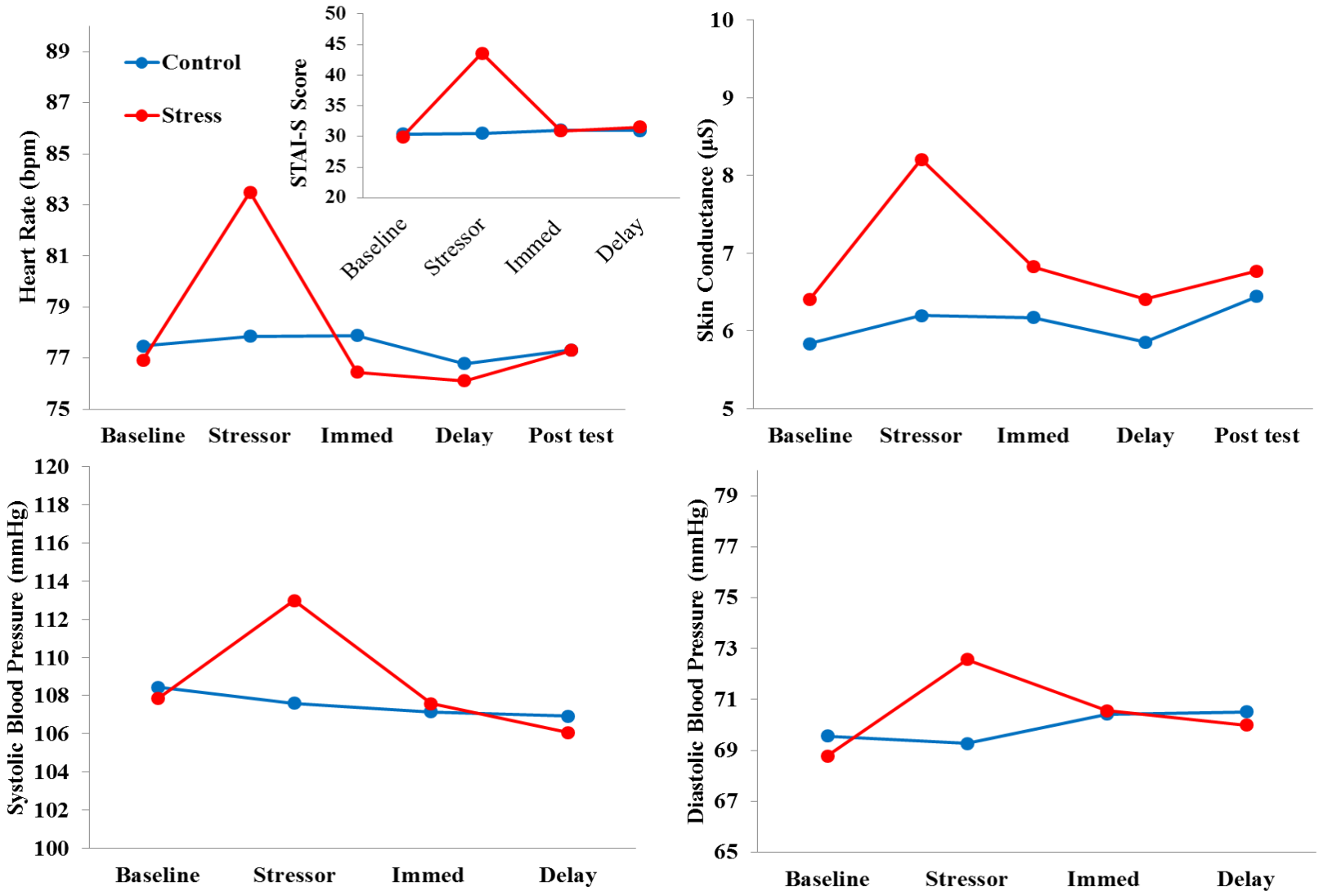


Figure 16. Experiment 2: Time course of the PASAT effect on physiological measurements.
Y-axes denote: Heart Rate (HR), Skin Conductance (SC), Systolic Blood Pressure (BP-Sys), Diastolic Blood Pressure (BP-Dia), and State-Trait Anxiety Inventory-State Portion (STAI-S; inset).

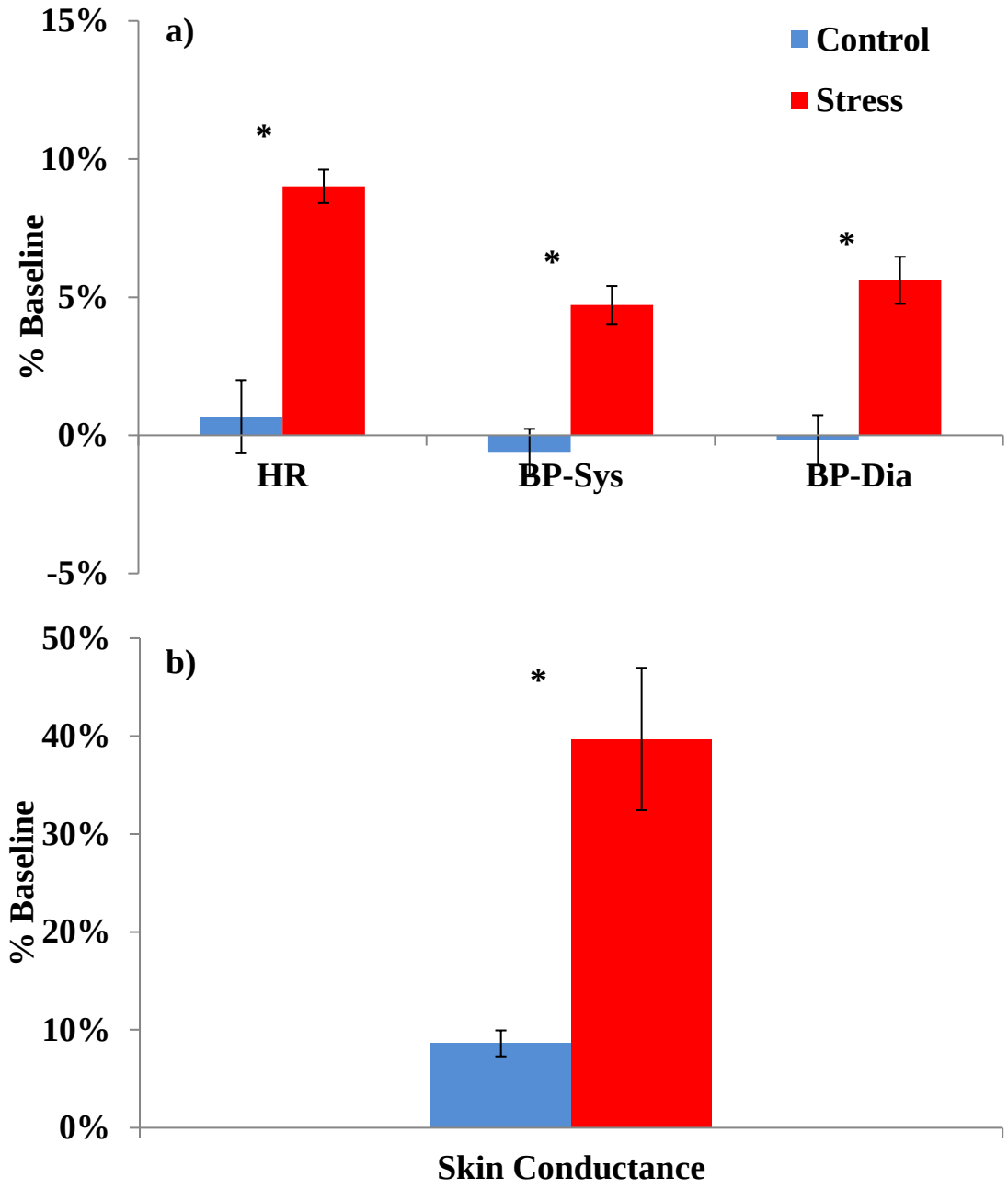


Figure 17. Experiment 2: The effect of the PASAT on SAM measures.
The effect of the PASAT on a) heart rate (HR), systolic (Sys) and diastolic (Dia) blood pressure, and b) skin conductance. Values are expressed as mean % of baseline. Error bars are SEM. *p < .01.

Stress and navigational strategy.

In terms of strategy selection, stress had a very specific effect on participants' tendency to navigate allocentrically. Here stress caused a shift in strategy selection away from allocentric navigation. This is the opposite of Experiment 1, in which the stress caused a shift towards allocentric navigation. This shift was greater when testing was delayed by 20 minutes (i.e., 30 minutes after stressor onset) than when testing was immediate. Unexpectedly, there was little or no effect in females regardless of delay (Figure 18).

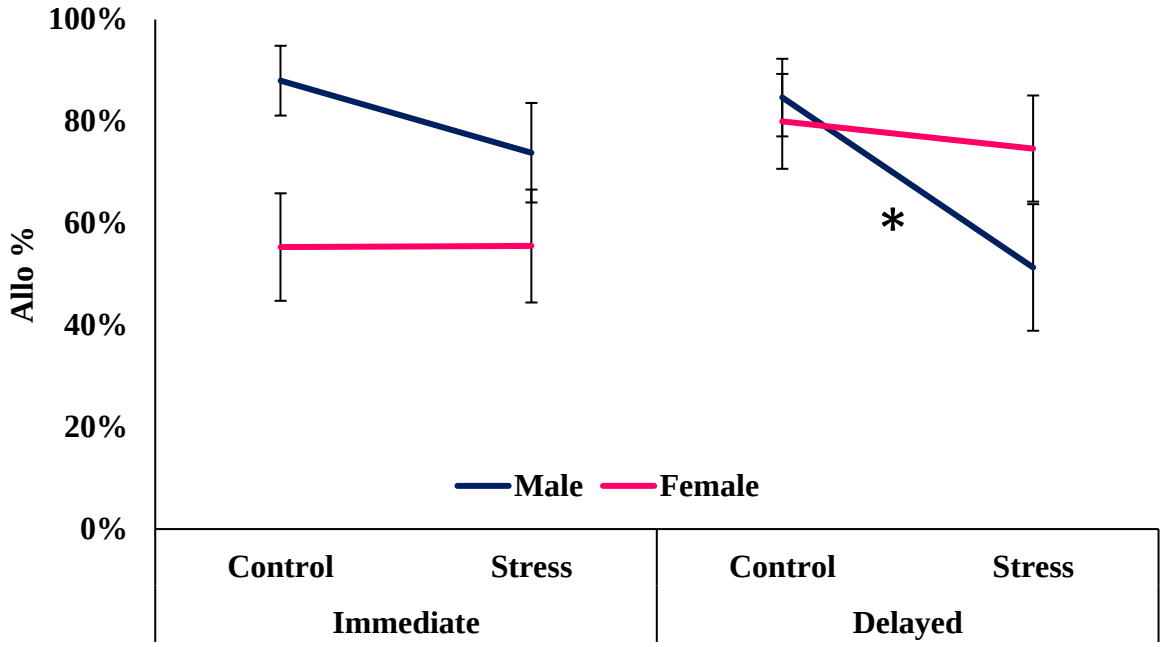


Figure 18. Experiment 2: Effect of PASAT stress on strategy selection.
The effect is expressed as average Allo%. Error bars are SEM. *p < .01 (simple effect).

Although the effect of stress on navigation appeared to be confined to males at delayed testing, unfortunately, an ANOVA showed that neither the Condition x Delay interaction, nor the Condition x Delay x Sex interaction were significant (Condition x Delay $F(1,110) = .76$, $p = .39$, $\eta^2 = .007$; Sex x Delay x Condition $F(1,110) = .23$, $p = .63$, $\eta^2 = .002$). The only significant main effect or interaction was a Sex x Delay interaction ($F(1,110) = 6.06$, $p = .02$, $\eta^2 = .05$) which

appeared to be due to females being more likely to navigate allocentrically at the delay (regardless of stress condition) whereas males were less likely to navigate allocentrically. Because the effects of stress appeared to depend, at least to some extent, on both Delay and Sex, simple main effects and simple interactions were analysed separately for immediate and delayed testing and separately for males and females. These analyses revealed that there were no significant simple main effects or interactions between Condition and Sex at the immediate testing (p's > .47) or at the delayed testing (though the simple main effect of Condition was close, p = .06). There was, however, a significant simple main effect of Sex at immediate testing, such that across stress groups, males had higher overall Allo%.

Simple effects analysis of the effect of stress on strategy selection among males and females revealed that stress generally reduced male tendency to navigate allocentrically, but had no effect on females. For males, there was a significant simple main effect of Condition (stress) across immediate and delayed testing ($F(1, 53) = 6.13$, $p = .02$, $\eta^2 = .10$), and no simple interaction ($p = .32$). That is, stress generally decreased Allo% for males across testing times. However, planned contrasts reveled a significant effect of stress in males at delayed testing ($t(28) = 2.29$. $p = .03$, $d = .84$; Figure 18, right side), but not at immediate testing ($p = .25$). For females, there was only a simple main effect of delay ($F(1, 57) = 4.56$, $p = .04$, $\eta^2 = .07$), showing a greater tendency to navigate allocentrically in the delayed uDS maze (across stress groups). There were no other simple main effects, simple interactions or significant planned pairwise contrasts.

In sum, the only statistically significant effects of stress was found in delayed testing and was a reduced tendency by males to navigate allocentrically. Even though the overall analysis revealed no significant interaction between Sex, Stress Condition and Delay, both descriptive

and inferential statistics indicated that stress had little or no effect on females regardless of whether they were tested immediately or at a delay.

Stress and navigational performance.

In terms of navigational performance, PASAT stress worsened latency in the Place maze, but only in females, and only at delayed navigation (Figure 19a). Analysis of mean latency to find the platform revealed only the expected main effect of Sex ($F(1,116) = 4.20$, $p = .04$, $\eta^2 = .04$), with females having higher latency than males overall. There was no significant main effect of Condition or Delay (p's > .27). There was, however, a significant Condition x Sex interaction, such that across delay intervals, stress increased female latency and slightly decreased it in males ($F(1,116) = 5.68$, $p = .02$, $\eta^2 = .05$). There was also a Sex x Delay interaction, such that across stress and control groups, delayed testing increased female latency, and slightly decreased it in males ($F(1,116) = 5.92$, $p = .02$, $\eta^2 = .05$). There was no Condition x Delay interaction ($p = .11$), and critically, no Condition x Delay x Sex interaction ($p = .15$). In terms of distance, an ANOVA revealed only a significant main effect of Delay ($F(1,116) = 6.23$, $p = .01$, $\eta^2 = .06$), such that participants generally took less direct routes to the platform in the delayed Place maze, regardless of sex or stress group (Figure 19b). There were no other significant main effects or interactions. Similarly, for place error an ANOVA revealed a significant main effect of Delay ($F(1,116) = 5.67$, $p = .02$, $\eta^2 = .05$), such that participants generally made less accurate estimates of the platform location in the delayed Place maze, regardless of sex or stress group (Figure 19c). Once again, there were no other significant main effects or interactions with respect to place error. As with strategy selection, because the effects of stress on performance seemed to depend on both Delay and Sex, simple effects analysis was conducted separately for immediate and delayed testing and separately for males and females.

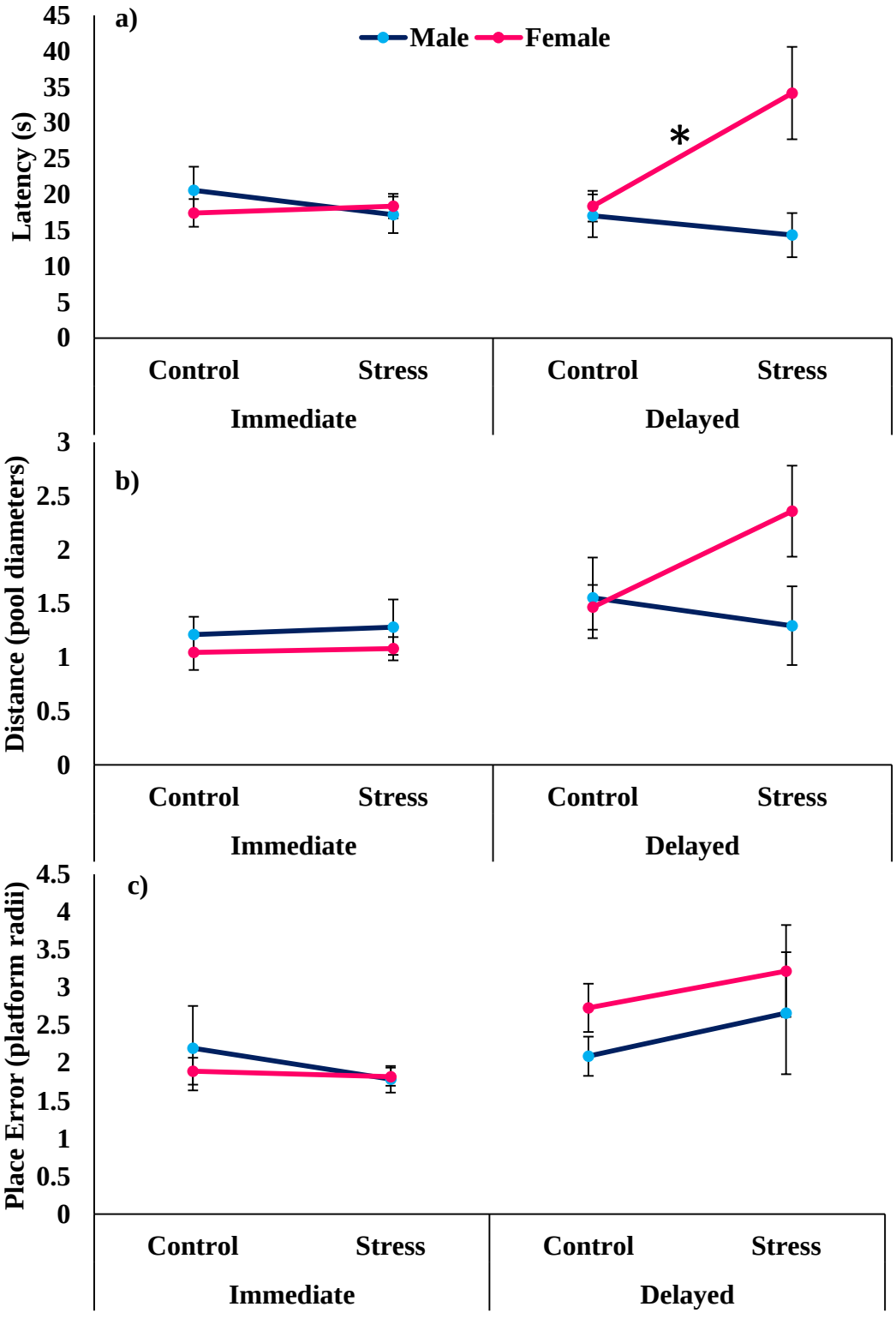


Figure 19. Experiment 2: The effect of PASAT stress on immediate and delayed performance. Performance is represented in terms of average a) latency (in seconds), b) distance (in pool diameters) and c) place error (in platform radii). Error bars are SEM. *$p < .05$ (simple effect).

Splitting the analysis by Delay revealed that stress worsened performance in females and slightly improved it in males when tested at the delay, but not immediately after stress. ANOVAs on delayed performance in the Place maze revealed a significant simple main effect of Sex ($F(1, 52) = 6.51$, $p = .01$, $\eta^2 = .11$) and a significant simple Sex by Condition interaction ($F(1, 52) = 4.8$, $p = .03$, $\eta^2 = .09$), such that stress increased female and slightly decreased male latency (Figure 19a, right side). There were no simple main or interaction effects on distance or place error at delay (all p's > .12). Similarly, simple effects analysis on immediate performance in the Place maze revealed no simple main or interaction effects for latency, distance, or place error (all p's > .38; Figure 19, left side).

Splitting the analysis by Sex revealed that stress worsened performance for females when tested at the delay, but not immediately after stress. ANOVAs on female performance in the Place maze revealed a significant simple main effect of Condition ($F(1, 55) = 5.12$, $p = .03$, $\eta^2 = .09$) and Delay ($F(1, 55) = 5.16$, $p = .03$, $\eta^2 = .09$), as well as a significant simple Condition x Delay interaction ($F(1, 55) = 4.04$, $p = .049$, $\eta^2 = .07$) on latency. This indicates that stress generally increased (rather than decreased, as expected) the time required for females to find the platform in the delayed Place maze. Planned t-tests revealed a simple effect of Condition at delayed navigation ($t(27) = -2.24$. $p = .03$, $d = -.85$; Figure 19a, right side), but not at immediate navigation ($t(28) = -.37$. $p = .72$, $d = -.14$; Figure 19a, left side). There were also significant simple main effects of Delay on distance ($F(1,55) = 9.69$, $p = .003$, $\eta^2 = .15$) and place error ($F(1,55) = 9.50$, $p = .003$, $\eta^2 = .15$), indicating that females generally took less direct routes and were less accurate at identifying the platform location in the delayed maze, regardless of stress condition. There were no other significant simple main or interaction effects for females (all

other *p*'s > .08). ANOVAs on male performance in place maze revealed no significant simple main or interaction effects for any performance dependent variable (all p's > .28).

In sum, although the overall analysis revealed no significant main or interaction effects of stress on navigational performance, statistically significant simple effects indicated that stress increased female latency to find the platform, but only at delayed navigation. In contrast, stress appeared to have virtually no impact on male navigational performance (either immediate or delayed).

Navigation and SAM axis activation.

The physiological underpinnings of these effects of stress on navigation appeared to be indirectly related to SAM activation. As described above, PASAT stress appeared to affect strategy selection only in males and only at the delay. The most meaningful relations between SAM and strategy selection would thus be found among males at the delay. Presumably, in females or at immediate testing, if SAM activation was correlated with Allo%, and SAM elevation (by the stressor) did not change Allo%, then a correlation would not be expected, and mixing correlated and uncorrelated data together would mask the presence of a correlation that existed in one sex, at one time of testing. Surprisingly, however, despite the behavioral effect of PASAT stress on delayed Allo% in males, there were no significant correlations between Allo% and any of the measures of SAM activity (HR, BP, or SC; all *p*'s > .09) or the SAM Composite score ($p = .15$).

Somewhat surprisingly, as described above, PASAT stress appeared to affect navigational performance only in females and only at the delay. Here, the most meaningful relations between SAM and performance would thus be found among females at the delay. Pearson correlations revealed no significant relationships between any performance measure and

Composite SAM score (all *p*'s > .07). However, when examining relationships between performance measures and individual SAM measures, significant positive relationships between latency and systolic (r = .61, p = .001) and diastolic blood pressure (r = .40, p = .04) appeared. That is, when stress increased blood pressure at the time of the PASAT, it also increased the time required for females to find the platform in the Place maze when tested at the delay. No other relationships between individual measures of female SAM activation and performance at this time of testing were observed (all *p*'s > .14).

However, an important question is whether the effect of stress on navigation is due to SAM activation at the time of the stressor, or SAM activation at the time of navigational testing. For example, given the correlation between SAM activation by the stressor and delayed navigation, it is possible that SAM activation lingered through the delay to the time of testing and therefore was acting directly and proximally on navigational cognition. An analysis of the relations between SAM activation at the time of navigation (as opposed to at the time of the stressor) and delayed navigational strategy selection in males revealed no significant correlations (all *p*'s > .32). Interestingly however, an analysis of the relations between SAM activation at navigation and delayed performance in females revealed significant negative relations between distance and HR (r = -.41, p = .03), as well as systolic (r = -.41, p = .03) and diastolic (r = -.42, p = .03) BP. That is, SAM activation measured at the time of navigation was associated with shorter distance to find the platform. No other significant relationships between measures of SAM activation (including the SAM Composite Score) at the time of navigation and delayed navigational performance in females were found (all *p*'s > .12).

In sum, correlational analysis between strategy selection in males at the delay versus SAM activation revealed no significant relationships, either at the time of the stressor or the time

of testing. This was despite the significant simple effect of stress on male strategy selection at delayed testing. In contrast, correlational analysis of navigational performance in females at the delay versus SAM activation revealed that SAM activation at the time of the stressor was associated with impaired performance (i.e., longer latencies). However, when SAM was measured at the time of navigation, the relation between SAM activation and navigational performance flipped, such that SAM was associated with improved performance (i.e., shorter distances).

Discussion

As in Experiment 1, the PASAT produced a robust physiological response, and once again, there was no sex-based difference in physiological reactivity. However, the effects of stress on strategy selection were essentially the opposite and sex-specific. New findings with respect to the optimal timing of the stress effects, and the effects of stress on navigational performance were more consistent with previous findings than with Experiment 1. Most of the hypotheses for this experiment were based on findings from Experiment 1, and most of these hypotheses were either not confirmed or were disconfirmed. The first such hypothesis was that stress would increase allocentric navigation. This hypothesis was disconfirmed, as stress was found to decrease selection of an allocentric strategy and did so only in males. The second hypothesis, that stress would increase allocentric strategy selection immediately after the stressor was not confirmed in either the magnitude or the direction of the effect: stress had no effect on strategy selection whatsoever when tested immediately. The third hypothesis, that stress would enhance navigational performance in the Place maze, was either disconfirmed or not confirmed. Instead, stress impaired delayed (but not immediate) navigational performance in females, and had no effect on male performance (immediate or delayed). The fourth hypothesis was confirmed

– that is, the stress effect on navigational strategy selection and its effect on performance had the same time-course (occurring after a 20 min delay, or 30 min post-stressor onset, but not immediately after stress). However, these results also revealed an interesting puzzle: although the timing of the effects of stress on strategy and performance was the same, there was a double dissociation between sex and spatial cognitive domain. In males, stress reduced allocentric strategy selection, but had no effect on allocentric performance. In females, stress impaired allocentric performance, but had no effect on strategy selection. Finally, the hypothesis that the magnitude of the effects of stress on navigational strategy selection and performance would be related to the magnitude of its effect on SAM axis activation was partly confirmed. Correlations revealed relatively strong relationships between stress-induced SAM activation and female performance at delay. Surprisingly, however, and in contrast to Experiment 1, no relationships were revealed between SAM activation and delayed male strategy selection.

Each of these hypotheses and their findings deserves to be discussed in turn.

The first hypothesis was that stress would have the same effect in Experiment 2 as the surprising effect it had in Experiment 1: that it would increase the likelihood of selecting an allocentric navigational strategy. Instead, the strategy selection results from Experiment 2 not only did not replicate, but contradicted the results from Experiment 1. This was true even though both experiments had the same stressor, stress-to-test delay, and navigational task requirements. Despite this apparent contradiction, the effect of stress on navigational strategy selection that was observed in Experiment 2 is in line with previous empirical findings. Acute stress, stress hormones, and anxiogenic drugs bias rodents to solve dual-solution navigation tasks egocentrically (Elliott & Packard, 2008; Kim et al., 2001; Schwabe et al., 2010a). In humans, there has been no previous research on the effects of acute stress on navigational strategy

selection to date. However, similar to what was observed in Experiment 2, some studies have shown that acute stress biases humans to solve non-navigational dual-solution tasks using caudate nucleus-dependent strategies rather than hippocampus-dependent strategies (Schwabe et al., 2007; Schwabe & Wolf, 2012); See Chapter 1 for additional details on these studies). Furthermore, the effects of stress on strategy selection after a delay in Experiment 2 are in line with the original hypothesis of Experiment 1. This was based on all 4 models of the effects of acute stress on hippocampal function (TDM, Uniform Shift, MYD and Hot/Cool Systems), all of which predict that stress should bias navigational strategy selection from allocentric to egocentric.

The contradictory findings between Experiment 1 and 2 with respect to strategy selection give rise to the question: why did the findings differ, and which is a better reflection of the effect of acute stress on human navigational strategy selection? There were several differences between the two experiments that could conceivably have contributed to the differing results.

One factor which might have led to differences in results could have been baseline anxiety and/or cortisol levels in the participants. There could have been differences due to sampling, though the ages, sex balance and education levels were similar. There could have been differences due to time of year: Experiment 1 was collected mostly (51% of participants) between January and March and in the morning whereas Experiment 2 was collected mostly (45% of participants) between September and December and in the afternoon as well as the morning. Given that the participants were mostly first-year students, and that the sun rose later in the fall, baseline anxiety and circadian cortisol levels could have been different in the participants very first term at university (Experiment 2) than in their second term (Experiment 1). These differences are not only difficult to weigh, but seem unlikely explanations because cortisol did not appear to contribute to the effects seen in Experiment 1.

A more likely explanation for the difference between experiments was the intentional difference between the activities given during the delay period. In Experiment 1, participants read magazines and completed the FAPA and IGT. The latter were felt to be potentially anxiogenic, and possibly causing stress in the control group or augmenting it in the stress group. Experiment 2 attempted to achieve better psychological and physiological differentiation of stressed and control groups by giving participants a task previously found not to be stressful: navigation in the Place maze. One drawback to this procedure was that while participants in the "immediate testing" condition were experiencing the maze for the first time, the "delayed testing" participants were learning and selecting their strategies after having been exposed to the same environment, and after training in allocentric navigation. Presumably this training would increase the likelihood of choosing to navigate allocentrically in the uDS maze. This effect was observed in females: more females in the control group navigated allocentrically in the delayed condition than navigated allocentrically in the immediate test condition (80% versus 55%, see Figure 18). However, given that there was no effect of stress on strategy selection at either time, it would be non-parsimonious to argue that the training "protected" the females against a delayed effect of stress that was not present at immediate testing. The effect was not observed in males. That is, males who were given training in the Place maze prior to (delayed) testing in the uDS maze, were slightly less (not more) likely to choose to navigate allocentrically than those who did not have Place maze training prior to (immediate) testing (see Figure 18). It is difficult to know whether the prior Place maze training had any amplifying or dampening effect on the effect of stress on strategy selection. The effect of stress on males was somewhat greater at the delayed, post-Place-training testing. Although these two factors (delay and training) are confounded, it seems unlikely that additional allocentric training would amplify the supressing

effects of stress on allocentric strategy choice. Thus, a Place-training-induced predilection for navigating allocentrically seems an unlikely explanation for the difference between the allocentric suppression seen in Experiment 2, and the allocentric enhancement seen in Experiment 1.

An even more likely contributor to the contradiction between the findings between the two experiments was the different mazes used. As discussed in the previous chapter and the introduction of this experiment, the uDS maze in Experiment 2 was designed to improve upon the original Dual-Strategy maze from Experiment 1 by establishing cleaner separation between egocentric and allocentric navigation. It did this in several ways. First, the uDS maze was redesigned in three ways to reduce or eliminate the possibility of "impulsive" egocentric navigation being mischaracterized as allocentric: a) the key cue object was always in the field of view at the start of the ITSP trial, so that participants did not need to pan left or right to find it, b) flanker cues were eliminated by making all non-cue objects identical, and c) the cue-objects were larger and more obvious than they were in the original DS maze, reducing the likelihood that the target cue was overshadowed by a "snapshot" of the window. Second, the uDS maze was designed to eliminate the spatial displacement between the cue object and the platform, thereby making the maze more purely egocentric. In sum, whether the contradiction between the findings of the two experiments was caused by differences in sampling, "rest" activities, the construction of the mazes, or some other factor, it is clear that the effect of stress on navigational strategy selection needed to be tested once again (if only to break the tie).

The next three hypotheses all had to do with the main objectives of this experiment: examination of the timing and generality of the effects of stress on navigation. Specifically, were

the effects of stress apparent only after a delay, and were the effects of stress on just strategy selection or did they extend to navigational performance as well?

The second hypothesis of Experiment 2 proposed that the effects of stress on strategy selection would be present immediately after the stressor, and not just after a delay. Specifically, that stress would bias participants towards the use of more allocentric strategy selection when tested immediately after the stressor. The TDM model predicts that the SAM axis is the primary driver of the effects of stress on hippocampal function. According to the model, stress, via the SAM axis, rapidly and for a short time enhances hippocampal function after a stress event, which leads to a refractory period during which hippocampal function is supressed at longer delays. Associations between strategy selection and measures of SAM axis activity (but not HPA activity) in Experiment 1 led to the prediction that, in line with the TDM model, SAM axis activity was mediating the effect, and therefore the effect should appear immediately after the stressor and not just at the delay. Instead, there were no detectable effects of stress on immediate strategy selection whatsoever (in males or females). While this again suggests that the stress effect on strategy selection in both experiments was in fact driven by stress-induced HPA activation (because the timing of the effect matches the timecourse of stress-induced cortisol release), it does not rule out some other, concurrent physiological event. Despite the fact that no effects were observed, it was nevertheless important to check the effects of stress on navigational strategy selection immediately after stress largely because this has never been checked before.

The third hypothesis proposed that the effects of stress would generalize from strategy selection to performance and would do so immediately and after a delay. Specifically, based on the TDM model and the results from Experiment 1, stress was expected to enhance allocentric navigational performance in the Place maze, both immediately after stress or 30 minutes post

stressor onset. Instead, results indicated that stress had no effect on performance when tested immediately, no effect on males when tested after a delay, but impaired one aspect of performance (latency) by females after a delay. The latter result is consistent with findings in the rodent literature, with acute stress typically impairing performance on hippocampus-dependent spatial tasks (Xiong et al., 2003, Park et al., 2008), although in rodents, this impairment is often enhanced with much longer delays after stress (e.g., 24hrs; Diamond et al., 2006; Kim, Koo, Lee, & Han, 2005) and with one exception (Park et al., 2008) only male rodents are typically tested. However, these results partly contradict the first human study to examine the effects of acute stress on allocentric navigational performance which found that stress enhanced performance in an all-male sample (Duncko et al., 2007). The results also partly replicate the findings of this same study: no effect of stress was found in the traditional measures of water maze performance (distance and latency, as used here) but were only present in two variables not generally examined (heading error and failures to find the platform). The present findings also replicate those of Thomas et al., 2010, who found that stress caused impairment in female, but not male, performance in a similar virtual MWM to the one used here.

To date, Experiment 2 is the first study to examine the effect of stress on allocentric navigational performance immediately after the stressor. Given that no effect was observed, no support was found for the TDM-based idea that SAM-mediated effects of stress would appear immediately after stress.

The fourth hypothesis proposed that the timing of the effects of stress on navigational strategy selection and performance would coincide. This hypothesis was confirmed. That is, in both behavioural domains, the effects of acute stress appeared only at delay, and not immediately after stress.

This result implies a common (or closely related) mechanism of the effects of stress on both navigational domains. Curiously, whether the effect appeared in strategy selection or performance was sex dependent – with stress reducing allocentric strategy selection in males (but not affecting performance) and impairing allocentric performance in females (but not affecting strategy). Thus, although the time-course of the effects of stress appears to be the same for both male and female participants, the specific nature of the effects differs. This is a potentially interesting finding because it suggests that the same stress-related physiological mechanism is acting on different navigational systems depending on sex. However, this idea is tempered by the fact that sex did not seem to have an impact on strategy selection in Experiment 1. Thus, it would be premature to speculate much further about the interaction between stress, sex, and navigational domain before the results of Experiment 2 are re-tested using an improved design.

The fifth hypothesis of Experiment 2 was that there would be relationships between the effects of stress on SAM activation and on its effects on navigation. This hypothesis was partly confirmed. In females, SAM activation during the stressor matched the behavioral effect of stress – that is, stress increased SAM activation at the time of the stressor and impaired allocentric navigational performance when tested at a 30 min delay (after stressor onset). However, when SAM activity was measured at the time of navigation, the associations between SAM activity and navigational performance flipped (i.e., higher SAM was associated with improved, not impaired, performance). This suggests that the effect of stress was not mediated by persistence in SAM activation over the 20-min delay, but rather was due to some other persisting secondary or correlated physiological effect of SAM activation. Interestingly, in contrast to Experiment 1, there were no associations between stress-induced SAM activity and delayed strategy selection (despite finding a behavioural effect), casting doubt on the relationships observed in Experiment

1. Again, the contradictory results raise the question of which experiment more accurately measured the effects of stress on strategy selection, and again, they suggest the need for replication.

A key question in this dissertation so far has been whether the effects of stress on navigation are primarily mediated by the HPA axis (MYD model), the SAM axis (TDM model) or both (Hot/Cool Systems, Uniform Shift models). In Experiment 1, a lack of stress effect on HPA activity (salivary cortisol) and associations between navigational strategy selection and measures of SAM activity (but not HPA activity) pointed towards the SAM axis as the key mediator. However, in Experiment 2, two findings point back to HPA mediation. First, in Experiment 2, the only detectable effects of acute stress on navigation (both strategy and performance) occurred at the 30 min post-stressor onset delay. This is coincident with the putative time-course of stress-induced cortisol release (i.e., HPA activity; Dickerson & Kemeny, 2004), and not with SAM activity, which should peak immediately after stress. In other words, if the lingering influence of SAM activity was the key driver of the effect seen in Experiment 1, then a similar effect should have occurred when navigation was tested immediately after the stressor, when SAM activity should be at its peak. The lack of effects at this time points back to an HPA (i.e., cortisol) mediated effect. Second, the relationship between SAM activity and navigation was between SAM at the time of stressor and *impaired* navigation after a 30 minute delay. In contrast, SAM at the time of delayed testing was associated with *enhanced* allocentric performance. That is, the relationships disappeared or changed direction when SAM activity was measured at the time of delayed navigation. This makes it difficult to argue, as I did in the introduction of this chapter, that the effects of stress on strategy selection were mediated by the persisting influence of the SAM axis.

In conclusion, Experiment 2 was an important step in the study of the relationship between acute stress and navigation behaviour, and was able to provide insight into several key issues. First, Experiment 2 not only did not replicate, but contradicted the surprising finding of Experiment 1. Despite having the same stressor, stress-to-test delay, and navigational task requirements, in Experiment 1 stress tended to increase the likelihood of allocentric navigation whereas in Experiment 2 it tended to decrease the likelihood of allocentric navigation (but only in males). Possible explanations for this difference include different samples, different activities during the rest period, and (most likely) different maze designs. These possibilities should be explored, for example, by testing the effects of stress on navigation using the same mazes as Experiment 2, but with an improved experimental design that would allow for cleaner interpretations. Second, Experiment 2 showed that the effects of acute stress on strategy selection extend to performance – that is, stress tends to impair allocentric navigational performance (in females). Third, Experiment 2 showed that the effects of stress on navigation are time-dependent. That is, they appear only after a 30 min post-stressor onset delay (coincident with HPA related cortisol release) and not immediately after stress (coincident with SAM-axis activity). Curiously however, stress acted on different navigational domains depending on sex, with stress reducing male allocentric strategy selection and impairing female allocentric performance. Although this surprising finding is potentially interesting, it needs to be verified in another experiment before being considered further.

Bridge to Experiment 3

Although the findings of Experiments 1 and 2 seem to suggest that acute stress can affect delayed spatial navigation, the findings are somewhat confusing and at times contradictory. Experiment 3 sought to clarify these results by again retesting the effects of acute stress on

navigational strategy selection and performance, but this time using a cleaner design that addresses some of the methodological issues in the first two experiments. Experiment 3 also sought to see whether the effects of stress extend to egocentric navigation, by testing its effects in a new maze that forces participants to navigate egocentrically.

Chapter 4: Experiment 3

Introduction

Background.

The results of Experiment 1 showed that acute stress can shift navigational strategy selection in humans, and in the absence of HPA activation. The surprising direction of this effect led to Experiment 2, which had three main goals: 1) to see whether the findings of Experiment 1 would replicate in an improved virtual maze, 2) to investigate whether this effect is driven mainly by the SAM axis or the HPA axis, and 3) to see if the effect of stress on strategy selection would extend to allocentric navigational performance. Unfortunately, the results from Experiment 2 did not replicate, and were in fact opposite, those from Experiment 1. However, stress did influence allocentric navigational performance, although there was a puzzling sex dependency. That is, stress reduced allocentric strategy selection, but only in males, and impaired allocentric navigational performance, but only in females. Interestingly, for both spatial cognitive domains and for both males and females, the effects of stress only appeared when navigation was tested after a delay commensurate with the putative time course of an HPA-driven mechanism, and not a SAM-driven one. The key goals of Experiment 3 were to a) re-test the effects of acute stress on delayed strategy selection and allocentric navigational performance using a design that is easier to interpret, b) test the effects of stress on egocentric navigation, and c) examine the relationship between the effects of stress on strategy selection and egocentric and allocentric navigational performance.

Despite having the same stressor, delay before testing, and navigational task requirements, Experiments 1 and 2 revealed opposite effects of stress on navigational strategy selection. In Experiment 1, stress increased the likelihood that participants would solve the maze

allocentrically, whereas in Experiment 2, stress *decreased* the likelihood that it would be solved allocentrically (at least in male participants). There are two likely explanations for this discrepancy. First, the two experiments used different activities to occupy participants during the delay period. In Experiment 1, participants read magazines and conducted two cognitive tasks (the FAPA and the IGT). While these tasks were thought to be relatively benign, data from some physiological measurements as well as self-report suggested that they may have been moderately stressful. If this was the case, these tasks may have inadvertently stressed the control participants, reducing the separation between the two groups (in both physiological and psychological dimensions). With this in mind, participants in Experiment 2 were instead given a non-stressful navigation task. However, this task amounted to training in the use of an allocentric strategy, which may have confounded the strategy selection results from the uDS maze that followed. That said, such training would be expected to increase, not decrease, the likelihood of selecting an allocentric strategy (even under stress).

A more likely explanation for the difference in strategy selection findings between the two experiments is the difference in maze construction. As discussed in Chapters 2 and 3, the original Dual-Strategy maze (used in Experiment 1) may have miscategorised strategy selection by making the impulsive, but egocentric, choice appear allocentric. Specifically, participants may have impulsively navigated to the "allocentric" quadrant on ITSPs by either heading towards a flanker cue (visible at the start of trials, unlike the target cue) or to a more salient "snapshot" of the window nearest to the platform. In both cases, an egocentric response would have been classified as allocentric. This was not a problem in the uDS maze, where the target cue was always visible at the start of all trials, and where the distractor cues were all identical grey spheres, making them difficult to distinguish and use as simple egocentric cues.

With respect to navigational performance, acute stress should affect allocentric and egocentric navigation differently. As described in Part 1, all four theories predict that acute stress, after a delay of at least 30 minutes post-onset, should impair hippocampal function, thereby impairing allocentric (hippocampus-dependent) navigation. Two of the theories (Hot/Cool Systems and Uniform Shift) suggest that acute stress should enhance caudate function, thereby enhancing egocentric navigation. These predictions are consistent with the findings of rodent studies, which have found that stress impairs navigation in allocentric (Cazakoff et al., 2010) but not egocentric (e.g., Schwabe et al., 2010b) mazes, and that anxiogenic drugs can enhance performance in egocentric mazes (Packard and Wingard, 2008).

However, the differential effect of acute stress on allocentric and egocentric navigation has never been demonstrated in humans. As discussed in Chapter 1, the effects of acute stress on human allocentric navigation have so far been mixed, with one study finding enhancement (in males; Duncko et al., 2007), one study finding impairment (in females; Thomas et al., 2010), and a third study finding no effects whatsoever (in males or females; Klopp et al., 2012). The only study that has examined the effects of acute stress on egocentric navigational performance also tested its effects on allocentric performance, but this study found no effect of stress on navigation using either strategy (Guenzel et al., 2014). One possible explanation was that the study tested performance using virtual radial arm mazes. Radial arm mazes are easier to navigate than MWMs because the arms guide the navigator to targets, eliminating the need to compute vectors (distances and directions). Thus, although radial arm mazes are useful tools for testing working memory or reference memory (e.g., which arms contain rewards; which arms have been visited, etc.), they are not the best tools for testing navigational performance. The authors of at least one study have argued that radial arm mazes are too insensitive to even measure the sex effect in

navigational efficiency (Levy, Astur, & Frick, 2005), which is one of the strongest and most robust effects known in the navigation literature (e.g., Astur, Ortiz, & Sutherland, 1998; Lawton, 2010). This suggests the need for an experiment that compares the effects of stress on egocentric and allocentric navigation using a paradigm that can better measure navigational performance. Experiment 3 did so using virtual Morris water mazes.

Research into the relation between navigational strategy selection and performance is minimal, and so too is research into the effects of stress on the relation between these spatial cognitive domains. To date, with the exception of Experiment 2, there has been no research into the relationship between the effects of stress on strategy and performance in humans, although there has been some related research in rodents.

In one study, Kim et al. (2001) examined the effects of stress on rats in a traditional allocentric MWM, and in a similar MWM in which the submerged platform was marked by a visible pole (providing a simple egocentric cue in addition to typical extra-maze allocentric cues). They found that stress impaired performance in the allocentric MWM, but had no effect on performance in the maze with both egocentric and allocentric cues. However, on a follow up probe trial in which they moved the cue-pole to a new quadrant, they were able to show that 50% of the stressed animals swam directly to the new pole location, indicating an egocentric cued strategy, whereas 100% of the unstressed animals swam to the original platform location first, indicating an allocentric strategy. In sum, they were able to show that acute stress impairs allocentric performance, and biases strategy selection towards egocentric in a congruent, seemingly adaptive manner. However, the authors did not examine the effect of acute stress on "pure" egocentric navigational performance, because the visible cue maze contained both types of cues.

In another study, Schwabe et al. (2010) tested stressed and unstressed mice in a novel navigational task in which the mice were required to find an escape hole (leading back to their home cage) amongst a circular array of false holes. Similar to Kim et al.'s (2001) cue-pole maze, the goal was marked by a proximal cue (a bottle) that could be used egocentrically, as well as an array of extra-maze cues that could be used allocentrically. A post-training probe trial, in which the bottle was moved to a hole opposite the original, was used to identify strategy. Similar to the Kim et al. (2001) study, they found that stress reduced the likelihood that the animals would use an allocentric strategy. The authors also grouped the animals by strategy to compare their performance, and found that stressed animals that used an egocentric strategy had comparable performance to controls, while stressed animals that continued to use an allocentric strategy were impaired (in terms of latency to exit the maze). The authors suggested that the switch from hippocampus-based to caudate-based navigation under stress "rescued" performance, and was therefore an adaptive switch. However, this study suffered from a similar drawback that the Kim et al. (2001) study did – namely, they did not test the effect of acute stress on "pure" allocentric or egocentric navigational performance.

The dual-system theories discussed in Part 1, together with the rodent studies described above, suggest that the effects of acute stress on navigational strategy selection and performance should be congruent. The Hot/Cool Systems and the Uniform Shift models both maintain that acute stress should impair hippocampal function, and in doing so, shift behavioral dominance to the caudate. This should translate into impaired hippocampus-based allocentric navigation and a compensatory shift from allocentric to (caudate or amygdala-based) egocentric navigation. However, to date, the relationship between the effects of stress on navigational strategy selection and navigational performance has never been investigated using egocentrically or allocentrically

"pure" mazes, and never in humans. Experiment 3 separately tested the effects of acute stress on navigational performance and its effects on navigational strategy selection in order to compare them directly.

Key Issues.

After showing that acute stress can affect navigational strategy selection and allocentric performance in Experiments 1 and 2, Experiment 3 sought to verify these effects, extend the investigation to egocentric performance, and examine the relationship between the effects of stress on each of these spatial cognitive domains. In particular, Experiment 3 sought to explore 2 sets of key issues. The first set of issues was related to replication. The first of these was whether acute stress increases (like in Experiment 1) or decreases (like in Experiment 2) allocentric strategy selection. The second was whether the effect of acute stress on allocentric performance in Experiment 2 would replicate using a protocol that allows for easier interpretation. The third was whether stress has a sex-dependent effect on navigational cognitive domains, as it did in Experiment 2. The second set of issues was discovery oriented. The first of these was whether acute stress has any effect on human egocentric navigational performance. The second was whether stress affects navigational strategy selection and performance in a congruent manner. In other words, is there an adaptive de-selection of allocentric strategies when allocentric navigation is impaired by stress? The last issue was whether the effects of stress on allocentric and egocentric navigation are mediated by SAM axis activation.

Approach and hypotheses.

Experiment 3 is based on 2 key premises. The first premise, based on the literature and the findings from Experiments 1 and 2, is that the key mediator of the effects of stress on spatial navigation behaviour (either HPA or SAM) acts primarily by inhibiting the function of the

hippocampus, and at a 30 minute post-stressor onset delay. This suggests that stress should lead to a shift away from allocentric navigation when other options are available, and impair it when other options are not available. In contrast, stress should have little impact on navigation that is not dependent on the hippocampus. The second premise is that the differences in strategy selection results between Experiment 1 and Experiment 2 were caused by either a) differences in the construction of the mazes (the construction of the maze in Experiment 1 may have been problematic) or b) differences in the activities used to occupy participants during the delay period (the activities used in both experiments may have been problematic).

To examine the effects of stress on subsequent navigational strategy selection and performance, in Experiment 3, as with Experiments 1 and 2, participants were exposed to a stressor and had their navigation tested in virtual MWMs. Half of the participants were exposed to the PASAT (the stressor) and half were exposed to the U-PASAT (the non-stressful control task). One third of each of these groups was tested in one of either the uDS maze, the Place maze, or a new maze designed to test "pure" egocentric navigation, the "Cue" maze. In the Cue maze, participants had to find a hidden target egocentrically, by associating it with a single visible cue (a la Livingstone-Lee, Zeman, Gillingham, & Skelton, 2014; McDonald & White, 1994; see Methods for details). Experiment 3 used the same 30 min stress-onset-to-test interval at which effects were observed in Experiments 1 and 2. However, as with Experiment 2, an important question was how to occupy participants during this delay period, without causing unintentional stress or providing navigational training. Because it has been successful in previous research (Thomas et al., 2010), Experiment 3 used simple magazine reading to fill the stress-to-test delay period. Finally, similar to Experiment 2, SAM axis activity (heart rate, blood pressure, and skin conductance) was measured throughout the experimental sessions and activity at key points was

compared to pre-stress baseline in order to relate the physiological effects of stress to navigation behaviour.

The findings from Experiments 1 and 2, together with the empirical and theoretical literature on the effects of acute stress on hippocampal function and navigational cognition, led to several hypotheses in Experiment 3. First, it was expected that the effect of stress on strategy selection would be to decrease the likelihood of navigating allocentrically, as it did in Experiment 2. In other words, it was expected that the strategy selection results in Experiment 3 would replicate those of Experiment 2, especially given that Experiment 3 used the same uDS maze and a delay period free of potentially confounding activities. Second, it was expected that stress would impair allocentric navigational performance in the Place maze 30 minutes after stressor onset, again replicating Experiment 2 and consistent with the most common findings in the literature. Third, also based on the findings of Experiment 2, it was expected that there would be a sex-specific effect of stress on navigational strategy selection and allocentric performance. Fourth, it was expected that stress would have no effect on, or possibly enhance, egocentric performance in the Cue maze. This is based on the premise that stress affects navigation via the hippocampus, and that the hippocampus is not required for egocentric navigation. Fifth, because they are thought to share a common mechanism, it was expected that stress should affect strategy selection and navigational performance in a congruent way. Specifically, if stress causes a reduction in allocentric (in favour of egocentric) strategy selection in the uDS maze, it should also cause an impairment in allocentric performance in the Place maze, while having little effect on or enhancing egocentric performance in the Cue maze. Finally, consistent with Experiments 1 and 2, it was expected that the effects of stress on navigation in the uDS and Place mazes, but not the Cue maze, would be related to the level of stress-induced SAM activation.

Summary of hypotheses.

Replication:

1. Stress will lead to decreased allocentric strategy selection in the uDS maze.
2. Stress will lead to impaired allocentric performance in the Place maze.
3. Stress will have different sex-specific effects on strategy selection and allocentric performance.

Discovery:

4. Stress will have no effect on or will enhance egocentric performance in the Cue maze.
5. Stress will affect strategy selection and performance in a congruent way
6. Stress effects on the level of SAM activation will be related to its effects on navigation in the Dual and Place mazes, but not the Cue maze.

Method

Participants.

Two hundred and twenty seven participants were recruited from the University of Victoria undergraduate pool. As in Experiments 1 and 2, participants were pre-screened for English as a first language and normal (or corrected to normal) vision. Using a demographics questionnaire, participants were screened for a history of psychological or neurological problems, with particular attention paid to the exclusion of participants who may be unusually sensitive to stress (e.g., those who suffer from generalized anxiety disorder). Eight participants were excluded on this basis, including seven participants who reported suffering a serious concussion (i.e., hospitalized overnight) within the last 5 years, and one participant who reported suffering from severe anxiety. Three participants were excluded from the data after reporting that they were suffering from transient illness (the flu (2) and a urinary tract infection (1)). Ultimately, 216 (101 male) participants were included in the data analysis. The average age of participants was 20.15 years (SD = 2.43 years). Participants were required to provide informed written consent. Ethics approval was obtained from the University of Victoria, Human Research Ethics Committee.

Materials.

Stressor.

Experiment 3 used the same acute stressor task as Experiments 1 and 2: the PASAT (for task details, see Chapter 2: Method). Once again, the PASAT requires participants to add single-digit numbers serially, as quickly as possible, within a very limited period of time. The control version (the U-PASAT) consists of pairs of sing-digit numbers that have to be added together, with no time constraint or audience.

Manipulation checks.

Experiment 3 used the same measures (HR, SC, and BP) and equipment as Experiment 2 to assess the effectiveness of the PASAT to induce a physiological stress response (see Chapter 3: Method for details). As with Experiments 1 and 2, Experiment 3 used the State-Trait Anxiety Inventory to measure the psychological experience of stress.

Spatial navigation.

Experiment 3 will use three different virtual mazes to test spatial navigation. Navigational strategy selection and allocentric navigational performance were tested in the same way they were in Experiment 2, using the uDS and Place mazes (for a full description of these mazes, see Chapter 3: Method). Briefly, the uDS maze consisted of a circular arena inside a larger cylindrical room with windows that looked out onto distal landscape. A circular platform within the arena (invisible until stepped on) represented the navigational goal. The uDS maze contained an array of spheres, floating at head level, one of which (distinguished by colour) was always directly above the invisible platform. The Place maze was identical to the uDS maze in all respects except that the Place maze did not contain floating spheres that could be used egocentrically to find the fixed platform. Thus, because there were no single cues that could

predict its location, participants in the Place maze had to find the hidden platform allocentrically, using a configuration of distal landscape cues.

Egocentric navigational performance was tested using a new "Cue" maze (see Figure 20). The Cue maze derived from the uDS maze but had two key differences. First, unlike the uDS maze, there were no windows in the Cue maze. Rather, the arena was enclosed inside the cylindrical room with walls that were visually uniform, with no view to the landscape outside. Second, in the uDS maze, all spheres were identical in colour except the target sphere (which is a darker grey). However, pilot testing in the Cue-maze revealed that, without competing cues visible through the windows, such a color arrangement made finding the platform too easy for participants because the target sphere was the only salient cue. Thus, the Cue maze was revised so that all 8 cue-spheres had a distinct colour (red, green, brown, white, grey, mottled grey, blue, and orange). This meant that participants needed to learn the color of the target sphere (orange), and not just identify which sphere differed from the others. Because the cue-spheres in the Cue maze were all distinct, it was important that they not be spatially fixed so that participants could not form a cognitive map. Thus, with the exception of the orange target sphere, all spheres swapped positions with each other according to a fixed but complex pattern, on every trial. Although the target sphere and platform location were actually fixed, they appeared to move from trial to trial, relative to other spheres and the changing start position because there were no other spatial reference points (e.g., windows). In sum, the Cue maze prevented the formation of an (allocentric) cognitive map by eliminating all stable spatial relationships, both inside and outside of the arena.

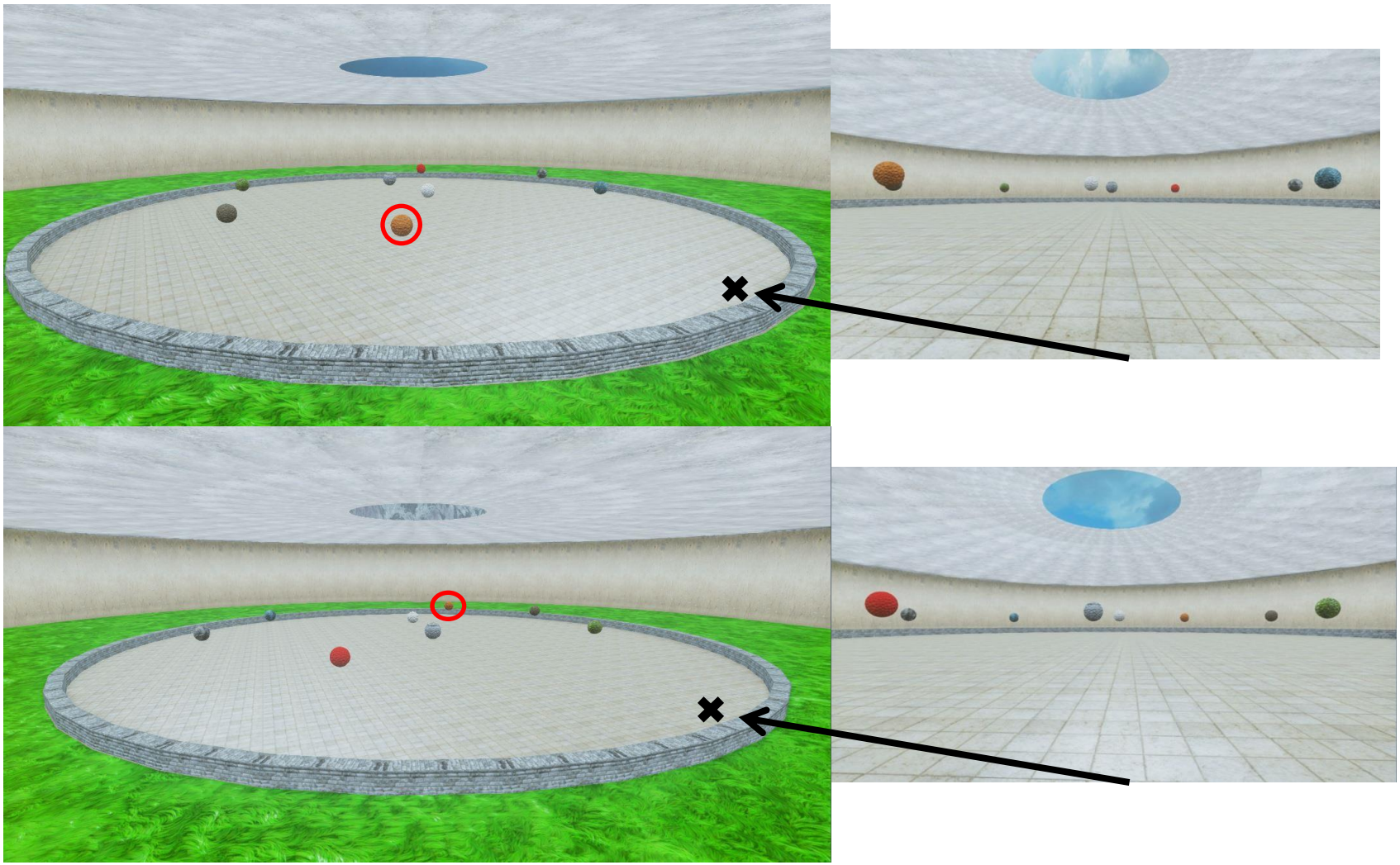


Figure 20. Experiment 3: Standard trial views of the Cue maze.
Left: Views from the northeast (top) and southwest (bottom), from above normal eye-level to highlight the layout of the cue-spheres. The orange sphere (circled in red in the larger views) marks the position of the platform. Black X's show the starting location for the corresponding starting views on the right. Right: North (top) and south (bottom) starting views. Note that all spheres (except the orange sphere) swap positions relative to each other on all Standard and ITP trials.

The trial procedure for all three mazes was the same and identical to that for the uDS and Place mazes in immediate testing in Experiment 2 (see Chapter 3). As a reminder, the trial procedure went as follows: 1 Exploration trial, 4 visible platform trials, 1 Pre-test Probe and 10 pairs of alternating Standard and Inter-trial probes (see Chapter 2 for details on each of these trial types). Performance in the Place and Cue mazes was measured in the usual way, as the distance and latency taken to reach the platform on each Standard trial. Whereas in the uDS maze Inter-Trial (Strategy) Probes (ITSPs) provide a measure of strategy selection, in the Place and Cue mazes Inter-Trial Probes (ITPs) provide an indicator of the accuracy of participants' knowledge of the platform location. This "Place" or "Cue Error" (in the Place and Cue mazes, respectively) variable is calculated as the Euclidean distance between the actual platform location (centre) and the participants' estimate, averaged over trials.

Procedure.

The procedure for Experiment 3 was similar to Experiment 1 (see Figure 21). Testing was conducted in a quiet, distraction-free room, with one experimenter. Participants completed all computer-based tasks on a desktop computer with a 19" LCD monitor set to a resolution of 800 x 600. Experimental sessions were run between 8:00am – 6:00 pm. Overall the procedure took about 85 minutes.

After consent was obtained, the session began with an initial ~30 minute "pre-stress" resting period. This period included a short introduction, after which participants were equipped with the HR monitor, BP cuff, and SC electrodes, then completed a short demographics questionnaire, in the same manner as Experiments 1 and 2. After participants had completed the questionnaire, the experimenter left the room and participants were allowed to relax and read magazines. When the experimenter returned after 10 minutes, participants completed the STAI

(both “state” and “trait” portions) and then gave baseline blood pressure measurements (BP1). This marked the end of the “pre-stress” phase.

Immediately following the “pre-stress” phase, participants completed either the PASAT or the UPASAT, using procedures identical to Experiments 1 and 2 (see the Method sections of those chapters).

A second ~20 minute “post-stress” resting period immediately followed the PASAT/UPASAT task. This phase began with participants completing the “state” portion of the STAI a second time (STAI-S B) while BP measurements (BP2) were taken. Next, participants were again allowed to relax and return to their magazine reading while the experimenter left the room for 15 minutes. When the experimenter returned, participants completed a third STAI (“state” portion; STAI-S C), while again having their BP measurements taken (BP3). This marked the end of the post-stress period.

Participants were tested in the one of the three mazes (uDS, Place, or Cue) immediately after the second rest period. Maze trials were presented in the order described above, and participants were given detailed instructions prior to starting each new trial type. The entire maze procedure, including all trials and instructions, required approximately 15 minutes.

After navigation testing, participants completed a final STAI (STAI-S D) and provided final BP measurements (BP4). To end the session, participants completed a short (3-4 minute) post-test questionnaire. The main purpose of the questionnaire was to gather information about possible factors that might have influenced navigational strategy selection, such as video-game experience.

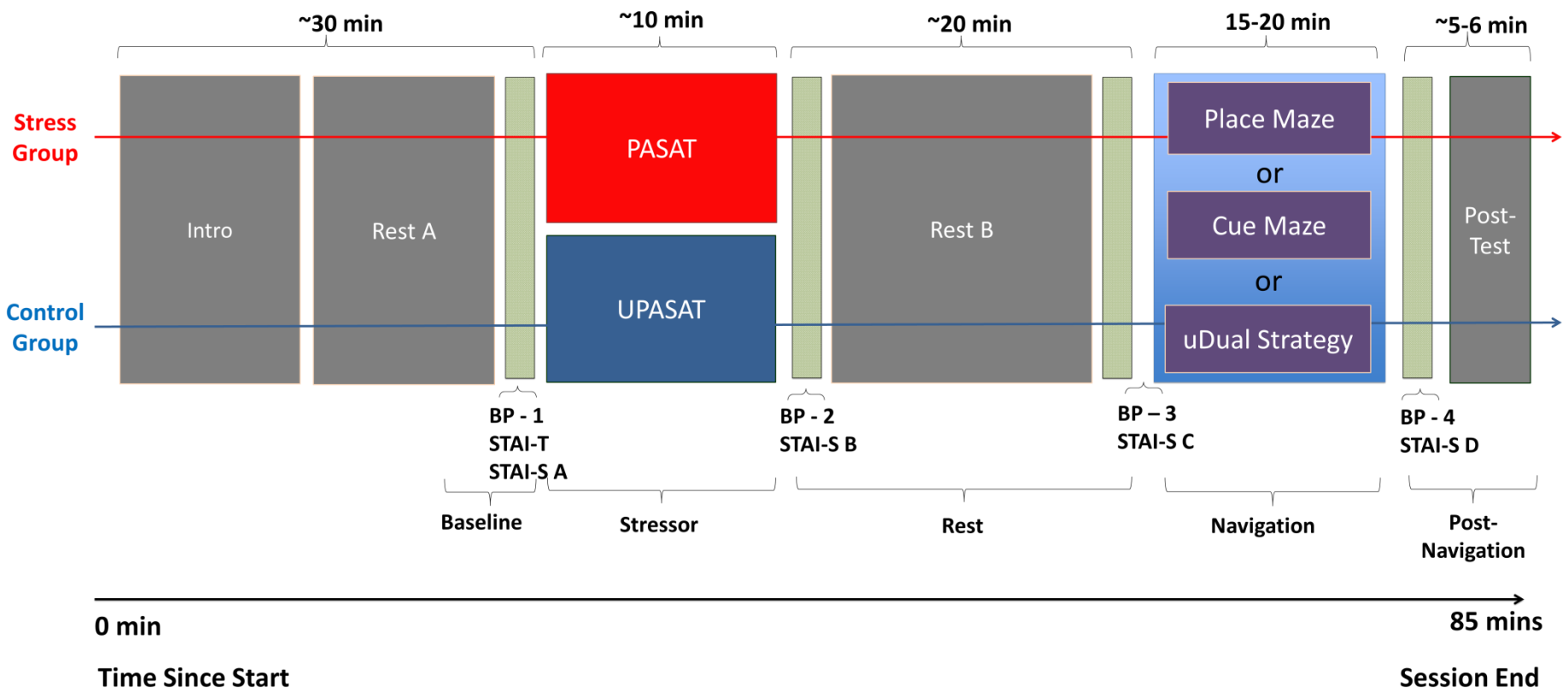


Figure 21. Experiment 3: Procedure.
Brackets at the top of the figure show estimations of task length (e.g. 30 min); brackets at the bottom specify the physiological and psychological measurement time points. Heart rate (HR) and skin conductance (SC) were measured continuously through the periods indicated (then averaged) whereas blood pressure (BP) and STAI-trait (STAI-T) and STAI-state (STAI-S) were measured as single points.

Data analysis.

Statistical analyses were similar to those used in Experiment 2, and were conducted using Microsoft Excel and SPSS version 21.

As with the previous 2 experiments, to assess the effectiveness of the PASAT to induce stress, I used 2 x 2 ANOVAs[6] for each of the physiological measures, the composite SAM score (see Experiment 1), as well as STAI score. Measures of SAM axis activity (HR, SC, systolic and diastolic BP) in response to stress and the psychological experience of stress (STAI score) were calculated as percentage of baseline, in the same manner as Experiments 1 and 2. For HR and SC, percentage of baseline was based on the average of all measurements taken during the stressor period (PASAT/UPASAT) divided by the average of all measurements taken over the 5-minute baseline period immediately prior to the stressor. For BP and STAI, percentage of baseline was based on the measures taken immediately after the stressor divided by the measures taken immediately before the stressor (See Figure 21: BP 2/BP 1). Similarly, measures of SAM axis activity during navigation were also calculated as a percentage of baseline using measurement periods comparable to those of SAM activity during the stressor.

I assessed navigational strategy selection by conducting a 2 x 2 (Stress Condition x Sex) analysis of variance (ANOVA) on average Allo % in the uDS maze. Allo % was calculated in the same way as in Experiments 1 and 2, by taking the number of ITSP trials that were designated as "allo" and dividing that by the total number of ITSP trials in which the participant chose a strategy. I assessed navigational performance in the Place and Cue mazes by conducting 2 x 2 (Stress Condition x Sex) ANOVAs on average latency and distance to the platform on standard trials, and on Place or Cue Error on ITPs. Finally, relationships between physiological

[6] Note: Like Experiments 1 and 2, Experiment 3 used a quasi-experimental design and that Sex was a predictor variable. However, throughout the rest of the chapter and in the general discussion I will be referring to sex as a variable in a manner consistent with the literature (e.g., du Plooy et al., 2014; Wolf et al., 2001).

measures of stress and navigational strategy selection and performance were assessed using Pearson correlations.

Results

Manipulation checks.

Both physiological and psychological measures indicated that the PASAT was effective at inducing stress (Figures 22-23). Condition x Sex ANOVAs revealed a main effect of Condition on each of the 4 measures of SAM activity. Stress elevated both systolic ($F(1, 212) = 16.13$, $p < .000$, $\eta^2 = .07$), and diastolic ($F(1,212) = 27.01$, $p < .000$, $\eta^2 = .12$) BP, as well as HR ($F(1,199) = 14.98$, $p < .000$, $\eta^2 = .07$) and SC ($F(1,210) = 10.66$, $p = .001$, $\eta^2 = .05$) (Figure 23). However, as with Experiments 1 and 2, Sex did not have any main or interaction effect on any of the SAM measures (all p's > .07). This pattern was also reflected in the SAM Composite score, with a main effect of Condition ($F(1,216) = 71.47$, $p = .000$, $\eta^2 = .25$), but no main or interaction effects for Sex (all p's > .13). (Figure 23). Regarding the subjective experience of stress, the PASAT caused participants in the Stress group to report higher levels of anxiety on the STAI than those in the Control group ($F(1,216) = 147.16$, $p = .000$, $\eta^2 = .41$). Here too, Sex had no main or interaction effects (all p's > .09). .

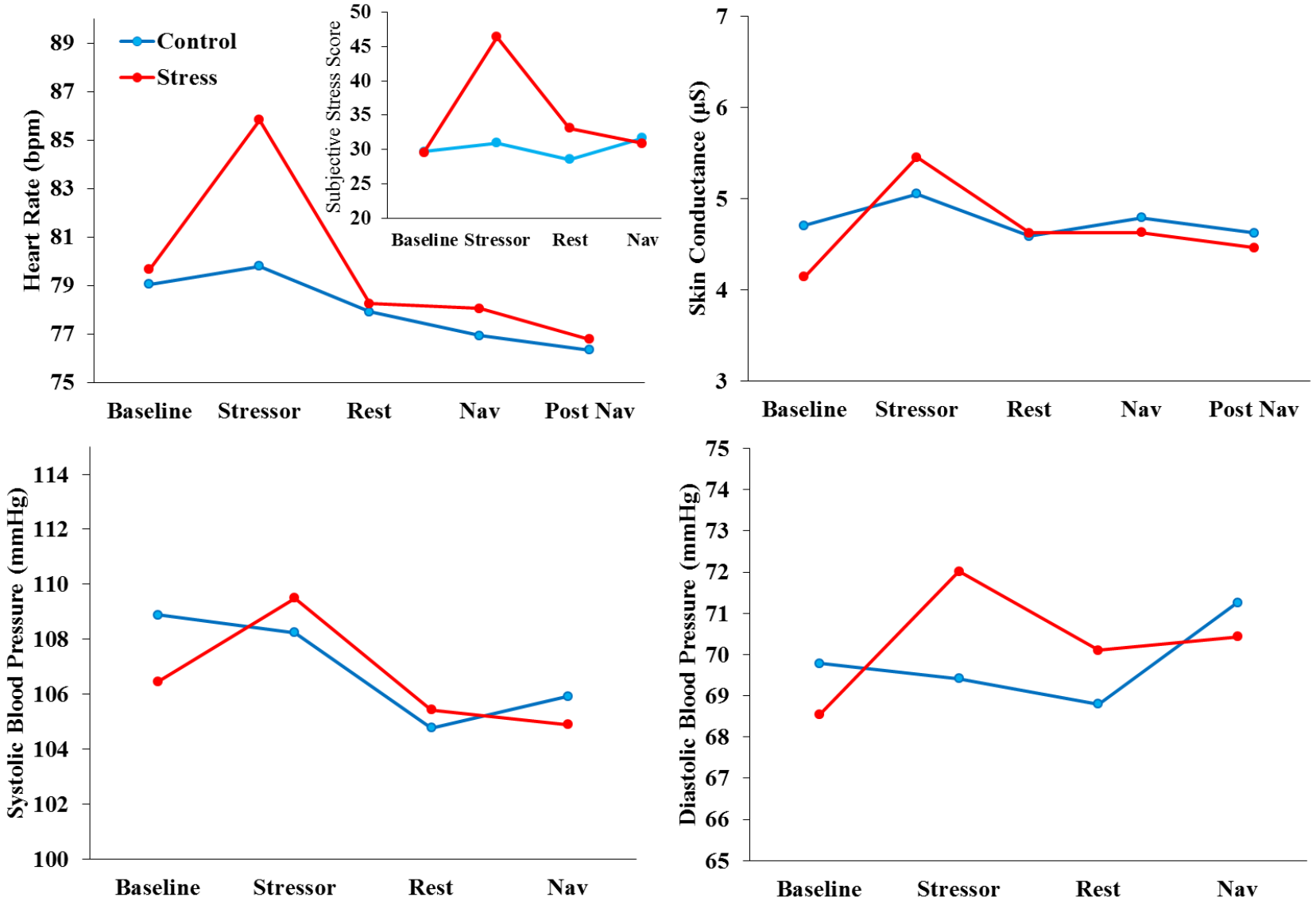


Figure 22. Experiment 3**:** Time course of the PASAT effect on physiological measurements.
Y-axes denote: Heart Rate (HR), Skin Conductance (SC), Systolic Blood Pressure (BP-Sys), Diastolic Blood Pressure (BP-Dia), and State-Trait Anxiety Inventory-State Portion (STAI-S; inset).

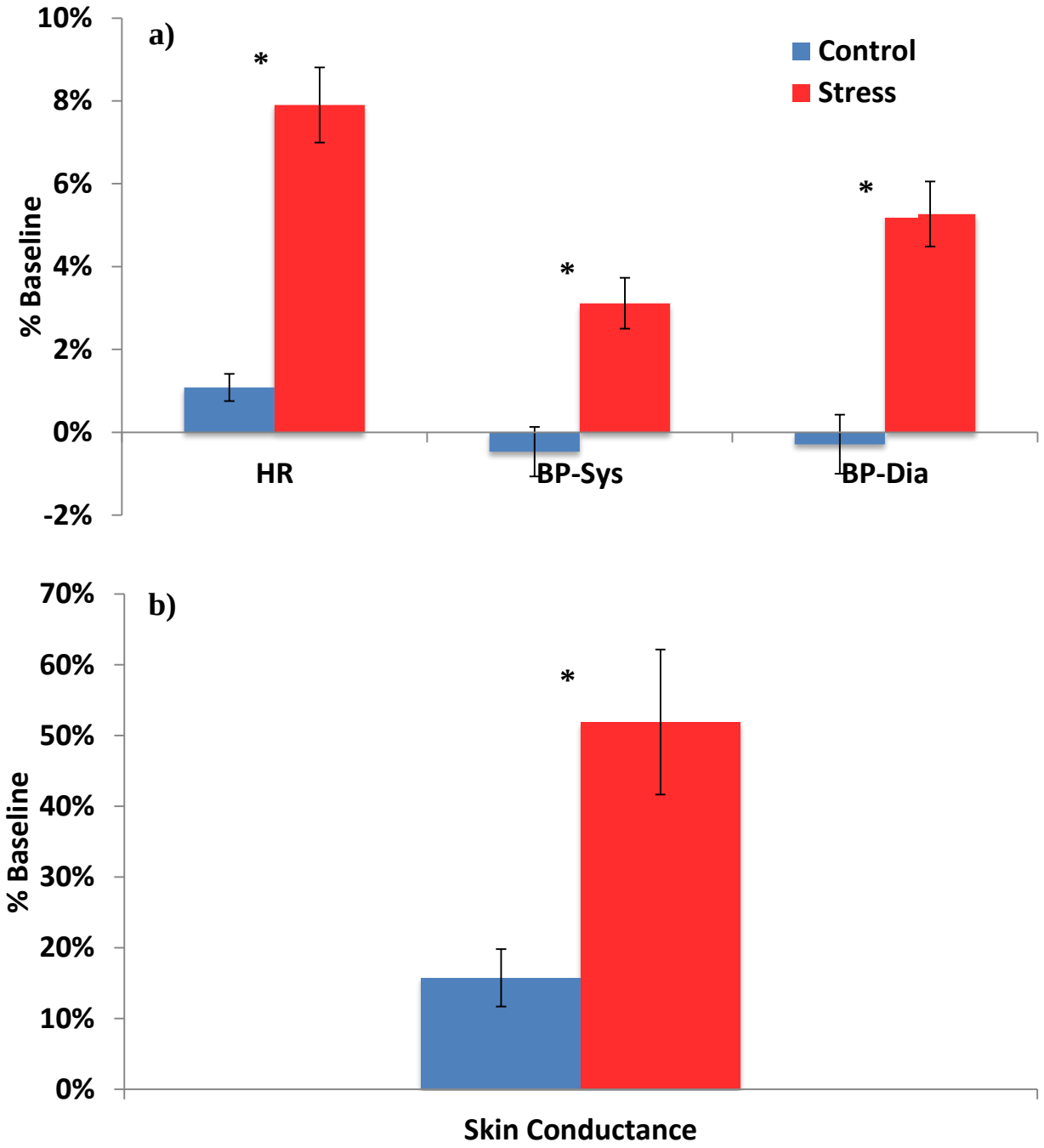


Figure 23. Experiment 3: The effect of the PASAT on SAM measures.
The effect of the PASAT on a) heart rate (HR), systolic (Sys) and diastolic (Dia) blood pressure, and b) skin conductance. Values are expressed as mean % of baseline. Error bars are SEM. *p < .01.

Stress and navigational strategy.

Similar to Experiment 2, and consistent with hypothesis 1, the present results revealed that stress caused a shift away from allocentric, and toward egocentric navigation. Unlike Experiment 2, however, the effect in Experiment 3 was not sex dependent (in contrast to hypothesis 3; See Figure 24). A Condition x Sex ANOVA revealed a main effect of Condition ($F(1, 80) = 4.62$, $p = .04$, $\eta^2 = .06$), indicating that stress reduced the likelihood that participants would select an allocentric strategy in the uDS maze. Furthermore, a significant main effect of Sex ($F(1, 80) = 7.12$, $p = .01$, $\eta^2 = .08$), indicated that males tended to navigate allocentrically more often than females, regardless of stress condition. Moreover, a lack of a Condition x Sex interaction suggests that stress reduced allocentric strategy selection similarly in males and females ($p = .61$). Simple effects analysis using planned *t*-tests revealed no significant effects within Sex (p's > .08).

Figure 24. Experiment 3: Effect of PASAT stress on strategy selection.
Values are expressed as average Allo%. Error bars are SEM. The black arrow indicates the main effect ($p < .05$).

Stress and allocentric performance.

Also similar to Experiment 2, and consistent with hypothesis 2, PASAT stress worsened performance in the Place maze, although the effect appeared to be more robust in males (consistent with hypothesis 3; Figure 25). In contrast to Experiment 2, however, in which stress increased latency in females and slightly decreased it in males, in the present experiment, stress led to an overall increase in latency across Sex (Figure 25a). A Condition x Sex ANOVA revealed a main effect of Condition ($F(1, 80) = 7.12$, $p = .01$, $\eta^2 = .08$) and a main effect of Sex ($F(1, 80) = 7.12$, $p = .01$, $\eta^2 = .08$), but no Sex x Condition interaction ($p = .61$). This indicates that although females had longer latencies than males overall, stress increased latency in males and females in a comparable way. However, simple effects analysis on the effects of stress within Sex revealed that stress significantly increased latency in males ($t(30) = -2.50$, $p = .02$, $d = 0.86$) but not females ($p = .41$). In terms of distance, there were no main, interaction, or simple effects (all p's > .08; Figure 25b). Similarly, there were no main or interaction effects for place error (all p's > .08; Figure 25c). However, simple effects analysis using planned t-tests revealed that, as it did with latency, stress significantly increased place error in males ($t(30) = -2.26$, $p = .03$, $d = 0.80$) but not females ($p = .98$; Figure 25c).

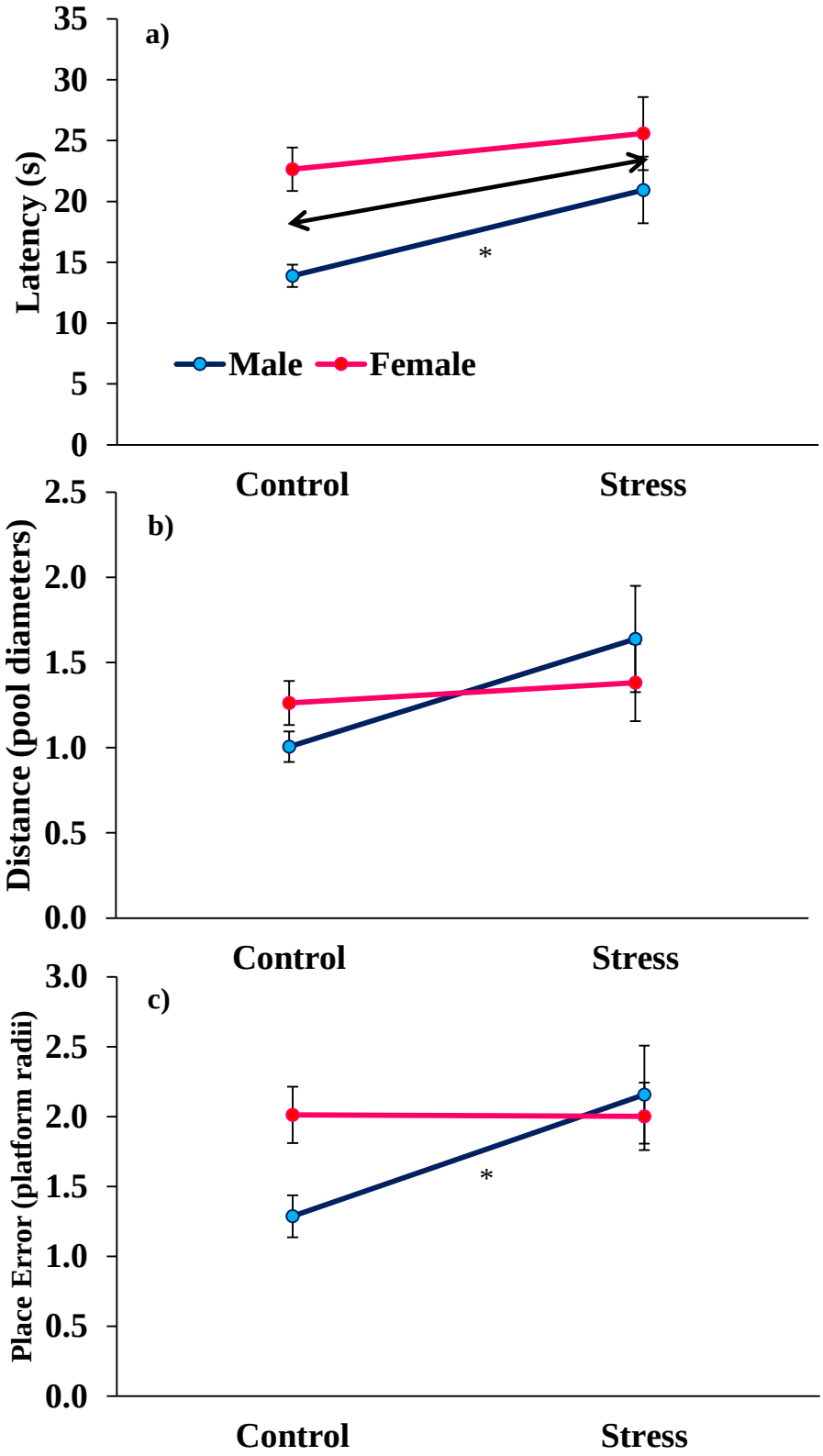


Figure 25. Experiment 3: The effect of PASAT stress on performance in the Place maze. Performance is represented in terms of average a) latency (in seconds), b) distance (in pool diameters) and c) place error (in platform radii). Error bars are SEM. The black arrow in a) shows the main effect of condition (p = .01). Asterisks (*) in a) and c) show simple effects (p < .05).

Stress and egocentric performance.

In terms of the egocentric performance, PASAT stress again tended to have sex-specific effects (Figure 26). In contrast to hypothesis 4, stress tended to worsen male performance in the Cue maze, as it did in the Place maze. For females, the effects were more complex, with stress either slightly enhancing or slightly impairing performance, depending on the measure. With respect to latency to the platform, a Condition x Sex ANOVA and planned *t*-tests revealed no main, interaction, or simple effects (all *p*'s > .08; Figure 26a). In other words, both stressed and control males and females required a similar amount of time to reach the platform. However, although there were no main effects of Condition or Sex on the distance required to reach the platform (*p*'s > .58), there was a significant interaction ($F(1, 62) = 6.69$, $p = .01$, $\eta^2 = .10$; Figure 26b). This indicates that stress caused males to take less direct, and females to take more direct routes to the platform. Simple effects analysis within Sex showed that this interaction was driven largely by a significant increase in distance in males under stress ($t(30) = -2.39$, $p = .02$, $d = 0.85$). The decrease in distance for females under stress did was not significant ($p = 18$). Finally, in terms of cue error, there was a significant main effect of Condition ($F(1, 62) = 5.21$, $p = .03$, $\eta^2 = .09$), such that stress caused an overall increase in the distance between participants' estimates of the platform location and its actual location (i.e., they were less accurate; Figure 26c). However, there was no main effect of Sex or interaction, suggesting that males and females had statistically equivalent cue error, and that stress impacted males and females in a similar way. However, simple effects analysis again revealed that the effect of stress on was significant in males ($t(30) = -2.17$, $p = .04$, $d = 0.78$) but not in females ($p = .29$).

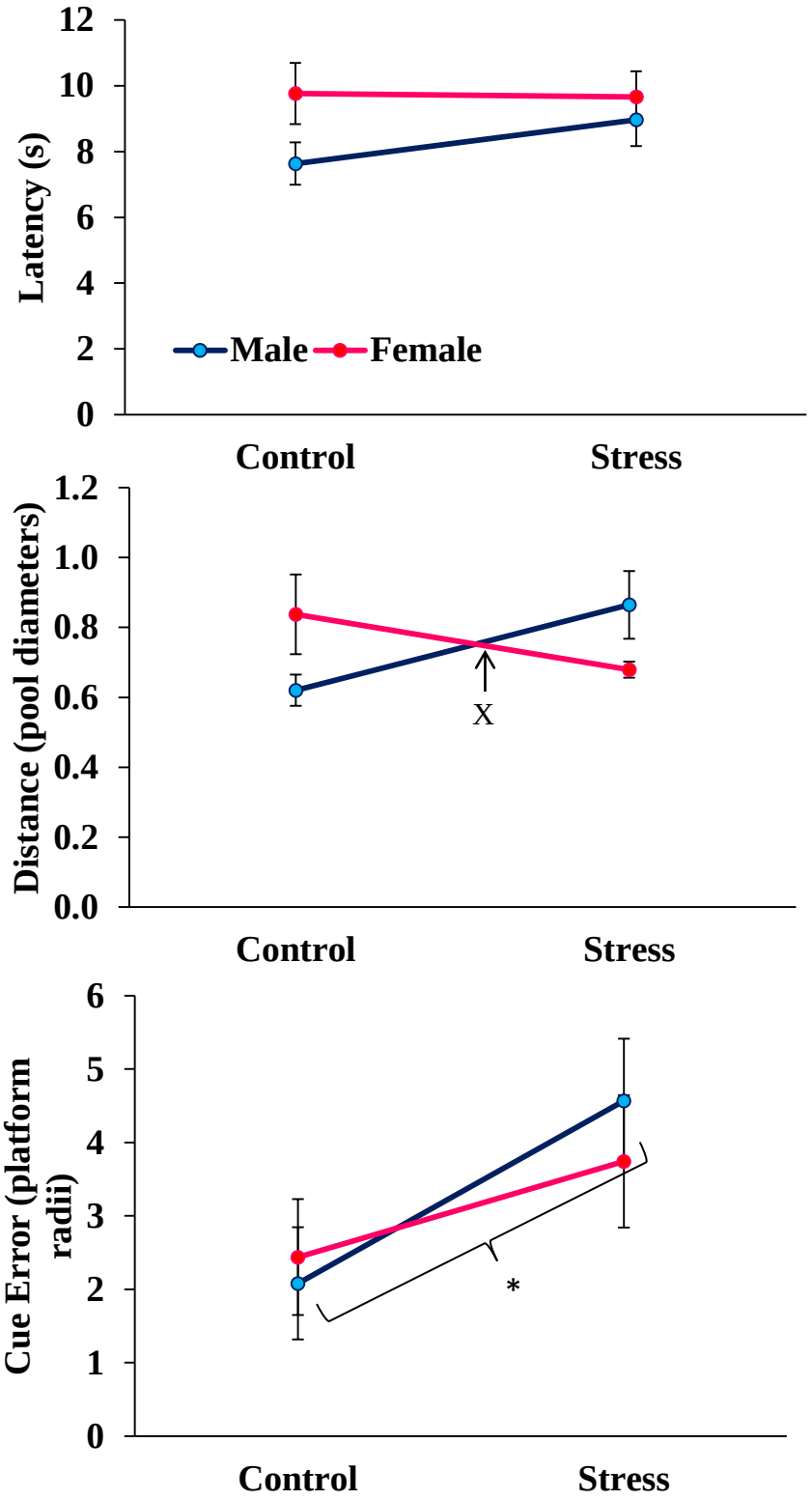


Figure 26. Experiment 3: The effect of PASAT stress on performance in the Cue maze. Performance is represented in terms of average a) latency (in seconds), b) distance (in pool diameters) and c) cue error (in platform radii). Error bars are SEM. The "X" in b) shows the Sex x Condition interaction (p = .01). The asterisk (*) in c) shows a main effect of stress ($p < .05$).

In sum, stress reduced the likelihood that participants would solve the uDS maze allocentrically, as expected. In contrast to hypothesis 5, despite reducing allocentric strategy choice, stress also tended to worsen allocentric navigational performance in the Place maze. However, by some measures (latency and place error), simple effects analysis revealed that this effect was significant for males but not females. In the Cue maze, stress caused males to take less direct routes to the platform, but females to take *more* direct routes. Stress also caused participants to make less accurate estimates of the platform location overall, although a simple effect within Sex was only significant in males.

Navigation and SAM axis activation.

Unlike Experiment 2, no sex-dependency appeared with respect to navigational strategy selection. Thus, correlational analysis of the relations between SAM activity and strategy selection were conducted using combined data from males and females. Like Experiment 2, however, the effects of stress on navigational performance appeared to be largely sex-dependent. For most behavioural measures of performance, stress worsened navigation in males, but had little effect or enhanced it in females. Thus, in order to avoid obscuring relationships by mixing data that is only weakly correlated, or correlated in the opposite direction, correlational analysis of the relations between SAM activity and performance was performed separately for males and females.

Despite the behavioral effect of PASAT stress on navigational strategy selection in the uDS maze, there appeared to be no link between the physiological correlates of stress and navigational strategy selection. Pearson correlations revealed that there were no significant

correlations between Allo% and any of the measures of SAM activity (HR, BP, or SC; all *p*'s > .27).

Analysis of the relations between measures of SAM activity and performance in the Place maze revealed several significant relationships. In males, both HR and SC were significantly positively related to latency and distance to find the platform in the Place maze (HR x latency: $r = .45$, $p = .01$; HR x distance: $r = .39$, $p = .04$; SC x latency: $r = .43$, $p = .00$; SC x distance: $r = .62$, $p = .00$). In other words, when stress increased HR and SC at the time of the PASAT, it also increased the time and distance required for males to find the platform in the Place maze. Similarly, in females, significant relations appeared between measures of SAM activity and performance in the Place maze. Specifically, HR was positively related to latency ($r = .36$, $p = .04$), and SC was related to both latency ($r = .58$, $p = .00$) and distance ($r = .38$, $p = .03$). This indicates that when stress increased HR and SC, it also increased female latency and distance (in the case of SC) to find the platform in the Place maze.

With respect to navigational performance in the Cue maze, in males, correlational analysis revealed a significant positive relationship between diastolic BP and cue error ($r = .36$, $p = .04$). That is, when stress increased diastolic BP at the time of the PASAT, it also increased the error in males' estimates of the platform location in the Cue maze. Regarding female performance in the Cue maze, correlations uncovered only a significant negative relationship between diastolic BP and latency ($r = -.43$, $p = .01$). That is, when stress increased diastolic BP, it decreased the female latency to find the platform location in the Cue maze.

As with Experiment 2, it was important to check whether the effect of stress on navigation was due to SAM activation at the time of the stressor, or SAM activation at the time of navigational testing. An analysis of the relations between SAM activation at the time of

navigation and navigational strategy selection in the uDS maze revealed no significant correlations (all *p*'s > .14). In terms of the relations between SAM activation at navigation and male performance in the Place maze, there was no relation between the SAM Composite Score and any performance measure (all *p*'s > .07). However, when looking at individual measures, a significant negative correlation appeared between HR and place error (r = -.50, p = .00), indicating that increased HR during navigation was related to reduced error. Puzzlingly, a significant positive correlation between diastolic BP and place error also appeared (r = .48, p = .01), indicating that increased diastolic BP at navigation was related to increased error. In females, although there were no significant relations between performance measures and the SAM Composite Score (all *p's* > .45), there was a significant negative correlation between SC and latency (r = -.39, p = .03), indicating that as SC increased during navigation, females needed less time to find the platform. In the Cue maze, significant negative correlations appeared for males between the SAM Composite Score and latency (r = -.38, p = .03). This was likely driven by significant correlations between HR and latency (r = -.36, p = .05) and distance (r = -.43, p = .02), indicating that increased HR at the time of navigation was associated with reduced times and path lengths to the platform. Similar associations, in the same direction, were also found for females in the cue maze (HR x Composite Score: r = -.40, p = .02; HR x latency: r = -.52, p = .00; HR x distance: r = -.42, p = .02).

In sum, contrary to hypothesis 6, the effect of stress on SAM activation appeared to have little relationship to its effect on strategy selection. However, consistent with hypothesis 6, stress-induced SAM activity was related to a worsening of performance in the Place maze for both males and females. With one exception (diastolic BP in males), the direction of the relations flipped when SAM was measured at the time of navigation, as they did in Experiment 2.

Surprisingly, a similar relationship appeared for males in the Cue maze: increased SAM activation was related to a worsening of performance (i.e., increased cue error) at the time of the stressor, and improved performance (i.e., reduced latency and distance) at the time of navigation. For females in the Cue maze, consistent with hypothesis 6, increased SAM activation was associated with improved performance when SAM was measured either at the time of the stressor (i.e., reduced latency) or navigation (reduced latency and distance).

Discussion

In the present experiment, as in Experiments 1 and 2, the PASAT produced a robust overall physiological and psychological response in the absence of sex differences. This caused an interesting, but complicated, collection of effects on navigational strategy selection and performance. These effects supported some of the hypotheses and challenged others. The first three hypotheses related to the consistency of findings between Experiments 2 and 3. The first of these was that stress would reduce allocentric strategy selection in Experiment 3 as it did in Experiment 2. This hypothesis was confirmed – stress caused an overall decrease in the allocentric strategy selection (across Sex). The second hypothesis was that stress would impair allocentric performance in the Place maze in Experiment 3 as it did in Experiment 2. This hypothesis was also confirmed. Stress generally impaired performance in the Place maze. The third hypothesis – that there would be a sex-specific effect of stress on strategy selection and allocentric performance – was partially confirmed. Stress reduced allocentric strategy selection in males and females to a similar extent. However, although it did impair one aspect of female performance (latency), the effects appeared to be more robust in males (as evidenced by simple effects on latency and place error). The remaining hypotheses focussed on the generality of the effects of stress on navigation, the relationship between its effects on different spatial cognitive

domains, and the relations between the physiological correlates of stress and its effect on navigation. The first of these was that stress would either have no effect on or enhance egocentric performance in the Cue maze. This hypothesis was partially confirmed and partially disconfirmed. Although stress had no effect on latency, and improved female distance, puzzlingly, it impaired male distance and impaired accuracy (cue error) in both males and females (though the simple effect was only significant in males). The second of these hypotheses was that the effect of stress on strategy and performance would be adaptive – that is, that stress should bias strategy selection towards the strategy that is not impaired by stress. This hypothesis was partially confirmed. Stress biased participants to navigate egocentrically in the uDS maze, and impaired allocentric performance in the Place maze. Meanwhile, in the Cue maze, stress still impaired male performance. Paradoxically, stress improved female performance on one measure (distance) but tended to impair it on another (cue error, though this simple effect was not significant). Finally, the hypothesis that the magnitude of the effects of stress on navigational strategy selection and allocentric, but not egocentric, performance would be related to the magnitude of its effect on SAM axis activation was partly confirmed. Similar to Experiment 2, there were no correlations observed between stress-induced SAM activation and strategy selection in the uDS maze. However, there were relatively strong relationships between stress-induced SAM activation and performance in the Place maze (both male and female), and surprisingly, also in the Cue maze.

Each of these hypotheses and their results will be discussed in turn.

First, the results confirmed my expectation that stress would reduce allocentric strategy selection in the uDS maze, as it did in Experiment 2. These findings are consistent with limited rodent and human empirical studies on the effects of acute stress on navigational strategy

selection. There are only two (all male) rodent studies that have examined the effects of stress on strategy selection to date (Kim et al., 2001; Schwabe et al., 2010a), and both of these found that acute stress caused rodents to solve dual-strategy spatial tasks egocentrically. There is no human research (outside the present dissertation) on the relationship between stress and navigational strategy selection. However, the present findings are consistent with studies of the effects of stress on related cognitive domains (Schwabe et al., 2007). In the Schwabe et al. (2007) study, stress biased participants to solve a (non-navigational) spatial cognition puzzle using a stimulus-response (i.e., egocentric) strategy rather than a mapping (i.e., allocentric) strategy, similar to its effects in the present experiment. In a related study by the same lab, Schwabe and Wolfe (2012) showed that acute stress can also bias humans to solve non-spatial dual-solution tasks using caudate nucleus-dependent strategies rather than hippocampus-dependent strategies. Moreover, the effects of stress on strategy selection (30 min after stressor onset) in Experiment 3 are consistent with the original hypothesis of Experiment 1 – i.e., that acute stress would reduce allocentric strategy selection. This was based on all 4 models of the effects of acute stress on hippocampal function (TDM, Uniform Shift, MYD and Hot/Cool Systems), all of which predict that stress should bias navigational strategy selection from allocentric to egocentric.

Second, my expectation that stress would impair allocentric navigational performance in the Place maze was also confirmed. These findings parallel those from the rodent literature, in which the most common finding is that acute stress impairs hippocampus-based, allocentric navigation (e.g., Schwabe et al., 2010a, Park et al., 2008, Xiong et al., 2003). With respect to the effect of stress on human navigational performance, the present findings replicate and extend those of Thomas et al., 2010. While Thomas et al. (2010) were able to show that acute stress can impair female navigational performance in a similar virtual MWM to the one used here, the

present results revealed that stress can also impair male performance. In contrast, the present results contradict those of Duncko et al. (2007), who found that stress enhanced performance in an all-male sample. However, in that study, no effect of stress was found in the traditional measures of water maze performance (distance and latency, as used here), but were only present in two less-commonly used variables (heading error and failures to find the platform). Moreover, Klopp et al., 2012, attempted but failed to replicate the findings of Duncko et al. (2007) using the same maze but a different stressor (they used the Trier Social Stress Task instead of the Cold Pressor Task). This casts some doubt on the veracity of the Duncko et al. (2007) findings. In addition to empirical research, the performance findings of the present experiment are consistent with the 4 theoretical models discussed in Chapter 1. Each of these models holds that stress, via either the HPA or SAM axis (or both) should impair hippocampal function, and thus predicts that stress should impair hippocampus-based (allocentric) navigation.

Third, my expectation that stress would have sex-specific effects on strategy selection and allocentric navigational performance, as it did in Experiment 2 was only partially confirmed. That is, there was no sex-specific effect of stress on strategy selection, but there were sex-specific effects in the performance domain. Although stress did impair one aspect of female performance (latency), its effects were more robust in males (as evidenced by simple effects on latency and place error). Despite the importance of sex as a factor in the navigation literature, sex has played only a relatively minor role in the stress/navigation literature. Hence, the interaction between sex, stress, and navigational strategy and performance has been an unexpected finding in the present dissertation, and is difficult to compare to the literature. By and large, rodent studies study only male animals. One exception (Park et al., 2008) studied only navigational performance (latency) and spatial memory (arm entries) in a radial arm maze. As is typical in

radial arm mazes, they found no effects of stress or sex on latency, although they did find that stress caused males to make more memory errors than females 24 hrs after stress induction. Both of the rodent studies that examined the effects of stress on strategy selection used male animals exclusively (Kim et al., 2001; Schwabe et al., 2010a). In humans, one study found that stress impaired female, but not male navigational performance in a similar virtual MWM (Thomas et al., 2010), in contrast to the results of Experiment 3. Two other studies found no impact of sex on the effects of stress on navigational performance in an allocentric virtual MWM (Klopp et al., 2012) or either an egocentric or allocentric virtual radial arm maze (Guenzel, 2014). The only other study to examine the effects of stress on navigational performance used an all-male sample (Thomas et al., 2010). In terms of strategy selection, as mentioned previously, there is no human study to date for direct comparison (not including Experiment 2). One study (Schwabe et al., 2007) examined the effects of stress on strategy selection in a non-navigational spatial puzzle. In this study, although stress was found to reduce allocentric strategy selection overall, there was no impact of sex. Importantly, however, none of these studies compared the effects of stress on performance *and* strategy selection in males and females. The question remains as to why the sex dependencies of the effects of stress on navigational cognitive domains differed between Experiments 2 and 3. This will be considered further in the General Discussion (Chapter 5).

Fourth, my expectation that stress would have no impact on or enhance egocentric navigational performance in the Cue maze was partly confirmed and partly disconfirmed. On the one hand, stress had no effect on latency (for either sex), and improved female distance. On the other hand, stress impaired male distance and impaired accuracy (cue error) in both males and females (though the simple effect was only significant in males). These findings are in contrast to the only other human study to examine the effect of stress on egocentric navigational

performance (Guenzel et al., 2014), in which stress had no influence on egocentric performance in a virtual radial arm maze. However, as mentioned previously, radial arm mazes are better suited to test memory than performance. In fact, in this same study, males, but not females, were impaired in their ability to recall the correct route through the maze when tested one week later in a single probe trial. This apparent sex dependency in the effects of acute stress on caudate-based spatial cognition (i.e., that the effect appears in males, not females) is generally consistent with the present results. However, the present results only partly match theoretical predictions. Both the Hot/Cool Systems model and the Uniform Shift model suggest that stress should either have no effect on or enhance egocentric navigational performance. This was true only for females (though stress led to slightly worse accuracy (cue error)). In contrast, stress caused males to take less direct routes to the platform and to be significantly less accurate in their location estimates. Unfortunately, neither the Hot/Cool Systems nor the Uniform Shift (nor the single-system models: TDM or MYD) consider sex as a factor in the effects of stress on hippocampal function.

Fifth, my hypothesis was that the effects of stress on strategy and performance would be parallel was partly confirmed and partly disconfirmed. In line with this hypothesis, stress biased navigation away from allocentric and towards egocentric strategy selection, and impaired allocentric performance in the Place maze. However, in males, it also impaired egocentric navigation in the Cue maze, contrary to hypothesis 5. In females, stress either had no effect (latency), or enhanced (distance) or slightly impaired (cue error; non-significant effect) performance in the Cue maze. It is not clear why the effects of stress on strategy selection and performance were, by and large, parallel for females but not for males. Suffice it to say (for now)

that sex complicates the interaction between stress and navigation, and more research is needed to clarify the relationships. Possible future research will be considered in the general discussion.

The last hypothesis was that the magnitude of the stress effect on SAM axis activation would be related to the magnitude of the effects of stress on navigational strategy selection and on allocentric performance, but not egocentric performance.

This hypothesis was not confirmed with respect to strategy selection. SAM activity during the stressor or at the time of navigation was not related to selection of one strategy or the other. The hypothesis was partly confirmed with respect to allocentric performance. SAM-activity during the stressor related to poorer performance in male and females. However, SAM at the time of navigation was related to better performance in males and females. Results from the Cue maze were complex. In males, SAM activity at the time of the stressor was associated with impaired performance, but SAM activity at the time of navigation was associated with improved performance. In females, SAM activity was related to improved performance, regardless of whether it was measured during the stressor or navigation. Taken together, it appears that a) SAM activity is not associated with navigational strategy selection, b) stress-induced SAM activity is related to allocentric navigational performance, but this relation does not persist, and c) the association between SAM activity and egocentric performance is complex, may be influenced by sex, and requires further study.

Experiment 3 shed light on several key issues of the present dissertation. The first set of issues was related to the replicability of findings within the dissertation. The first of these was whether (and how) acute stress influences navigational strategy selection. Although both Experiments 1 and 2 showed that stress can affect navigational strategy selection, it was unclear whether its effect was to bias strategy selection towards allocentric (as Experiment 1 suggested)

or away from allocentric (as Experiment 2 suggested). The findings of Experiment 3 (that stress reduces allocentric strategy selection) agree with Experiment 2, and together with other empirical findings (discussed above) and the predictions of theoretical models, suggest that Experiment 1 was anomalous. The most likely reason for the contradictory findings of Experiment 1 was that the original Dual-Strategy maze may have miscategorised strategy selection by making the impulsive, but egocentric, choice appear allocentric. Specifically, participants may have impulsively navigated to the "allocentric" quadrant on ITSPs by either heading towards a flanker cue (visible at the start of trials, unlike the target cue) or to a more salient "snapshot" of the window nearest to the platform. In both cases, an egocentric response would have been classified as allocentric. This was not a problem in the uDS maze (used in Experiments 2 and 3), where the target cue was always visible at the start of all trials, and where the distractor cues were all identical grey spheres, making them difficult to distinguish and use as simple egocentric cues.

A second replication issue was related to whether (and how) acute stress affects allocentric performance. The results of Experiment 2 suggested that acute stress can impair allocentric navigation. However, in Experiment 2, performance in the delayed maze was confounded with navigational experience in the uDS maze, making interpretation of this result difficult. Experiment 3 avoided this potential confound with a cleaner protocol: instead of navigation, participants read magazines during the delay period. Consistent with Experiment 2, the results of Experiment 3 also indicate that stress can impair allocentric navigational performance.

The third replication issue was related to the interaction between stress, sex, and navigational cognitive domain. In Experiment 2, there was a surprising sex-dependency in the

effects of stress on strategy selection versus allocentric performance. Specifically, stress shifted male strategy selection away from allocentric but did not affect male allocentric performance, and impaired female allocentric navigational performance but did not shift their strategy selection. In contrast, in Experiment 3, sex was not a factor in the effect of stress on strategy selection (i.e., stress similarly reduced allocentric strategy choice in both sexes), but sex did appear to matter with respect to allocentric performance, such that stress had a more robust (impairing) effect on males. In sum, although sex dependencies did appear for the effects of stress on navigation in Experiment 3, they did not replicate, and even contradicted those that appeared in Experiment 2. This casts some doubt on the idea that stress had sex-dependent effects on navigation, and calls for further investigation in future research.

The second set of issues in Experiment 3 was discovery-oriented. The first of these was whether (and how) stress can affect egocentric navigation. Experiment 3 was the first to show that stress can have sex-dependent effects on performance. Unexpectedly, stress impaired male performance, as it did in the Place maze. However, depending on the measure, stress sometimes enhanced and sometimes impaired female performance. This suggests either a) males and females depend on different brain structures for egocentric navigation, and stress impacts these structures differently, or b) males and females rely on the same structures, but the consequences of the physiological stress-response systems on the brain are modulated differently by sex-specific dominant gonadal hormones. For example, some authors have suggested that female gonadal hormones can either dampen (Wolf et al., 2001) or amplify (Andreano & Cahill, 2009) the cognitive effects of cortisol. However, future research should re-examine and verify these results before speculating further.

The second discovery-oriented issue was whether stress would lead to a shift in navigational strategy selection that was adaptive. In other words, do people select the strategy that is least impaired by stress? With respect to males, the results of Experiment 3 suggest that the answer is “no”. Although stress biased males to select egocentric navigation more often, male navigational performance was impaired by stress in general, regardless of whether it was egocentric or allocentric. For females, the answer is “maybe, but more testing is needed”. Stress also biased female navigation to egocentric, but the impairing effect of stress on female allocentric navigation was less pronounced that it was in Experiment 2, and although stress did enhance female egocentric performance by one measure, it impaired it by another (although the simple effect did not reach significance).

The third discovery-oriented issue was whether the effects of stress on navigation are mediated by SAM-axis activation. Consistent with Experiment 2, Experiment 3 found strong relationships between the magnitude of the effects of stress on both egocentric and allocentric navigation and its effects on SAM activation. However, also like Experiment 2, these associations were found at a time that should coincide with the putative timecourse of stress-induced cortisol release (Dickerson & Kemeny, 2004), and not SAM activity (Diamond et al., 2007). Given the correlation between measures of SAM and HPA activity in Experiment 1, it is possible that HPA activity is the true driver of the effects, and that SAM activity is acting merely as an indicator. This suggests that the influence of HPA activity (i.e., cortisol release) on spatial navigation should be tested again in the future.

In conclusion, the present experiment answered some questions but raised others. First, Experiment 3 resolved the disparity between Experiments 1 and 2 by showing that, in line with Experiment 2, stress reduces allocentric strategy selection. Experiment 3 also strengthened the

evidence from Experiment 2 that stress impairs allocentric navigational performance. However, whether there are sex-dependent effects of stress on strategy selection and allocentric performance remains unclear, as the present findings contradicted those from Experiment 2. A novel finding in Experiment 3 was that stress can sex-dependently impair or enhance egocentric navigational performance. The present findings suggest that sex may also be an important factor in the question of whether the stress effects on the relation between navigational strategy selection and performance are adaptive. It seems that the stress-induced shift from allocentric to egocentric navigation may be compensatory for females (i.e., they switch to an unimpaired strategy) but not for males (i.e., they switch from one impaired strategy to another). Finally, the present findings support the idea that the effects of stress on spatial navigation may be mediated by SAM activity, or another physiological process closely related to SAM activity. Importantly, the relationship between stress-induced SAM activity and navigational performance extends beyond allocentric navigation to hippocampus-independent, egocentric navigation.

Chapter 5: General Discussion

Overview

The present dissertation has revealed interesting findings about the relationship between acute stress and spatial navigation in humans. In this section, I will first briefly revisit the overarching rationale for the dissertation and review the rationale, approach and main findings of each of the three experiments. Next, I will discuss the meaning of the findings with respect to the effects of stress on navigational strategy selection, performance, and the relation between the two, and offer possible explanations for internally conflicting findings. Following this, I will discuss the implications of the findings with respect to each of the four models of the effects of stress on hippocampal function (outlined in Chapter 1). I will then discuss possibilities for future research that may help to resolve some of the questions raised in the present work.

Dissertation rationale and review of main findings

The present dissertation sought to investigate the effects of acute stress on human navigation and navigational strategy selection, and to reconcile these effects with contemporary models of the effects of acute stress on the function of the hippocampus. The initial premise was that acute stress activates a complex physiological stress response that involves the release of neuromodulators, via the HPA and SAM stress-axes, and that these neuromodulators influence hippocampal function, which is reflected in navigation behaviour. Which neuromodulators are considered to be the primary mediators of the stress effect, and the exact nature of their influence, depends on the model. In general, evidence from rodent studies has suggested that acute stress or stress hormones impair hippocampus-dependent allocentric navigation, have little effect on (or enhance) caudate-dependent egocentric navigation, and switch navigational strategy selection from allocentric to egocentric. However, research on the effects of acute stress on human

navigation has been sparse and mixed, and no research (prior to this dissertation) has examined the effects of stress on human navigational strategy selection.

Recap and logic of the 3 experiments.

The main objective of Experiment 1 was to study the effect of stress on human navigational strategy selection and to link this effect to the physiological correlates of the stress response. It was expected that, in line with theoretical models and empirical findings, stress-induced cortisol release (via HPA activation) would suppress hippocampal function and reduce allocentric strategy selection. Surprisingly, exposure to an acute psychological stressor apparently led to increased, rather than decreased, tendency to navigate allocentrically in a virtual dual-strategy maze. Furthermore, there was no evidence of HPA activation in response to the stressor. However, there was strong SAM-axis activation which was correlated with strategy choice, suggesting that SAM activity may have mediated the effect of stress on navigation. These findings had implications that led to the next experiment. First, the selection of an allocentric strategy may be promoted, rather than hindered by stress, either because the mechanism of strategy selection is independent of the hippocampus, because the stress was not intense enough to inhibit hippocampal function, or because there was something about the design of the maze that yielded anomalous results. Second, SAM activity, rather than HPA activity (i.e., cortisol), may be the key mediator of the effects of stress on strategy selection.

The main objectives of Experiment 2 were a) to verify the surprising findings of Experiment 1 using an updated virtual maze, b) to see if the effect of stress on strategy selection extends to allocentric navigational performance, c) examine the time-course of the stress effects, and d) examine the notion that the effects of stress observed in Experiment 1 were in fact driven by SAM axis activation. Based largely on the findings from Experiment 1, I expected that stress

would increase allocentric strategy selection and improve performance when tested either immediately or 20 min after stress (30 min after stressor onset). By and large, the results contradicted my predictions. When participants had their strategy selection tested in the updated Dual-Strategy maze (uDS maze), stress reduced (rather than increased) allocentric strategy selection, but only in males and only after a delay. When participants had their allocentric performance tested in a traditional virtual Morris water maze (the Place maze), stress impaired (rather than enhanced) allocentric performance, but only in females and only after a delay. Interestingly, the effects of stress on SAM activity (at the time of the stressor) were associated with its effects on performance but not strategy selection. Several implications followed from these findings which led to the next experiment. First, with respect to the effect of stress on strategy selection, the findings of Experiments 1 and 2 were in direct conflict. Likely explanations included different maze designs and interim "delay" activities. Second, it appeared that the stress effects did extend to allocentric navigation. Third, the time-course of the stress effects were not consistent with SAM-mediation, despite the appearance of some SAM-navigation correlations. This was surprising, given that cortisol did not appear to mediate the behavioural effect observed in Experiment 1. For this reason (and because of the expense), cortisol was not measured in Experiment 2. The findings also raised a new issue: whether the effects of stress on navigational strategy selection and performance were sex-dependent.

Experiment 3 attempted to resolve some of the differences between the results of Experiments 1 and 2 by using an experimental design that was as free as possible from factors that would confound the interpretation of the results. The objectives were to a) re-test the delayed effect of acute stress on strategy selection and allocentric performance using a stress-free, non-navigational activity during the delay, b) test the effect of acute stress on egocentric navigation,

c) check again for sex-dependent stress effects, and d) re-examine the relationships between navigation and stress-related SAM activity. To test the effect of stress on egocentric navigation, I used the Cue maze, which forced egocentric navigation by eliminating all spatially stable cues except the one that flagged the hidden platform. Once again, cortisol was not measured in Experiment 3, largely due to the cost and its lack of influence in Experiment 1. Based on all 4 theoretical models, the results from Experiment 2, and findings from rodent studies, I expected that stress would reduce allocentric strategy selection in the uDS maze, impair allocentric performance in the Place maze, and enhance or have not effect on egocentric performance in the Cue maze. Furthermore, I expected that the effects of stress on navigation in the uDS and Place mazes, but not the Cue maze, would be related to the level of stress-induced SAM activation. Results showed that, consistent with Experiment 2, stress reduced allocentric strategy selection in the uDS maze, but this time it was an overall effect and not sex dependent. Also consistent with Experiment 2, stress generally impaired allocentric performance in the Place maze. However, stress had a sex-dependent effect on performance in the Cue maze. In females, stress enhanced navigation (as expected) but in males, stress impaired it. Finally, similar to Experiment 2, there were no correlations observed between stress-induced SAM activation and strategy selection in the uDS maze. However, there were relatively strong relationships between stress-induced SAM activation and performance in the Place maze (both male and female), and surprisingly, also in the Cue maze.

Limitations

There are several potential limitations to the present dissertation. The first relates to the fact that the effects of stress on strategy selection were different (in fact opposite) for the original

and the uDS mazes. These mazes were both designed to test the same construct (navigational strategy selection), and yet the original Dual-Strategy maze suggested that stress increased allocentric strategy selection, while the uDS maze suggested the opposite. The upshot of this is that a clean interpretation of the strategy selection results of the first two experiments is difficult. However, because of its improved separation of egocentric and allocentric cues (the details of which have already been discussed more specifically; see Chapter 2: Discussion, Chapter 3: Introduction & Discussion), at this point I am confident that the results from the uDS maze (and not the original) are reliable, and would recommend using this design in future research. Perhaps the next steps in future research should be to explore why stress affects strategy selection differently in these two mazes.

This brings up a second limitation: because there were multiple differences in the designs of the two dual-strategy mazes, it is not clear which design change was most critical. Future research could try to isolate the key design elements to improve maze design methodology. Key manipulations might include varying the visibility of the target cue and reliable flankers at the start, or varying the salience of the target cue by changing its size or contrast. However, it is not clear whether isolating these factors is worth the effort. Knowing which maze more clearly isolates egocentric from allocentric navigation is a methodological issue which might not have much impact on how other researchers in this area conduct their research. However, understanding the difference between the mazes and why stress has different effects in the different mazes might provide new insights into the mechanisms by which stress affects navigational cognition. For example, is it a direct effect on performance, perhaps via hippocampal function, which then leads to a change in the selection of strategies? Alternatively, is it a direct effect on a strategy selection mechanism which is non-hippocampal and distinct

from navigational mechanisms? For example, maybe it is an effect on impulsivity which biases the selection of navigational cues at the outset of the trial. If this is true, then it would be interesting to relate the effects of stress on impulsivity with its effects on navigation. The IGT in Experiment 1 was partly an attempt to do this, but there are better tools to test impulsivity (e.g., the Stroop task).

The third limitation of the present dissertation relates to the design of Experiment 2. In Experiment 2, all participants first had their navigation tested immediately after stress in one of two visually similar mazes (either the uDS or the Place maze). The testing in the immediate maze served two functions. First, it provided a measure of the immediate effects of stress on navigation (i.e., acute SAM effects). Based on the findings of Experiment 1 and the predictions of the TDM model, the most interesting results were expected to appear at this time, and not after a delay. Second, it provided a non-stressful activity to occupy participants during the "rest" period before the effects of stress on delayed allocentric performance were tested and delayed strategy selection were re-tested in the second maze. Unfortunately, this meant that the effects of stress which appeared during delayed navigation were confounded with experience navigating in the first (immediate) maze, making interpretation of these findings difficult. The most important reason for this design was practical. Having another condition would have doubled the required *n* from 120 to 240[7], which did not seem practical (or necessary) when I started the experiment. However, this limitation on the interpretability of the delayed navigation results was largely overcome in Experiment 3, which used the same stressor and maze, but in which delayed navigation was not confounded with experience.

[7] To achieve a power of 1-*b* = .5, which is on par with the achieved power of similar studies in the literature (e.g., Duncko et al., 2007 (1-*b* = .57); Schwabe et al., 2007 (1-*b* = .49)), I needed a per-group *n* of ~15.

A fourth limitation in the present dissertation results from the limited number of cortisol measurements in Experiment 1. Salivary cortisol measurements were taken only twice – a baseline measurement immediately before the stressor, and a second measurement just before navigation, when stress-induced increases in cortisol levels should have been at or close to peak. Although there are many studies in which cortisol is only measured twice (e.g., Stawski et al., 2009; Wolf et al., 2001), it is more commonly measured at least 3 times during the experimental run (e.g., Zolatz et al., 2011; Nater et al., 2007). In Experiment 1, the reason behind the minimal number of measurements was largely financial. However, the limited number of measurements raises the possibility that, rather than being non-existent, the effect of the stressor on cortisol reactivity was simply missed. That is, a cortisol elevation might have occurred (and dissipated) before the second sample was taken, or alternatively, it might have been delayed until after the sample was taken. Thus, although purely speculative, it is possible that increased cortisol release (via HPA activation) could have played a mediating role in the effects of stress on navigation in Experiment 1 after all. As a consequence of this limitation, it is difficult to say whether HPA or SAM activity mediated the effects of stress on navigation in Experiment 1. Clearly, any future research on the relationship between stress and navigation behaviour should incorporate more than 2 cortisol measures.

The fifth limitation of the present dissertation relates to the lack of cortisol measurement in Experiments 2 and 3. The rationale for this was twofold. First, the measurement of salivary cortisol is expensive (by our standards, anyway). Second, based on the findings of Experiment 1, cortisol appeared to have little to do with the effect of stress on navigation behaviour. Thus, it seemed pointless to spend extremely limited funds on a measure that would have little explanatory power. However, there are reasons to believe that cortisol may have played a role in

the effects of stress on navigation after all. For example, the timing of the stress effects in Experiment 2 was consistent with the time-course of stress-induced cortisol release. Furthermore, measures of SAM activity (HR, BP) and salivary cortisol were correlated in Experiment 1, suggesting that SAM activation may be an indirect index of cortisol release after stress. As mentioned above, one possible explanation for the puzzling lack of a stress-induced increase in salivary cortisol in Experiment 1 was that it was missed due to the minimal number of measurements taken. Another possibility was measurement error. In sum, it is possible that increased cortisol (via HPA activation) could have played a mediating role in the effects of stress on navigation in Experiment 1, and thus measuring salivary cortisol in Experiments 2 and 3 might well have been worthwhile. Once again, the consequence of this limitation that it is difficult to say whether HPA or SAM activity mediates the effects of stress on navigation. This highlights the value in incorporating cortisol measures in any future research on the relationship between stress and navigation behaviour.

A sixth limitation relates to the lack of screening for female menstrual cycle and contraceptive use. Although there is some evidence that changing levels of gonadal hormones throughout the menstrual cycle may modulate (or even attenuate) the female stress response (for both the HPA (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999) and the SAM axis (Kajantie & Phillips, 2006), it is inconsistent (e.g., Zoladz et al., 2014). However, there is also some evidence that some types of contraceptive use might suppress the release of cortisol (Kirschbaum et al., 1999). Thus, it is possible that tracking female menstrual phase and contraceptive use may have helped to explain some of the variability between males and females in the present work. Although sex differences in the effects of stress on navigation were not expected based on the literature (only 1 out of 3 human studies that looked found sex differences,

Thomas et al., 2010), future research would do well to at least track menstrual cycle and contraceptive use as possible mediating factors.

Discussion of the main issues and synthesis of the findings

The effect of stress on human navigational strategy selection.

Introduction.

A key objective of the present dissertation was to understand how stress affects human navigational strategy selection. To date, only two rodent studies, and no human studies, have examined the effect of acute stress on navigational strategy selection (Kim et al., 2001; Schwabe et al., 2010a). The results of Experiment 1 suggested that stress increased the likelihood that participants would navigate the maze using a hippocampus-based, allocentric strategy. However, the findings of both Experiments 2 and 3 suggested the opposite, that is, that stress reduces allocentric strategy selection after a delay. Furthermore, Experiment 2, but not Experiment 3, indicated that the effects of stress on strategy selection are sex-dependent – that is, that stress reduces allocentric strategy selection in males but not females. It is important to consider what led to the differences in the effects of stress on strategy selection between these experiments.

Does stress shift strategy selection to egocentric or allocentric?

Although it seemed clear that, based on the findings of Experiment 1, stress increased allocentric strategy selection, this surprising finding was contradicted by Experiments 2 and 3. There are several possible explanations for the difference in findings between Experiments 1 versus 2 and 3 (see also Chapter 3: Discussion). One possibility was differences in sampling. Although the ages, sex balance and education levels were similar between experiments, differences in the time of year at which data collection took place could have resulted in different physiological and psychological manifestations of stress. Experiment 1 was collected mostly

between January and March (51% of participants) and in the morning whereas the majority of Experiment 2 was collected between September and December (45% of participants) and in the afternoon as well as the morning. Given that the participants were mostly first-year students, and that the sun rose later in the fall, baseline anxiety and circadian cortisol levels could have been different in the participants very first term at university (Experiment 2) than in their second term (Experiment 1). However, this idea does not measure well with the results of Experiment 3, for which, like Experiment 1, the majority of the data was collected between January and March (71% of participants). Despite this, the effect of stress on strategy selection observed in Experiment 3 still contrasted that of Experiment 1. This, in combination with the fact that cortisol did not appear to contribute to the effects seen in Experiment 1, makes sampling differences an unlikely explanation for the contradictory findings.

Another explanation for the difference in strategy selection results between Experiments 1 versus 2 and 3 was the intentional difference between the activities given during the delay period. In Experiment 1, participants completed the FAPA and IGT during the delay period. These were felt to be potentially anxiogenic, and possibly causing stress in the control group or augmenting it in the stress group. Experiment 2 attempted to achieve better psychological and physiological differentiation of stressed and control groups by giving participants a task previously found not to be stressful: navigation in the Place maze. One drawback to this procedure was that while participants in the "immediate testing" condition would be experiencing the maze for the first time, the "delayed testing" participants would be learning and selecting their strategies after having been exposed to the same environment, and after training in allocentric navigation. Thus, delayed testing was confounded with training in the Place maze. However, this methodological issue was eliminated in Experiment 3, in which participants only

read magazines during the delay period. Despite this, the effect of stress on strategy selection in Experiment 3 agreed with Experiment 2. In other words, stress reduced allocentric strategy selection in the uDS maze, regardless of the delay activity. Given this, there is some doubt as to whether the differences in the delay activity between the three experiments can explain the different results.

The most likely contributor to the contradiction between the findings of Experiment 1 versus Experiments 2 and 3 was the different mazes used. As discussed previously, the uDS maze (used in Experiments 2 and 3) was designed to improve upon the original Dual-Strategy maze from Experiment 1 by establishing cleaner separation between egocentric and allocentric navigation. It did this in several ways. First, the uDS maze reduced possibility of "impulsive" egocentric navigation being mischaracterized as allocentric by: a) ensuring that the key cue object was always in the field of view at the start of ITSP trials, so that participants did not need to pan left or right to find it, b) eliminating flanker cues by making all non-cue objects identical, and c) making the cue-objects larger and more obvious than they were in the original Dual-Strategy maze, reducing the likelihood that the target cue was overshadowed by a snapshot of the window. Second, the uDS maze eliminated the spatial displacement between the cue object and the platform, thereby making the maze more purely egocentric. Although it is difficult to know which of these elements, or combination of elements, was the key contributor to the difference in findings between the two mazes, there is clear consistency in the effects of stress on strategy selection in the uDS maze between Experiments 2 and 3. This is evidence that the design of the original dual-strategy maze led to a mis-categorization of strategy selection, such that under stress, participants who were navigating egocentrically appeared to be navigating allocentrically. These maze design flaws were overcome in the updated uDS maze.

In sum, it appears that the surprising finding of Experiment 1 – that stress increases allocentric strategy selection – was anomalous. After addressing some methodological issues related to the design of the maze, Experiments 2 and 3 consistently found the opposite, that stress decreases allocentric strategy selection. This finding is in-line with the predictions of all four theoretical models and with previous empirical findings in rodents (Kim et al., 2001; Schwabe et al., 2010a) and one non-navigational human study (Schwabe et al., 2007). This leads to the conclusion that the true effect of stress is to shift strategy selection from allocentric to egocentric.

Are the effects of stress on strategy selection sex-dependent?

Across the experiments in the present dissertation, the findings with respect to the influence of sex on the effects of stress on navigational strategy selection have been mixed. Sex appeared to interact with stress in terms of its effect on strategy selection in Experiment 2, but not Experiments 1 or 3. However, given that the strategy selection findings of Experiment 1 are questionable (see discussion above), the focus of the present discussion will be on comparing the findings of Experiments 2 and 3. In Experiment 2, stress reduced allocentric strategy selection, but only in males, raising the possibility that sex is an important factor in the effects of stress on strategy selection. However, in Experiment 3, stress reduced allocentric strategy selection for males and females in a similar way, casting doubt on the idea that stress influences male and female strategy selection differently.

It is once again worth considering methodological differences between the two experiments in order to explain the conflicting findings. The discrepancy between the two experiments appeared despite using the same stressor, delay before navigational testing, and the same mazes. Indeed, the only major difference between the two experiments with respect to the measurement of delayed strategy selection was that, in Experiment 2, participants navigated in

the Place maze before navigating the uDS maze, whereas in Experiment 3 participants read magazines. However, it is possible that this difference may have led to the different findings. Training in the Place maze may have prevented females from switching to an egocentric strategy under stress. For example, it may be that stress biased females to "do what worked before" (cf. habit memory, Packard & Goodman, 2012), leading them to employ the same strategy they used in the Place maze and ignore other options when they navigated the uDS maze. However, if this were the case, it is not clear why stress would not have had the same effect on males, or if it did, why the stress effect was sufficient to overcome the training effect in males but not females.

One possibility is that males and females reacted differently to the stressor in Experiment 2, and that this interacted with training in the Place maze to lead to different strategies in the uDS maze. There are two ways in which this might have occurred. The first is that there might have been a different physiological reaction to the stressor. In general, males tend to exhibit stronger physiological responses to acute psychological stress (Kajantie & Phillips, 2006; Clemens Kirschbaum, Wüst, & Hellhammer, 1992; Kudielka & Kirschbaum, 2005; Nicolson, Storms, Ponds, & Sulon, 1997), although not always (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008). By this logic, it is possible that males' physiological reaction to the stressor was so strong, and the resulting suppression of hippocampal function was so significant, that they were less able to solve the uDS maze allocentrically, despite allocentric training in the Place maze. In contrast, females, having had a less robust physiological reaction, may have been able to continue to solve the maze allocentrically, as they did in the Place maze. This explanation is seems unlikely,

however, because males and females did not differ with respect to their physiological reactivity to the stressor in Experiment 2[8].

The second way in which males' and females' reactions to the stressor might have interacted with training in the Place maze is that there might have been a different psychological reaction to the stressor. While males tend to react more strongly physiologically, females have been shown to exhibit stronger psychological reactions to psycho-social stressors (Kelly et al., 2008). By this logic, it is possible that females were either a) so distracted by the stressor that they were unable to fully attend to the uDS maze, and did not notice (or care) that an alternative strategy was available, or b) felt vulnerable as a result of the stressor and selected the "safer" strategy (i.e., the one that worked well in the Place maze). Males, in contrast, may have been better able to "put the stressor behind them", and tended to solve the maze egocentrically under stress simply because it seemed to be the easier strategy (perhaps because their hippocampus was suppressed by physiological correlates of stress). However, this explanation also seems unlikely, because males and females did not differ with respect to their psychological reactivity to the stressor in Experiment 2^4, as measured by the STAI.

Taken together, it is not clear what led to the sex-based differences in the effects of stress on strategy selection in Experiment 2, but it may be related to training in the Place maze. At the very least, Place maze training in Experiment 2 makes the strategy selection findings in the delayed uDS maze difficult to interpret. In Experiment 3 there was no training confound and no sex difference in the effects of acute stress on strategy selection. Therefore, these results are more credible.

Interim Conclusion.

[8]A MANOVA on HR, SC, Systolic and Diastolic BP, and STAI revealed a significant effect of Condition for all of these dependent variables (all p's<.000), no significant effect of Sex for any dependent variable (all p's >.13), and no interactions (all p's > .07).

Two key points can be taken from the results of the present dissertation about the overall effects of acute stress on navigational strategy selection. First, the answer to the question of whether stress affects human navigational strategy selection (at all) is clear enough: yes. Determining the direction of this effect has been complex and difficult, but Experiments 2 and 3 provide good reason to believe that stress biases navigational strategy selection from allocentric to egocentric in humans (as it does in rats). Second, based on the results of Experiment 3, the effect of stress on strategy selection does not appear to be sex dependent. However, perhaps because of its flawed design, the results of Experiment 2 (serendipitously) raise the possibility that there may be a differential influence of experience on the delayed effects of stress on males and females. This interesting idea could be explored further in future research.

The effect of stress on allocentric performance.

A second key objective of the present dissertation was to examine the influence of acute stress on allocentric navigational performance. Although stress appears to have no impact on allocentric performance immediately after stress-exposure, the findings of Experiments 2 and 3 were in general agreement that stress impairs allocentric navigation after a delay.

However, the findings of Experiments 2 and 3 were somewhat complicated by sex dependencies. Specifically, in Experiment 2, stress impaired female, but not male allocentric performance. In Experiment 3, stress impaired allocentric performance for both males and females (although the effects appeared more consistently across measures for males). Once again, this raises the question of what led to the difference in sex-dependencies between Experiments 2 and 3. It is interesting to speculate that this difference was due to training effects in the immediate uDS maze in Experiment 2. However, exploring this possibility more deeply would require further splitting the groups in Experiment 2 based on the strategies that participants

selected in the immediate uDS maze (i.e., to determine whether participants had practice using an egocentric or allocentric strategy before navigating the Place maze). Unfortunately, doing so makes the group *n*'s too small for meaningful analysis (mean $n = 5.63$, with only 1 participant in 2 of the 8 groups). Given this, and the fact that Experiment 3 had a cleaner design (i.e., delayed navigation was not confounded with training), it is best to rely on the findings of Experiment 3.

The findings of Experiment 3 are in general agreement with the animal literature and add to the human literature on the effects of stress on allocentric navigation. In Experiment 3, stressed participants required more time to find the goal overall, and stressed males had less accurate location estimates than controls. These findings mirror those from the rodent literature, in which stress (e.g., Schwabe et al., 2010a, Park et al., 2008, Xiong et al., 2003), stress hormones (Schwabe et al., 2010a; Snihur, Hampson, & Cain, 2008), or anxiogenic drugs (Wingard and Packard, 2008) commonly impair hippocampus-based, allocentric navigation. The findings in the 4 human studies to date have been less consistent, with one study finding that stress impaired performance (in females; Thomas et al., 2010), one finding that it enhanced performance (in males; Duncko et al., 2007), and two others findings no effect (Guenzel et al., 2014; Klopp et al., 2012; See Chapter 1: Background for fuller descriptions of these studies).

The relationship between the findings of Experiment 3 and the findings of the 4 previously existing studies on the effects of stress on navigation merits consideration. The present findings are consistent with and extend those of Thomas et al., 2010, who also showed that acute stress can impair female navigational performance in a similar virtual MWM to the ones used in this dissertation. Experiment 3 extended this finding by showing that stress can also impair male allocentric performance, possibly to a greater extent than females. In contrast, the present results contradict those of Duncko et al. (2007), who found that stress enhanced

performance in an all-male sample. However, in that study, no effect of stress was found in the traditional measures of water maze performance (distance and latency, as used here), but were only present in two less-commonly used variables (heading error and failures to find the platform). Moreover, Klopp et al., 2012, attempted but failed to replicate the findings of Duncko et al. (2007) using the same maze but a different stressor. Klopp et al. (2012) were unable to detect any effects of stress on navigational performance whatsoever, despite eliciting a strong stress response. This casts some doubt on the ability of this particular virtual MWM to accurately detect the effects of stress on allocentric navigational performance. A 4th study (Guenzel et al., 2014) revealed no effect of stress on allocentric performance in a virtual RAM. However, as noted previously (see Chapter 4: Introduction), RAM's are better suited to the measurement of memory than they are to navigational performance.

In conclusion, the findings of the present dissertation, especially Experiment 3, suggest that stress impairs hippocampus-dependent, allocentric navigational performance. This is generally in agreement with the rodent literature, and extends findings with rodent models to humans. Furthermore, it extends the human literature, showing that stress not only impairs female, but also male allocentric performance after a delay.

The effects of stress on egocentric performance.

A third key objective of the present dissertation was to determine the effect of acute stress on human egocentric navigation. The present work represents the first time stress effects on human egocentric navigation have been observed. Until now, direct tests have been unable to show that stress has any effect on egocentric navigation, in humans (Guenzel et al., 2014) or in animals (Schwabe et al., 2010b; although anxiogenic drugs have been shown to enhance egocentric navigation, Wingard and Packard, 2008). Surprisingly, stress had complex, sex-

dependent effects on egocentric navigation. Stress impaired male performance (distance and cue error), but it enhanced (distance) or impaired (cue error) female performance depending on the measure. These findings raise several considerations.

The first consideration is the differences between males and females with respect to the direction of the stress effects. Specifically, stress caused females to navigate more efficiently (take more direct routes) in the egocentric Cue maze, but caused males to navigate less efficiently. That stress enhanced female egocentric navigational efficiency (distance) was not particularly surprising, and is in line with theoretical models (Hot/Cool Systems and Uniform Shift) and the animal literature (Schwabe et al., 2010b). However, the stress-induced impairment in male egocentric navigation was a surprise, and was not predicted by theory or previous empirical studies.

One explanation for this could be that the consequences of the physiological stress-response systems on the brain are modulated differently for males and females. For example, at least one author has suggested the possibility that female sex hormones, especially estradiol, may shield female hippocampal neurons from the modulatory effects of stress-induced glucocorticoids (Wolf et al., 2001). However, the evidence for this idea is weak; it is based on a single rat study in which male, but not female rats, showed significant dendritic atrophy in the hippocampus after chronic stress (Galea et al., 1997). In any case, this possibility does not explain why stress made females *more* efficient at finding the platform in the egocentric Cue maze. A more likely explanation for the difference in stress effects on egocentric navigational efficiency between males and females is that they rely on different brain structures for egocentric navigation, and that stress impacts these structures differently. For example, it is possible that, while females tended to rely on the caudate to find the platform, males relied more on frontal

cortex. There is plenty of evidence to show that frontal lobe function is impaired by stress (Dias-Ferreira et al., 2009; Liston et al., 2009; Porcelli & Delgado, 2009; van den Bos et al., 2009; Young et al., 1999; Youssef et al., 2012). However, this would seem to contradict neuroimaging evidence that suggests that females tend to rely more heavily on the frontal lobes during navigation, while males tend to rely more on the hippocampus (Gron et al., 2000).

The second consideration relates to performance differences within sex. Although the effects of stress on male egocentric performance were generally consistent across measures (impairment), they were not consistent across measures for females. Specifically, stress caused females to take more direct routes to the goal on standard trials (as measured by distance) but to make less accurate estimates of the platform location on ITPs (as measured by cue error). This indicates that, in females, stress affected the systems involved in navigating to a find-able place differently that the systems involved in identifying the exact location of the place. Why it did so in females, and did not do so in males is a bit of a puzzle. Perhaps it indicates that males and females used different anatomical systems, at least for one of the two tasks. Perhaps it indicates that the physiological effects of stress and the interaction with brain areas were different for males and females. Alternatively (but not mutually exclusively) it could indicate that males and females interpreted the instructions or the tasks differently or were differentially affected by the stressor, or both.

The third consideration relates to the effect of stress on participants' ability to estimate the platform location in the Cue maze. The data suggest that stress generally reduces accurate knowledge of the goal location when navigating egocentrically (for both sexes). This was surprising, given that in the Cue maze, participants could see the cue that flagged the platform. However, on ITP trials, some participants occasionally followed the same spatial trajectory (e.g.,

short sweeping left turn) that they had just followed on the preceding Standard trial, winding up under the cue-sphere marking the location of where the platform would have been if the start position had not changed. This strategy is based on a combination of body-based movements (i.e., a "praxis" strategy; Sutherland & Dyck, 1984) and homing in on a visible cue (i.e., a "taxon" strategy, O'Keefe & Nadel, 1978). Hence, the strategy is still egocentric (because it does not require knowledge of the spatial relations between multiple cues). However, this strategy ignores the color of the cues, and also explicit instructions that start positions move from trial to trial. Of course, because participants' start positions were changing, a praxis/taxon strategy would have led them to the wrong sphere, and greatly inflated their cue error. It is possible that participants under stress were unable to suppress the impulse to respond habitually and automatically, based on the movements that were successful in the preceding Standard trial. In other words, stress may have caused participants to simple "do what worked before". This idea is in line with both the Hot/Cool Systems and the Uniform Shift models, as well as recent empirical studies (e.g., Schwabe and Wolf, 2011; Ellenbogen et al., 2010), all of which suggest that stress shifts cognition from a controlled, reflective mode to an automatic, reactive mode. This might have caused more stressed participants to estimate the platform location on ITPs using praxis/taxon navigation, and thus account for the large stress effect.

I examined this possibility by calculating a new variable – *minimum cue error*. This variable represents the lessor of the distance between participants' location estimate and the location of the cue that marked the platform location on the preceding Standard trial, or the location of the cue that was at the end of the trajectory that matched the correct trajectory of the preceding Standard trial (See Figure 27 for an example).

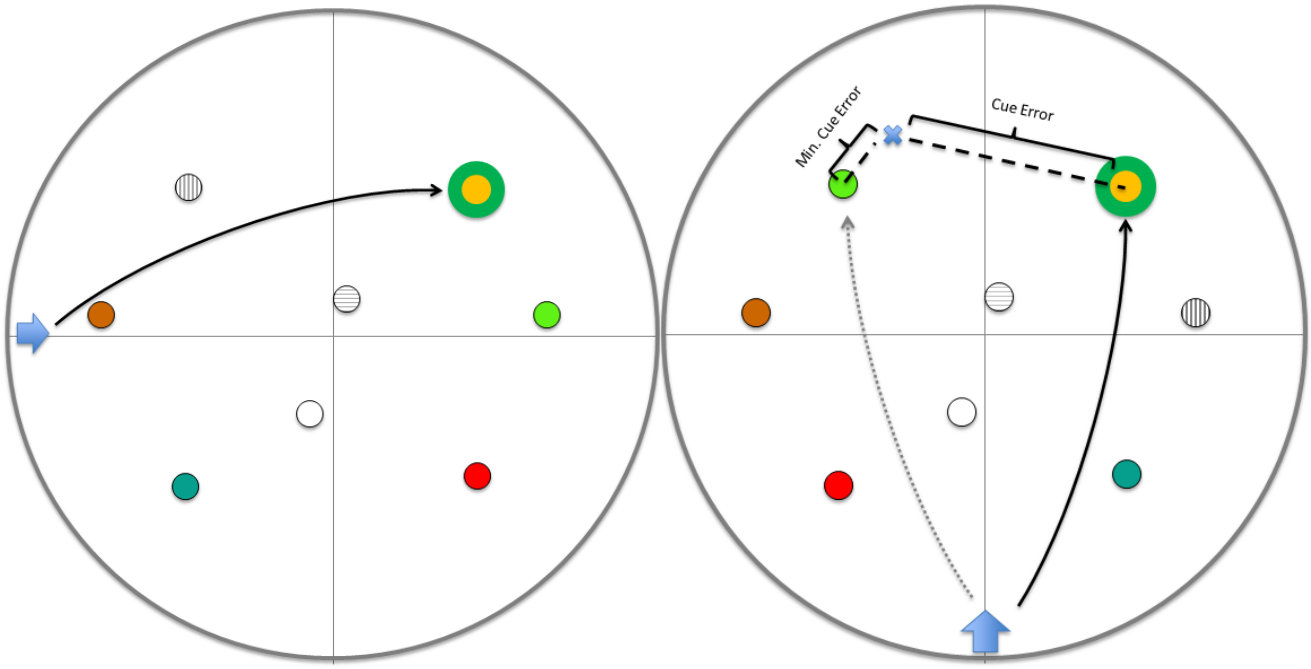


Figure 27. Minimum Cue Error example.
In this example, the left side represents a Standard trial, and the right side an Inter-trial Strategy probe. The blue arrows represent start positions. Smaller circles represent the locations of the 8 cue spheres, with the target sphere (orange) overlaid on the invisible platform (larger green circle). The black arrows represent the correct path from the start to the target sphere. On the right side, a blue "X" represents a participant's estimate of the platform location. The dashed lines represent the displacement between the estimate and the correct platform location (under the orange sphere) or the location of the cue at the end of the correct trajectory from the preceding Standard trial (green sphere).

However, although this variable greatly reduced the overall error magnitude, the difference between stressed and control participants was still significant[9]. In other words, the

[9] A 2x2 (Condition x Sex) ANOVA on minimum cue error revealed a main effect of Condition ($F(1, 62) = 8.7$, $p = .001$, $\eta^2 = .12$), but no effect of Sex and no interaction (p's > .49).

occasional use of praxis/taxon strategies on ITP trials did not explain the stress-induced impairment in location accuracy. Thus, the question remains as to why stress worsened participants' ability to accurately estimate the platform location egocentrically. This question could be investigated further in future research.

Taken together, the present work provides the first evidence that acute stress influences human egocentric navigation. These effects are complex and sex-dependent, again suggesting that sex is a key factor in the study of the effects of stress on navigational cognition. Furthermore, the present findings offer tantalizing hints males and females rely on different brain regions to solve egocentric tasks, and that these regions are differently affected by stress. This indicates that sex is a critical factor in the study of the effects of stress on navigation specifically, and on the brain in general. It remains unclear why stress caused participants to be less accurate in the platform location estimates. While stress appeared to change the nature of some participants' approach to estimating the platform location (i.e., they used a blended taxon/praxis strategy), this did not entirely explain the apparent stress-induced impairment. Future research should explore this effect more deeply.

Bringing together stress effects on strategy and performance.

A fourth objective for the present dissertation was to examine the relationship between the effects of stress on navigational strategy selection and its effects on egocentric and allocentric performance. Based largely on the Uniform Shift and Hot/Cool Systems theories (discussed in detail below and in Chapter 1), the central idea was that a stress-induced switch between navigational strategies would be adaptive. That is, if one cognitive system is impaired by stress (e.g., hippocampus-based allocentric navigation), than people should be able to switch to an alternate, unimpaired cognitive system (e.g., caudate-based egocentric navigation). Experiment 3

was the first study capable of comparing the effects of stress on navigational strategy selection against its effects on pure egocentric or allocentric navigation. Because the relationship between strategy selection and performance differed for males and females, they will be considered separately.

In males, stress shifted navigational strategy selection towards the use of more egocentric strategies and less allocentric strategies. However, it is difficult to argue that the stress-induced strategy shift in males was adaptive, given that stress impaired both egocentric and allocentric navigational performance. I suppose that it could be argued that males switched to the strategy that was *less* impaired by stress, and thus the switch was still adaptive. However, this argument is problematic, given that it is difficult to compare the magnitude of stress-induced impairment. First, the effects of stress on egocentric and allocentric navigation were tested in different mazes in Experiment 3, and second, males were impaired on multiple measures in both place and cue mazes. Taken together, the data suggests that stress shifts male strategy selection from allocentric to egocentric, but impairs navigational performance globally. It also suggests that stress impaired the brain regions that males had recruited for egocentric navigation. Again, because stress and stress hormones typically have no effect on or enhance caudate-based navigation, is seems likely that stress was affecting another area, possibly in combination with the caudate (e.g. frontal cortex).

In females, the idea that stress causes an adaptive shift from hippocampus-based allocentric navigation to caudate-based egocentric navigation was partly confirmed. Stress shifted female strategy selection from allocentric to egocentric and enhanced egocentric performance in the Cue maze (as measured by distance). Interestingly, despite the strategy shift towards egocentric navigation and improved efficiency on Standard trials in the Cue maze

(suggesting enhanced caudate function), females were still impaired in their ability to accurately estimate the platform location in ITP trials. This suggests that females recruited different brain areas for this aspect of the task, and that these areas were impaired by stress (e.g., frontal lobes).

Taken together, the evidence suggests that an adaptive shift in navigational strategy selection can occur. However, the occurrence of an adaptive shift may depend on sex, once again suggesting that sex is a critical factor in the study of the effects of stress on navigational strategy selection and performance, as well as its effect on the relation between the two. Why the effects of stress on navigation should be adaptive for females but not males is still an open question and worth exploring in future research.

The relationship between navigation and the physiological correlates of stress.

Introduction.

A fifth key objective of the present dissertation was to investigate the relationship between the physiological correlates of stress (i.e., SAM and HPA activity) and navigational strategy selection and performance. Past studies have found relationships between stress reactivity and performance on tasks that share a common neural basis with navigation, such as hippocampus-based declarative memory tasks (e.g., Domes et al., 2002; Elzinga et al., 2005; Zolatz et al., 2011). However, the studies in the present dissertation are the first to examine the relationship between measures of stress reactivity and navigation.

HPA Activity.

It is commonly thought that HPA activity, and its end product, cortisol, is the key mediator of the effects of stress on hippocampal function. Thus, it came as a surprise that increased HPA activity (as measured by salivary cortisol) was not detected in Experiment 1, despite the observed behavioural effect of stress on navigational strategy selection. Furthermore,

there was no correlation between strategy and cortisol. Interestingly, this is not the first time that behavioural effects of stress on human navigation have been observed without attendant stress-induced cortisol increases (Duncko et al., 2007). On the one hand, this suggests that HPA activity is not associated with navigational strategy selection. On the other hand, given the problems with the measurement of strategy selection in Experiment 1, this conclusion might be premature. This is especially true given the time-course of the effects of stress in Experiment 2, which were consistent with the time-course of HPA-based cortisol release (Dickerson & Kemeny, 2004). Unfortunately, largely due to expense, I was unable to assess the impact of HPA activation on navigational performance in Experiments 2 and 3. Thus, the opportunity to explore the relationship between HPA activity and allocentric and egocentric navigational performance is open for future research.

SAM activity.

The studies in the present dissertation revealed interesting relationships between stress-induced SAM activity (measured indirectly by HR, SC, and BP) and navigation behaviour. First, SAM activity correlated with increased allocentric strategy selection in Experiment 1, but did not correlate with strategy selection at all in either Experiments 2 or 3. Given the agreement between Experiments 2 and 3, and the noted problems with the measurement of strategy selection in Experiment 1, the evidence seems to indicate that SAM activity is not associated with navigational strategy selection. Instead, the behavioral effect of stress on strategy selection is likely mediated by some other mechanism, possibly HPA-based cortisol influence (discussed below).

Second, SAM activity was generally associated with impaired allocentric performance in Experiments 2 and 3. This suggests that the effect of stress on allocentric navigational

performance may be mediated by SAM activity. However, by and large, this relationship was limited to SAM activity at the time of the stressor, and not SAM activity during navigation. This raises the possibility (again) that a third variable, which is highly correlated with SAM activity and which is either persistent or has a delayed onset (e.g., cortisol release) may underlie the effect.

Third, SAM activity was also associated with impaired egocentric performance in Experiment 3, but only in males. Once again, this association was limited to SAM at the time of the stressor. In females however, SAM activity was associated with improved performance, regardless of when SAM activity was measured. Thus, SAM activity had different relationships with male and female egocentric navigational performance. This once again raises the possibility that males and females relied on different brain areas to navigate in the Cue maze, and that stress, possibly via the SAM axis, operates on these areas differently.

Taken together, this pattern of relationships suggests that a) SAM activity likely is not related to navigational strategy selection, and b) SAM activity either mediates or is closely associated with a mediator of the effects of stress on navigational performance. The direction of the association appears to be modulated by both sex and the strategy used.

Interim Conclusion.

The physiological correlates of the effects of stress on human navigation remain elusive. Although HPA-mediation seemed likely at the outset of this dissertation (based on theory and rodent literature), there was no evidence for this idea when HPA-activity was directly tested in Experiment 1. However, HPA-based mediation may be worth another look, given the time-course of the behavioural findings in Experiment 2, as well as the pattern of correlations between SAM variables and navigational variables in Experiments 2 and 3. SAM activity appears to have

little to do with strategy selection, but has sex-dependent relationships with performance – once again indicating that sex is a critical factor in the relationship between stress and navigation.

Reconciling the present results with the 4 models.

Introduction.

Chapter 1 outlined the key characteristics of 4 theoretical models, each of which posit physiological mechanisms for the effects of acute stress on the function of the hippocampus and related structures. These models served as part of the initial rationale for the dissertation. Presently, I return to these models to see how well they match the data from the three experiments. For each model, I will first briefly summarize its key elements (for further details, see Chapter 1: Background), then describe how the findings from the present dissertation either support or contradict it. The evidence for and against each model will then be integrated and discussed in the light of several key considerations.

Modified Yerkes-Dodson (MYD).

According to the MYD model (de Kloet et al., 1999; Metcalfe & Jacobs, 1998; Yerkes & Dodson, 1908), the key mediator of the effects of stress on navigation behaviour should be HPA activity. Specifically, stress should stimulate the HPA axis to release cortisol, which then modulates the activity of hippocampal neurons via two types of receptors there (glucocorticoid and mineralocorticoid receptors (MRs and GRs)). The direction of the cortisol (i.e., stress) effect depends on stress intensity (described by an inverted-U shaped function) and is related to the relative occupation of these receptors. Importantly, it takes 20-40 min for the cortisol response to reach peak levels (in blood or saliva; Dickerson & Kemeny, 2004), and it is generally assumed by proponents of this model that the effects of stress should appear in this time frame.

The present dissertation revealed some evidence that supports the MYD model. First, and most importantly, Experiment 2 showed that the effects of stress on navigation behaviour are delayed, not immediate. Stress impaired allocentric navigational performance and shifted strategy selection (from allocentric to egocentric) only after approximately 30 min post-stressor onset, and not immediately after the cessation of the stressor (10 min post-onset). This is consistent with the time-course of the HPA-based cortisol response (Dickerson & Kemeny, 2004), suggesting that it may have played a role in the behavioral effects, as is predicted by the model. Second, in both Experiments 2 and 3, stress reduced (hippocampus-based) allocentric strategy selection and impaired allocentric performance, consistent with cortisol mediated suppression of hippocampal function. Third, by and large, the correlation between SAM activity and delayed navigation behaviour in Experiments 2 and 3 was limited to the time of the stressor, suggesting that SAM activity may have been associated with a third variable, the effects of which either persisted or had delayed onset. Cortisol seems like a good candidate, given the known time-course of the cortisol response to acute stress and the strong correlations between measures of SAM activity and cortisol in Experiment 1.

Some results of the present dissertation are difficult to explain by the MYD model. Although in Experiments 2 and 3 the timing of the stress effects and correlations seemed to suggest mediation by cortisol, this is difficult to reconcile with the findings from Experiment 1. That is, despite observing a behavioural effect of stress on strategy selection in Experiment 1, there was no evidence that stress triggered cortisol release. Furthermore, salivary cortisol was not associated with navigation behaviour whatsoever. These findings suggest that the behavioural effect of stress was not mediated by HPA activity, contradicting the MYD model. Furthermore, even if cortisol did play a role in the behavioral effects of stress, but was not detected in

Experiment 1 (i.e., type 2 error), the model still cannot explain the sex-dependencies with respect to the effects of stress on hippocampus-based allocentric navigation in Experiments 2 and 3.

Temporal Dynamics (TDM).

According to the TDM model (Diamond et al., 2007), the key mediator of the effects of stress on navigation behaviour should be SAM activity. This model postulates that, primarily via the SAM axis, stress initiates a pattern of time-dependent shifts in hippocampal efficiency, which plays out in two phases. In the initial phase, beginning shortly after stress onset, hippocampal function is enhanced for a brief period (minutes). In the subsequent phase, the hippocampus enters a refractory period in which its function is supressed (for minutes to hours).

Evidence in favour of the TDM model comes from two sources. First, in both Experiments 2 and 3, stress reduced (hippocampus-based) allocentric strategy selection and impaired allocentric performance after a delay, consistent with suppression of hippocampal function in the 2nd phase of the model. Second, there were associations between navigation behaviour and measures of SAM activity in all 3 experiments. SAM activity was (somewhat paradoxically) correlated with increased strategy selection in Experiment 1, which would be consistent with hippocampal enhancement in the initial phase of the TDM model. This served as part of the rationale for Experiment 2. SAM activity also correlated with delayed allocentric (hippocampus-based) performance in Experiments 2 and 3 (consistent with the 2nd phase of the TDM model). In sum, is seems that SAM activity may be an important factor in the relationship between stress and navigation, in line with the TDM model.

The evidence against the TDM model comes primarily from Experiment 2, in which one goal was to test the TDM model by assessing the time-course of the effects of stress on

navigation. If Experiment 1 had tested navigation too soon, and so detected effects that were driven by SAM activity in the initial phase of the model, then those effects should also be present when navigation was tested immediately after stress in Experiment 2. They were not. Rather, the observed effects of stress in Experiment 2 were only detected after a delay, at a time more consistent with HPA, rather than SAM mediation. Furthermore, the model cannot account for the sex differences in the effects of stress on allocentric performance in Experiments 2 and 3.

Hot/Cool Systems.

According to the Hot/Cool Systems model (Metcalfe and Jacobs, 1999), both SAM and HPA activity mediate the effects of stress on navigation behaviour. Part of this model is virtually identical to the MYD model: stress enhances or suppresses hippocampus-based "Cool" system function via cortisol and according to a u-shaped function. The other part of the model proposes that the SAM axis enhances amygdala-based "Hot" system function in a linear fashion. In this way, sufficiently intense stress suppresses the hippocampus-based cool system, while stress continues to enhance the amygdala-based hot system up to high levels. This eventually shifts cognitive-behavioural dominance from a hippocampus-centred, controlled, reflective mode to an amygdala-centred, automatic, reactive mode. Although not explicitly part of the model, it has been suggested by other studies that when the amygdala is activated by stress or stress hormones, it in turn activates the caudate nucleus (Packard & Wingard, 2004; Schwabe et al., 2013; Schwabe & Wolf, 2012; Wingard & Packard, 2008).

Much of the evidence in favour of the Hot/Cool Systems model comes from Experiment 3. Consistent with the model, stress impaired hippocampus-based allocentric navigational performance, enhanced caudate-based egocentric performance (in females) and shifted strategy selection away from allocentric and towards egocentric navigation. Also consistent with the

model was the fact that SAM activity correlated with improved egocentric performance in females.

Some findings in the present dissertation that are difficult to explain using the Hot/Cool Systems model. For example, in Experiment 1, there was a behavioural effect of stress on hippocampus-based allocentric strategy selection (enhancement) without a concomitant change in HPA-activity, and no correlation between the two. Thus, it is difficult to argue, as the model does, that HPA activity mediates the effect of stress on hippocampal function (at least based on the findings of Experiment 1). Also difficult to explain using the Hot/Cool model (which does not dissociate sex) is that in Experiment 3, stress impaired both allocentric (i.e., "Cool") *and* egocentric (i.e., "Hot") navigation in males. This suggests that the Hot/Cool systems may need to be updated to include sex as a factor.

Uniform Shift.

According to the Uniform Shift model (Schwabe, 2013), both SAM and HPA activity mediate the effects of stress on navigation behaviour. According to this model, stress generally biases behavioural control from the hippocampus to the caudate, and both stress systems work together in two ways to do so. First, they act directly on the hippocampus and caudate, disrupting the balance of mutual inhibition between them and promoting caudate function at the expense of the hippocampus. Second, they act indirectly via the amygdala, which acts as a "conductor", further biasing behavioural control to the caudate under stressful conditions.

The findings in the present dissertation that fit well with the Uniform Shift model are similar to those that fit well with the Hot/Cool systems model. Specifically, as predicted by the Uniform Shift model, in Experiment 3 stress impaired allocentric (i.e., hippocampus dependent) performance, enhanced female egocentric (i.e., caudate dependent) performance, and biased

strategy selection towards egocentric navigation (and away from allocentric navigation). The Uniform Shift model also fits well with much of the correlational results. For example, by this model, which posits that both SAM and HPA generally suppress hippocampal and enhance caudate function, it makes sense that SAM activation would be associated with impaired allocentric performance (as seen in Experiments 2 and 3), in addition to improved egocentric performance (in Experiment 3).

However, there are findings in the present dissertation that are a challenge to explain by the Uniform Shift model. The most difficult is that, in Experiment 1, stress apparently increased, rather than decreased, allocentric strategy selection. This is opposite to what would be expected by the Uniform Shift model, which holds that stress biases behavioural control to the caudate, and thus stress should *decrease* the likelihood of solving the maze allocentrically. However, this apparent contradiction might be explained by problems with the measurement of strategy in Experiment 1 (as discussed previously). Another problem for this model is that stress impaired male egocentric performance in Experiment 3, rather than enhancing it, as the model predicts. This suggests that sex may be an important factor in the effects of acute stress on the brain function, and this factor is missing from the Uniform Shift model (as well as the other 3 models).

Integrating the evidence.

Taken together, none of the 4 models fit particularly well with the findings from the present dissertation. For the models that emphasize HPA activation as a key mediator of the stress effect on hippocampal function (MYD, Hot/Cool Systems), a key problem is that there was no evidence for HPA activation in Experiment 1, despite observing a behavioural effect of stress. For the Uniform Shift model (which also considers HPA activation to be important), additional problems were the direction of the behavioural effects in Experiments 1 (enhanced allocentric

strategy selection) and 3 (impaired egocentric performance). These effects are opposite the predictions of the model. For the TDM model, which emphasizes SAM activation as a key mediator of the stress effect on hippocampal function, a key problem was that in Experiment 2, stress effects appeared only after a delay, and not immediately after stress, which is inconsistent with the time-course of stress-induced SAM activity. Finally, all four models are unable to account for the sex-dependent effects of stress that appeared in Experiments 2 and 3. These inconsistencies raise several important considerations.

First, each of these models is founded primarily on rodent evidence. It is possible that differences in the anatomy and/or physiology between the rodent and human brain means that the effects of stress and stress hormones on brain function simply do not translate between these species. For example, in their review of the literature, Lupien and Lepage (2001) found evidence that MRs and GRs (thought to play a key role in the mechanism of stress effects on cognition) may have different distributions in the rodent and human brains. This led the authors to advocate for caution when assuming that corticosteroids (cortisol in humans, corticosterone in rats) affect the rodent and human brain in the same way, as each of the models do. However recent human studies have been able to mimic the effects of stress by modulating hippocampal function (declarative memory) via pharmacological manipulation of MRs and GRs (e.g., Lupien et al., 2002; see Het et al., 2005, for a review). Given this uncertainty in the literature, it is clear that models of the effects of stress on the function of the hippocampus (and related structures) may need to be updated as more human research is conducted.

This relates to a second consideration. Most of the evidence for each of the models comes from rodent studies that test the effects of stress or stress hormones using hippocampus-dependent navigation paradigms. In contrast, the human evidence for the models comes almost

entirely from tests of the effects of stress on declarative memory which is also known to be hippocampus dependent (Scoville & Milner, 1957). While it may be reasonable to assume that stress has the same effect on cognitive functions (i.e., spatial navigation and declarative memory) that share the same neuroanatomical substrate (even across species), this assumption may be too simplistic. For example, a recent meta-analysis of the functional MRI literature examined the pattern of brain activation during spatial navigation and declarative memory tasks in humans (Kühn & Gallinat, 2014). They found that spatial navigation tasks activated posterior regions of the hippocampus, and tended to be lateralized to the right side, whereas declarative memory tasks activated anterior regions and were less lateralized. The finding that declarative memory and spatial navigation tasks activate distinct regions of the hippocampus (with little overlap) raises the possibility that stress may also influence the function of these regions differently. Indeed, there is some evidence that MR expression differs significantly in the anterior and posterior regions of the human hippocampus (Medina et al., 2013), hinting at a possible mechanism for differential effects of stress on cognitive functions supported by different hippocampal regions. This suggests that new models may be needed, not only for the effects of stress on the rodent versus the human brain, but also for the effects of stress on cognitive functions that depend on different regions of the hippocampus.

A third consideration relates to sex. The possible role of sex as a modulator of stress effects on the hippocampus (and related structures) is not considered by any of the 4 models. This is likely because the vast majority of rodent studies only use male animals. However, the sex-dependent findings of Experiments 2 and 3 in the present dissertation suggest that the effects of stress on navigation behaviour (and thus the brain structures that underlie navigation) depend on sex. Indeed, many authors in the human stress/cognition literature have pointed out that sex

may be a critical biological factor (e.g., Espin et al., 2013; Gabriel, Hong, Chandra, Lonborg, & Barkley, 2010; Kajantie & Phillips, 2006; Kelly et al., 2008; Kudielka & Kirschbaum, 2005; Wolf et al., 2001). Given the likely importance of sex, the models of the effects of stress on brain function that are described in the present work may need to be updated to incorporate sex as a key factor.

It is worth noting two important caveats to the discussion of how the results of the present dissertation relate to the MYD, Hot/Cool Systems, and Uniform Shift models. First, the most important argument against the MYD, Hot/Cool Systems, and (to a lesser extent) Uniform Shift models is based on the fact that there was no evidence that cortisol mediated the effects of stress in Experiment 1. However, as discussed above (see Limitations), there is some reason to believe that this may have been a Type 2 error (e.g., the timing of the effects in Experiment 2 was consistent with the timecourse of cortisol). If cortisol did mediate the effects of stress on navigation behaviour in the present dissertation, then the evidence in favour of these models would be much stronger. Second, the most important argument against the Uniform Shift model is that, in Experiment 1, stress appeared to increase (rather than decrease) allocentric navigation. However, the measurement of strategy selection in Experiment 1 may have been unreliable. In fact, if we consider the findings of Experiments 2 and 3 alone, the Uniform Shift model fits much better.

Interim Conclusion.

It is difficult to explain the pattern of results from across the 3 experiments in the current dissertation using any of the 4 cognitive-neuropsychological models of the effects of stress on hippocampus-based cognition. Possible reasons for the discrepancy between the predictions of the models and the findings within the dissertation may include: a) there may be relevant

differences in brain morphology between rodents and humans (and the models are based on data from rodent brains), or b) if the models do apply to humans, they may only apply to some hippocampus-related cognitive domains (e.g., declarative memory) and not others; c) the lack of cortisol mediation in Experiment 1 was a Type 2 error and/or a mischaracterization of strategy selection, and d) the lack of cortisol measurement in Experiments 2 and 3 could have shown cortisol effects that would have supported the MYD, Hot/Cool Systems and (to a lesser extent) the Uniform Shift models, at least for males.

Despite the apparent lack of cortisol influence in Experiment 1, there is still some question as to whether HPA-activity (i.e., cortisol) played a role in the stress effects in the three experiments. If future research reveals that cortisol does mediate the stress effect on human navigation, than the MYD, Hot/Cool Systems, and Uniform Shift models may gain more support. Nevertheless, it is difficult to see how the TDM model can be reconciled with the lack of stress effects on immediate navigation, at least as far as it can account for stress effects on human navigation. Beyond this, none of the models deal well with sex differences in the effects of stress on navigation. Taken together, all 4 models need to be updated or revised to account for the effects of stress on human spatial navigation.

Other Considerations

Cortical contributions to the effects of stress on navigation

While the majority of this dissertation has focussed on the effects of stress on subcortical structures, it is important to remember that stress (and its physiological correlates) is also known to act on the cortex, and that the cortex is also a critical structure for spatial navigation (or indeed, any voluntary behaviour). A key site of action for stress appears to be the orbital and medial pre-frontal areas, both of which contain high concentrations of glucocorticoid receptors (Lupien et al.,

2007), and both of which are known to be impaired by high levels of stress (Dias-Ferreira et al., 2009; Liston et al., 2009; Porcelli & Delgado, 2009; van den Bos et al., 2009; Young et al., 1999; Youssef et al., 2012). Research in both rodents (Kolb, Buhrmann, McDonald, and Sutherland, 1994) and humans (Maguire, Burgess, Donnett, Frackowiak, Frith and O'Keefe, 1998; Spiers and Maguire, 2006) has suggested that these same areas are involved in the higher-order, executive components of spatial navigation, such as planning and top-down control of decision making. Kolb et al, (1994) showed that rats with lesions to these areas were significantly impaired on both egocentric and allocentric navigation tasks. The authors attributed these deficits to the rats' loss of the ability to select and execute the correct strategy in the right context, and not to an impairment in learning navigation strategies[10].

Given the impairing influence of stress on these areas, and their known role in spatial navigation, it is worth considering their possible role in the results of the present dissertation, particularly with respect to Experiment 3. The idea that stress, via actions on the prefrontal cortex, impairs the ability to execute the correct strategy in a given context fits well with the finding that stress generally impaired performance in the Place maze. However, it is difficult to explain the sex-based differential effects of stress on performance in Cue maze. For example, it is not clear why stress would enhanced performance for females, and impair it for males, if participants' ability to execute the correct strategy was impaired. Again, this raises the need for more future research into the effects of acute stress on human egocentric navigation, and the neurophysiological mechanism that underlie them.

[10] It is worth noting that these authors did not directly test strategy selection.

Stress, frontal cortex, top-down control, and navigation

It is more difficult to speculate on how stress-induced prefrontal cortex impairment might contribute to the general shift in strategy selection from allocentric to egocentric in the updated Dual Strategy maze. One possibility relates to a possible stress-induced loss of top-down control of the prefrontal cortex over subcortical areas (van den Bos et al., 2009). This idea is attractive because it fits well with the Hot/Cool Systems and Uniform Shift models. In this line of thought, allocentric navigation would be a reflective, "top-down strategy", requiring contributions from the prefrontal cortex as well as the hippocampus, while egocentric navigation would be a reactive, stimulus oriented "bottom-up strategy", mostly dependent on the caudate and amygdala. However, it is difficult to reconcile this notion with the results from the Iowa Gambling Task in Experiment 1. In the Iowa Gambling Task, impulsive choices based on immediate rewards are taken to reflect bottom-up processing, while considered choices based on the feedback from recent decisions is taken to reflect top-down processing (Bechara et al., 2005). As already noted (see Chapter 2, methods), stress had no effect whatsoever on Iowa Gambling Task performance. This suggests that either a) the updated Dual-Strategy maze is a more sensitive measure of shifts between top-down and bottom-up control of behaviour than the Iowa Gambling Task, or b) stress affects some aspects of top-down control (navigation strategy) and not others (decision making strategy), or c) top-down control is not particularly relevant (e.g., perhaps it is required for both navigational strategies) and the shift in strategy selection was simply due to an impairment in hippocampal function. Clearly, more research into the possible role of stress-induced shifts in top-down control can influence spatial navigation behaviour is needed.

Stress, attention and navigation

The effect of stress on attention has been well studied. According to Easterbrook's "tunnel hypothesis" (a.k.a., "cue utilization hypothesis"; Easterbrook, 1959), acute stress or arousal centralizes attentional focus by reducing cue utilization and reducing environmental scan Skosnik, Chatterton, Swisher, and Park, 2000; Booth and Sharma, 2009; see Staal, 2004, for a review). This likely occurs via the actions of stress hormones (e.g., cortisol) on orbital and medial prefrontal cortex (Radley et al., 2006; Petersen and Posner, 2012). It is not difficult to see how this effect of stress on attention might relate to the findings of the present dissertation. In Experiment 1, the design of the original Dual Strategy maze meant that the key egocentric cue was not in view at the beginning of trials, although spatially stable "flanker" cues were in view. According to the tunnel hypothesis, the effect of stress on attention might have led participants to the "allocentric" quadrant because either a) non-target (egocentric) flanker cues or b) allocentric cues were immediately visible and salient at the beginning of the trial. In contrast, finding and going to the target egocentric cue would have required participants to scan left or right. This might explain the apparently contradictory results in Experiments 2 and 3, in which the target egocentric cue was always in view at the start of the trial, and participants under stress tended to navigate more egocentrically.

A "tunneling" of attention under stress might also explain the impaired allocentric performance in Experiments 2 and 3. For example, reduced cue utilization would likely make the construction of a cognitive map (required for allocentric navigation) more difficult in the Place maze.

It is more difficult to explain the effects of stress on egocentric navigation, particularly with respect to sex differences, by its putative effects on attention. In Experiment 3, stress

improved performance for females in the egocentric Cue maze. This would make sense if stress reduced distracting stimuli (Booth and Sharma, 2009) and focused attention on a single salient cue (i.e., the target cue-sphere). However, stress impaired performance for males in the Cue maze. This suggests that, if stress effects on attention played a role in its effects on navigation behaviour, perhaps stress affects males and females differently. This suggests an interesting area for future research, as to my knowledge, there has been no research to date on sex differences in the effects of stress on attention.

Future Research

The present work suggests a number of issues that should be explored in future research. Some of these have already been alluded to (e.g., stress, impulsivity, and navigation; see Limitations). There are several areas of investigation that should take priority, however, in the interests of developing a model that can account for the effects of acute stress on human spatial navigation.

An obvious issue that should be further explored is whether the effects of stress on human spatial navigation (both strategy selection and egocentric and allocentric performance) are mediated by HPA activity (i.e., cortisol). As discussed previously, direct measurement of HPA axis activity in Experiment 1 failed to find any evidence of stress-induced HPA activation (or any relation between the behavioural effects and salivary cortisol). Other existing studies that detected behavioral effects of stress on human navigation were either also unable to show a stress-induced cortisol increase (Duncko et al., 2007), or did not test for cortisol changes at all (Thomas et al., 2010). Thus, as yet, there is little evidence from human research that stress-induced cortisol changes mediate the effects of stress on navigation. Still, there are reasons to believe that HPA-activity may have played a role in the behavioral effects of stress in the present

dissertation (see Limitations). Thus, it would make sense to re-examine the relationship between stress-induced HPA activity and navigation behaviour. A straightforward way to do this would be to simply test for salivary cortisol concentrations in a replication of Experiment 3 (perhaps with a slightly larger *n* to boost power). Settling this question would have important implications with respect to transferability of the MYD, Hot/Cool Systems, and Uniform Shift models from rodents to humans.

Another issue that should be explored in future research is whether stress has similar effects on declarative memory as it does on hippocampus-based spatial navigation. To date, no research has examined this possibility.[11] It is true that some have examined the relationship between the effects of stress on spatial recall and other forms of declarative memory (e.g., Elzinga et al., 2005), but these investigations have not involved navigation. This issue could be examined by extending the design of Experiment 3 to add additional groups that would be tested using a standardized declarative memory task, such as the commonly-used Rey Auditory Verbal Learning Task. Variations of this experiment could explore longer-term recall by testing participants' word and spatial navigation recall in a follow-up session (e.g., 48hr later). This research could provide insight into whether stress affects multiple functions of the hippocampus in a similar way (even those supported by different regions of the hippocampus), and help to improve theoretical models of the effects of stress on the hippocampus.

A third important issue is the impact of sex on the effects of stress on spatial navigation. The findings of the present research suggest that sex may be critical to understanding how acute stress and stress hormones affect brain function. A possible mechanism for these differences may

[11] Note: the FAPA task in Experiment 1 was partly an attempt to do this. However, the FAPA is a new, unstandardized declarative memory task, and the key variable in Experiment 1 was strategy selection (not performance), which is not a pure measure of hippocampal function.

be related to the influence of female gonadal hormones, which fluctuate throughout the menstrual cycle and with hormone-based contraceptive use (see Limitations for additional discussion). Surprisingly, relatively little attention has been paid to this issue. Nearly all of the rodent literature has tested the effects of stress on navigation in male animals only. There are two exceptions (Conrad et al., 2004; Park et al., 2008), but the effects of stress on female performance in these studies were contradictory. In humans, one study has found evidence that menstrual cycle and contraceptive use may be important in the relationship between stress and stress-hormones and hippocampus-dependent declarative memory (Espin et al., 2013), but this has not yet been extended to navigation. One study (Thomas et al., 2010) found sex differences in the effects of stress on navigation, but this study did not control for the effects of menstrual cycle or contraceptive use. Future work could investigate this issue by first grouping participants by sex, and in the case of female participants, by menstrual phase and contraceptive use, and then testing the effect of acute stress on navigation using a similar experimental design as Experiment 3. The findings of this research should reveal information about how gonadal hormones modulate the effects of stress on cognition that depends on the hippocampus and related structures, and inform theoretical models of the mechanism of stress effects on cognition.

There are a number of other cognitive dimensions that may be impacted by stress, but are beyond the scope of the present dissertation. For example, in hierarchical reinforcement learning, some research has shown that stress can shift the balance from "exploratory" (i.e., trying new things to gather more information about reward contingencies) to "exploitative" (i.e., using what has already been learned to acquire rewards) strategies (Luksys et al., 2009; Kaelbling et al., 1996). To date, no work has attempted to see how well these strategies align with navigation strategy, and it might be interesting to do so in future research – perhaps by correlating

performance on a reinforcement learning task with performance in a dual-strategy maze in stressful situations.

Another cognitive dimension that is impacted by stress is declarative memory. Stress is known to have differential effects on different phases of declarative memory. Stress tends to impair declarative memory recall (e.g., Kuhlmann et al., 2005), enhance consolidation (Beckner et al., 2006), and can impair (e.g., Schwabe et al., 2008) or enhance (Smeets et al., 2007) encoding. It is difficult to map declarative memory phase (i.e., encoding, consolidation, and retrieval) onto spatial navigation, because while with declarative memory, each phase can be examined as a discreet process, the same cannot be done for spatial navigation. Simply put, during navigational learning, encoding, consolidation, and recall are all happening at the same time, making it difficult to examine the effects of stress on any given phase in particular. Still, in future research, it might be worth considering applying the same approach to examining stress effects that declarative memory researchers do in a spatial navigation context. This would involve having participants learn to navigate to a navigational goal in one session, then return for another session in the same maze after a significant delay (e.g., 48hrs) to probe their knowledge. One experiment might apply acute stress before navigational learning in the first session (effect on encoding), anther might apply acute stress after navigational learning in the first session (effect on consolidation) and a third might apply stress immediately before navigating in the second session (effect on recall). The findings of this research might reveal information about the overlap between spatial learning and declarative memory.

General Conclusion

The present dissertation has improved our understanding of how acute stress influences the way we navigate space in four ways. First, stress shifts navigational strategy selection from

allocentric to egocentric, and impairs hippocampus-based allocentric navigation. Second, sex is an important modulating factor in the effects of stress on spatial navigation, and should be taken into account in future research. Third, the physiological underpinnings of the effects of stress on spatial navigation may be more difficult to elucidate than those of other cognitive tasks. Fourth, none of the current 4 models, though they might be well suited to explain the effects of stress on other species or cognitive domains, are able to explain the effects of stress on human spatial navigation.

Although the primary impact of the current findings is to highlight the need for future research, the finding that stress changes the way we navigated both qualitatively (i.e., it changes the way we navigate) and quantitatively (i.e., it changes how well we navigate) has important applications. This knowledge will help us understand human cognition and behaviour in many stressful circumstances requiring navigation or critical decision making. These conditions are faced by the military, police, people engaged in extreme sports, rush hour commuters (road rage) and people in crises like those who are lost, in a fire or a disaster, or in a vehicular accident on land, water or air. On an everyday level, it should help us understand navigation and decision making by tourists and others in a strange town, and could help improve the design of signs and roads. The expected findings should be also applicable to any educational circumstance that relies on tests (and fear of failure) to motivate students. Finally, the results should help us understand cognition and behaviour of those who have complete or partial loss of hippocampal function from stroke, incipient Alzheimer's, traumatic brain injury, and even normal aging. The findings of the present research may also benefit fields such as civil engineering and architecture. For example, it might inform the design of large-scale structures (e.g., airports) and infrastructure (e.g., highway systems) that people often navigate under stress. In addition, the

finding that men and women navigate differently under stress suggests that both male and female perspectives need to be involved in the design of our environment.

In conclusion, the present dissertation represents an important contribution to the study of the effects of stress on human spatial navigation and on cognition in general, if only to point to the desperate need for more research in this area. The discoveries made question current theoretical accounts of the effects of stress on cognition and highlight the need to improve methodological approaches to testing them.

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