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ABSTRACT

SHIFTING THE STRESS CURVE: USING “STRESS INOCULATION”
AND EXERCISE TO PROMOTE RESILIENCE

by

Mikyung Kim

Chair: Pamela S. Coburn-Litvak

ABSTRACT OF GRAUATE STUDENT RESEARCH

Thesis

Andrews University

College of Arts and Sciences

Title: SHIFTING THE STRESS CURVE: USING “STRESS INOCULATION” AND EXERCISE TO PROMOTE RESILIENCE

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Name and degree of faculty chair: Pamela S. Coburn-Litvak, Ph.D.

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Problem

Stress influences an organism’s physiological systems via an inverted u-shaped curve: An optimum amount of stress will optimize body functions, but too little stress or too much stress for long periods of time can impair body functions. Researchers have been very interested in exploring the mechanisms that may “delay the tipping point” between the positive and negative effects of stress. A rightward shift in the stress curve would allow one to maintain optimal performance even at higher or more prolonged stress levels. The molecular and cellular mechanisms that underlie this rightward shift could result in resilience, clinically defined as the ability to endure stress without sustaining damage, or even to benefit from experiencing stress. The experiments described in this thesis investigate two potential mechanisms of resilience. The first

mechanism is “stress inoculation,” in which previous exposure to a stressor “inoculates” an organism to respond more effectively to subsequent stressors. Recent studies suggest that controllable stress, even if the organism cannot predict when the stress will occur (thus called “unpredictable/controllable stress” or UST), may cause a rightward shift in the stress curve. The second mechanism is physical fitness that may improve the ability to cope with stress through molecular and cellular changes in the body.

Method

Experiment #1 (Stress inoculation): Thirty-three male Sprague-Dawley rats were in housing platforms for 21 days; 15 rats were exposed to unpredictable/controllable stress (UST), and 18 rats were not exposed to stressful stimuli as a control group. After 21 days, spatial memory and strategies were assessed on the Barnes maze under high stress conditions.

Experiment #2 (Physical Fitness): A pilot study was conducted on 22 subjects (12 females, 10 males). Thirty human subjects (15 females, 15 males) were recruited among the freshmen taking HLED 120, Fit for Life, at Andrews University. Physical fitness was assessed with the MicroFit[®] FAS-2 system, a FDA-registered medical device. The students’ chronic stress levels were assessed with ICSRLE (Inventory of College Student Recent Life Experiences), and their depression and anxiety levels with DASS 21 (Depression Anxiety Stress Scale). Cognitive performance was assessed with two memory tasks: an object location task thought to be dependent on the hippocampus, and an n-back test thought to be dependent on the prefrontal cortex. Finally, the physiological stress response to the acute, cognitive stressors (performing the n-back test) was assessed by changes in salivary cortisol, heart rate, and systolic/diastolic blood pressure.

Results

Experiment #1: UST rats took less time to find the goal box on the Barnes maze ($p<0.05$), and made fewer errors ($p<0.05$) and repeat errors ($p<0.01$). UST rats also took less time to find the goal box on reference memory trials ($p<0.05$) and on working memory trials ($p=0.05$). After a new goal position was introduced, UST rats visited the previous goal position as their first error at a rate of 46.67%, while CT rats visited the previous goal position at a rate of 27.78%. UST used spatial strategies more frequently ($p<0.01$) to find the goal box, while CT rats used random strategies more frequently ($p<0.01$).

Experiment #2 (Effects of physical fitness, stress, and depression and anxiety on memory): While higher fitness levels tended to be associated with better hippocampal memory scores ($p=0.15$, $d=0.7$), it did not affect prefrontal cortex-dependent memory (“n-back different”: $p=0.286$; “n-back same”: $p=0.411$). A significant, positive correlation was seen between ICSRLE and DASS 21 ($p<0.01$). Higher levels of self-reported stress were not associated with worse hippocampal memory ($p=0.389$), but subjects with higher self-reports of depression/anxiety tended to have better hippocampal memory scores ($p=0.075$, $d=0.8$). Subjects with lower self-reported stress levels got higher “n-back different” scores than those with higher self-reported stress levels ($p<0.05$), but no significant difference was found on “n-back same” memory scores between those two groups ($p=0.898$). Subjects with lower self-reports of depression/anxiety tended to get higher “n-back different” scores than those with higher self-reports of depression/anxiety ($p=0.066$, $d=0.87$). No significant difference was found between the “Top 50%” and “Bottom 50%” DASS 21 groups for “n-back same” memory scores ($p=0.661$).

Conclusion

Experiment #1 (Stress inoculation as an active resilience mechanism): The results of this study are consistent with the “stress inoculation hypothesis.” Exposure to unpredictable / controllable stress for 21 days causes “stress inoculation,” causing neural and behavioral adaptations that may represent a rightward shift of the stress curve. This would explain the optimal performance of UST with new environmental stressors on the Barnes maze.

Experiment #2 (Physical activity as an active resilience mechanism): The results of this pilot study partially support the original hypotheses, but they indicate directions for future studies. First, more subjects should be added (at least 54) to validate the current statistical results. Second, more rigorous spatial memory tasks may be needed in order to avoid “ceiling effects.”

Andrews University
College of Arts and Sciences

SHIFTING THE STRESS CURVE: USING “STRESS INOCULATION”
AND EXERCISE TO PROMOTE RESILIENCE

A Thesis
Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Mikyung Kim

2015

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APPROVAL BY THE COMMITTEE:

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A handwritten signature in black ink, appearing to be 'Brenda Anderson', with the date '3/10/15' written next to it.

Brenda Anderson, Ph.D.

Date approved

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CHAPTER 1

BACKGROUND

What Is Stress?

The term “stress” was first coined by Hans Selye in 1936 as “the non-specific response of the body to any demand for change” (Selye, 1936, p. 32). Dr. Selye also coined the term “stressor,” which is anything that causes stress on an organism (Selye, 1976). Today, many researchers agree that stress occurs when the homeostasis of an organism is threatened or at least is perceived to be so (Chrousos, 2009). When a human is stressed, the body shifts its energy resources to fight off the perceived threat. This is called the “fight or flight” response. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis to release stress hormones such as catecholamines (e.g., epinephrine) and glucocorticoids (e.g., cortisol in humans, corticosterone in rodents). When elevated for a short time these stress hormones help us deal with stress by increasing heart rate, raising blood pressure, boosting glucose levels in the blood stream, and suppressing non-emergency functions such as the digestive process. However, exposure to long-term stress can cause numerous emotional and physical disorders.

The Stress Response Is described With an Inverted U-Shaped Function

The relationship between stress and cognitive performance can be portrayed as an inverted u-shaped curve (Figure 1), first described by Robert Yerkes and John Dodson in 1908 (Yerkes & Dodson, 1908). In a rodent model for a learning task, Yerkes and

Dodson reported that the highest level of performance was found when the animal was under optimal stress, but was impaired under conditions below or above optimal levels. Many animal studies have confirmed this finding over the last several decades (Broadbent, 1965; Broadhurst, 1957; Mendl, 1999; Park et al., 2006; Sandi & Pinelo-Nava, 2007). Humans also exhibit an inverted u-shaped relationship between job-related stress and work productivity (Wilke, Gmelch, & Lovrich Jr., 1985).

How Stress Affects Brain Regions Involved in Memory

The Hippocampus Is Involved in Reference Memory and Spatial Navigation

The hippocampus is an area of the medial temporal lobe. It is critical when short-term working memory must be consolidated into long-term (also called reference) memory (Squire & Schacter, 2002; Yoon, Okada, Jung, & Kim, 2008). The hippocampus is also important for spatial memory (Squire & Cave, 1991), which is the ability to remember the relevance of spaces.

The Prefrontal Cortex and Hippocampus Are Involved in Working Memory

The prefrontal cortex (PFC) is located in the frontal lobe. It is involved in the highest-order cognitive functions, called “executive function.” Executive function includes mental flexibility, planning, execution of plans (Chan, Shum, Toulopoulou, & Chen, 2008), and problem solving (Monsell, 2003). The PFC is also involved in working memory, which is the temporary storage and use of information that lasts a few seconds. This memory function is considered an executive function because it requires monitoring

multiple sources of information and controlling subsequent behavioral responses (Monsell, 2003).

Spatial working memory is the ability to remember spatial information for a short period of time (Van Asselen et al., 2006). Studies in animals and humans indicate that both the hippocampus and the PFC are important in spatial working memory (Kesner & Churchwell, 2011; Van Asselen et al., 2006).

The Effects of Stress on Spatial Working and Reference Memory

A number of studies have examined the effects of stress on working memory, but with mixed results. Some animal studies reported impaired spatial working memory after exposure to chronic stress (Graybeal, Kiselycznyk, & Holmes, 2012). Collaborators in the research behind this thesis at Stony Brook University (Stony Brook, NY) reported that 3 weeks of chronic stress resulted in impaired spatial working memory when animals are tested under low stress conditions (D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). Similarly, human studies have reported stress-related impairments in spatial working memory (Liston, McEwen, & Casey, 2009; Qin, Hermans, van Marle, Luo, & Fernández, 2009). However, other studies have reported that stress does not affect or even facilitates working memory (Barha, Pawluski, & Galea, 2007; Yuen et al., 2009; Yuen et al., 2011).

The prefrontal cortex is rich in receptors for glucocorticoids, and chronic stress has been shown to alter structure and neuronal morphology in this brain area. Three weeks of chronic restraint stress reduces dendrite length, branching, and spine density in PFC neurons (Arnsten, 2009; S. M. Brown, Henning, & Wellman, 2005).

Similar to spatial working memory, studies have reported mixed results regarding the effects of stress on hippocampal-dependent function and spatial reference memory (Conrad, 2010). Some studies reported that chronic stress impaired spatial reference memory (reviewed in Conrad, 2010). Like the PFC, the hippocampus has a lot of glucocorticoid receptors and therefore is a primary target of glucocorticoids (McEwen, De Kloet, & Rostene, 1986). Chronic stress can alter the neurochemistry, neurogenesis, and neuronal morphology of the hippocampus (Bremner, 2006; Conrad, 2006, 2008). Several studies reported that 3 weeks of chronic stress or glucocorticoid exposure decrease dendritic length and branching of hippocampal neurons (McEwen et al., 1995; McEwen & Magarinos, 2001; McKittrick et al., 2000), although some also reported an increase in spine density (Rao & Raju, 1995). These animal studies are consistent with human studies that have reported reduced hippocampal volume and deficits in hippocampus-dependent memory tasks with chronic stress compared to normal-cortisol controls (Lupien et al., 1998). However, it should be noted that some studies reported no effect (Conrad, 2008, 2010; Luine, Martinez, Villegas, María Magariños, & McEwen, 1996; Luine, Villegas, Martinez, & McEwen, 1994; Williams, Baker, Gress, & Givens, 1998) or facilitation of stress on spatial reference memory (Gouirand & Matuszewich, 2005).

Researchers have offered various reasons for the discrepancies among these studies, such as differences in type and duration of stressors, and the interval between stress and behavioral testing. In this study, I focus on another possible reason to explain the discrepancies: a shifting of the stress curve that may promote resilience to stress (Figure 2).

Shifting the Stress Curve May Promote Resilience

Some studies reported that the inverted u-shaped stress curve can shift under certain conditions (Mendl, 1999; Russo, Murrough, Han, Charney, & Nestler, 2012). A rightward shift of the stress curve would move the peak of the curve further to the right, where the downside of the curve was originally located. This rightward shift allows the maintenance of optimal performance at higher levels of stress. This phenomenon is called *stress resilience*. “Resilience” is the ability to avoid deleterious changes in response to chronic or increased stress (Russo et al., 2012). According to Russo et al. (2012) two types of stress resilience are possible: *passive resilience* and *active resilience*. Passive resilience is the absence of molecular and neural processes that impair organisms coping effectively with stress. Active resilience is the presence of novel molecular and neural mechanisms that help organisms’ coping ability.

The two experiments in this thesis investigated two potential mechanisms of active resilience, because active resilience mechanisms have the potential to even counteract maladaptive changes. The first mechanism is *stress inoculation*, in which an exposure to earlier stressors helps an organism build resources to help it respond more effectively to subsequent stress (Russo et al., 2012). This potential mechanism was explored in Experiment #1. The second mechanism is exercise. Ironically, exercise itself is a physical form of stress. However, regular participation in exercise (which would presumably result in a higher level of physical fitness) also may cause molecular and cellular changes in the body that improve an organism’s ability to cope with stress. This potential mechanism was explored in Experiment #2.

Active Resilience Mechanism #1: Stress Inoculation

Exposure to Stress May Promote Resilience: Studies in Humans and Animals

Chronic stress is usually associated with increased susceptibility to mood and anxiety disorders (McEwen, 2004; Simon et al., 2006). However, although more than half of the general population experiences at least one traumatizing event in their lifetime, the prevalence of severe stress disorders, such as posttraumatic stress disorder (PTSD), is less than 10% (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). This indicates that stress resilience is a common occurrence.

“Stress inoculation” was first described by Levine and colleagues, who showed that infant rats exposed to mild, chronic stress early in life respond more effectively to a novel stressor than do their non-stressed counterparts (Levine, Chevalier, & Korchin, 1956). Since this initial finding, several more animal studies have reported that early exposure to stress results in better performance in subsequent tasks that were also stressful, but performed worse on less stressful tasks (Buwalda, Stubbendorff, Zickert, & Koolhaas, 2013; Champagne et al., 2008; Frankenhuis & Del Giudice, 2012; Laban, Markovic, Dimitrijevic, & Jankovic, 1995; Oitzl, Champagne, van der Veen, & De Kloet, 2010; Oomen et al., 2010). Although most of these studies focused on stress exposure during early life, some other studies have reported positive effects of stress inoculation during later stages in life, such as adolescence and early adulthood (Nederhof & Schmidt, 2012; Russo et al., 2012). This is good news because it means that stress inoculation potentially can be used to increase stress resilience throughout the life span.

Preliminary Data From an Animal Model of Psychological Stress

Collaborators in the research behind this thesis have developed a new animal model of psychological stress (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015). This animal model is unique in its ability to independently manipulate two important features of psychological stress: *predictability* and *control*.

Predictability occurs when organisms know when and under what situations stressors will occur (Miller, 1981). High predictability gives organisms more time to assess the potential risk of a stressor and to prepare a defense. Predictability can alter both the behavioral and physiological effects of stress: Animals exposed to predictable chronic stress showed fewer depressive and anxiety-related behaviors, had increased neurogenesis and dendrite growth in the hippocampus, and exhibited better spatial reference memory (Parihar, Hattiangady, Kuruba, Shuai, & Shetty, 2011).

Control is the ability to avoid an aversive stimulus (Averill, 1973; Folkman, 1984). Both human and animal subjects who believe they are in control of a stressor (even if the control is only an illusion) perform better on cognitive tasks compared to those who have little or no control (Glass, Reim, & Singer, 1971; Minor, Jackson, & Maier, 1984). A recent animal study reported that the PFC suppressed the stress response when the animal perceived itself to be in control (Amat, Paul, Zarza, Watkins, & Maier, 2006). The PFC and the hippocampus are connected to each other, therefore both the PFC and the hippocampus can suppress the HPA axis (Diorio, Viau, & Meaney, 1993). Animals exposed to controllable stressors tend to develop resilience, whereas control animals exposed to uncontrollable stressors tend to display a persistent state of anxiety

and learned helplessness (Graybeal et al., 2012; Maier & Watkins, 2010; Russo et al., 2012).

In the previous studies by collaborators of this thesis, rats exposed to unpredictable/controllable stress (UST) did not develop symptoms of depression or anxiety. UST rats also spent more time actively coping with novel stressors than control groups (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015). However, when the UST groups were tested in the Barnes maze under low stress conditions, they showed impaired spatial working memory (D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). This suggests that unpredictable/controllable stress develops resilience, but may do so at a cost to working memory.

Jacobson and Anderson (2013) suggested two possible hypotheses to explain these results: the *variable arbitration hypothesis (H1)* and the *stress inoculation hypothesis (H2)* (Jacobson & Anderson, 2013). The *variable arbitration hypothesis* suggests that unpredictable/controllable stress causes a neural shift in the control of behavior (Figure 3), away from the methodical and reflective control of the PFC, toward more rapid, reflexive actions associated with a subcortical structure: the caudate nucleus (Schwabe, Dalm, Schächinger, & Oitzl, 2008). Like a neuropsychological “reflex arc,” a stressful stimulus causes the caudate nucleus to respond directly (without input from higher cortical structures) to initiate the behavioral response. This is called a “stimulus response” mechanism. The caudate nucleus learns over time to respond to similar stressful stimuli in similar ways, and therefore builds a collection of “habit” memory responses. The advantage of habit memory is that it is a more rapid and efficient response (Schwabe et al., 2007; Seger & Cincotta, 2005). However, because habit memory does

not depend on explicit cognitive reflection in the cerebral cortex, it is a more rigid type of memory. Thus it poses the risk of missing important information about the current stressor. Consistent with this hypothesis, humans have reduced prefrontal activity during periods of acute stress (Arnsten, 2009), and both humans and animals have been reported to switch from spatial strategies to stimulus response strategies while under stress (Schwabe et al., 2008; Schwabe et al., 2007). If organisms need rapid responses in high threat conditions, transfer of control from higher to lower brain areas can be adaptive, but if the stressor is not imminent, it may be maladaptive. According to the variable arbitration hypothesis, the UST rats in the previous study (D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015) developed rapid, habit-based strategies to cope with their environmental stressors, but the habit-based strategies were not helpful for them in a spatial learning task when tested under low stress conditions.

Alternatively, the *stress inoculation hypothesis (H2)* would suggest that exposure to unpredictable/controllable stress causes “stress inoculation” in the UST rats causing neural and behavioral adaptations that facilitated coping with new stressors. According to this hypothesis, these adaptations may represent a rightward shift of the stress curve, and therefore optimal performance can be maintained with new stress. This could explain the UST rats’ facilitated coping with new environmental stressors (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015). This could also explain why the UST rats showed impaired spatial memory when tested under low or no stress conditions (D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). At low stress levels, the UST rats would be on the downward slope of the curve, while the control rats would be at the peak (Figure 4).

Goal and Hypotheses of Experiment #1

The goal of Experiment #1 was to determine which of the two hypotheses explains the observed increase in resilience but impaired spatial working memory following unpredictable/controllable stress (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015; D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). To do this, rats were exposed to the same type and duration of unpredictable/controllable stress used in previous studies. After 3 weeks, spatial memory was assessed in UST and control (CT) rats in the Barnes maze (Barnes, 1979), but this time the testing was performed under high stress conditions. The two hypotheses predicted opposite outcomes to spatial memory performance under higher stress conditions.

If the variable *adaptation hypothesis (H1)* is correct, the UST rats would show impaired memory compared to control rats, regardless of the stress levels during the memory testing. This is because chronic stress would shift learning strategies toward the more rigid, caudate-based “stimulus response” learning. The lack of methodical deliberation that is inherent in stimulus-response learning would make the stressed animals insensitive to changes in the aversive environment that should prompt a change in behavior.

Alternatively, if the *stress inoculation hypothesis (H2)* is correct, the UST rats would exhibit better spatial memory than controls under high stress conditions because of a rightward shift of the inverted u-shaped stress curve (Figure 5). Control rats would be relatively impaired compared to the UST rats under high stress conditions, because they would be on the downward, maladaptive side of their stress curve (Figure 5). In addition to spatial memory, the strategies used by the animals on the Barnes maze were also

analyzed. The use of stimulus-response-based strategies would support hypothesis H1, while the use of spatial strategies would support hypothesis H2.

Active Resilience Mechanism #2: Exercise and Physical Fitness

Stress in College-aged Adults

College students, especially freshmen, face several unique stressors because of their new college life. They are separated from home for the first time (Ross, Niebling, & Heckert, 1999), are adapting to a new scholarly environment and teaching methods, and are facing mounting financial responsibilities (Décamps, Boujut, & Brisset, 2011). The American Psychological Association (APA) recently conducted a “Stress in America” survey on 2,020 adults (American Psychological Association, 2012). When measured on a 10-point scale, the average, self-reported stress level of young adults ages 18-33 was 5.4 compared to the national average of 4.9. Young adults thus reported the highest stress levels in the nation. This age group reported that they are facing life challenges about their future including work, financial situation, relationships, and family responsibilities. This age group also felt that they are less likely to be successful in reaching their stress management goals.

Stress and Memory in College Students

Due to ethical constraints, few studies have investigated the effects of chronic stress in humans. However, a cross-sectional study on college students indicates that greater life event stress is associated with greater working memory impairment (Klein & Boals, 2001). Consistent with these results, pharmacological studies show that elevated glucocorticoid levels impair the memory processes of the PFC and hippocampus

(McAllister-Williams & Rugg, 2002; Wolf, 2008; Young, Sahakian, Robbins, & Cowen, 1999). College students exposed to psychosocial stress in the laboratory show impaired working memory on the n-back test compared to controls (Schoofs, Preuß, & Wolf, 2008). Chronic stress often causes reductions in brain volume and changes in cognition (McEwen, 2005).

How Exercise Affects Brain Regions Involved in Memory

According to the “Stress in America” survey (American Psychological Association, 2012), young adults aged 18-33 listen to music, play video games or surf the internet, and eat to cope with their stress. However, 51% use exercise to cope with stress. Exercise can be an effective stress-coping strategy for college students. For example, 60% of 275 Puerto Rican college students reported that physical activity was an effective coping strategy and 66% would use it again (Cruz et al., 2013).

Physical activity (commonly called “exercise”) is defined as “any bodily movement produced by skeletal muscles that requires energy expenditure” (World Health Organization, 2014). Physical fitness is defined as “a set of health and skill-related attributes associated with one’s ability to perform physical activities and includes muscular strength, muscle flexibility, and body composition” (Buckworth & Dishman, 2002). Many studies have reported a positive benefit of physical fitness and/or physical activity on cognition and mental health across the lifespan (Voss, Nagamatsu, Liu-Ambrose, & Kramer, 2011).

Physical Fitness Affects Learning

Relatively few studies have examined the effects of fitness or physical activity on cognition in young adults. Maybe this is because young adults are thought to be at their peak cognitive performance (Voss et al., 2011), with little room for fitness-related improvement. Studies in this area have been mixed. Some studies have failed to report a positive relationship between physical activity and cognition in young adulthood (Scisco, Leynes, & Kang, 2008) but others have (Åberg et al., 2009; Shay & Roth, 1992). For example, 6 weeks of aerobic exercise can improve the hippocampal-based memory at this age compared to controls (Stroth, Hille, Spitzer, & Reinhardt, 2009). Some studies have also reported a positive relationship between physical fitness and improved executive function in this age group (Themanson & Hillman, 2006).

More studies have been done on other age groups, such as children and the elderly. A meta-analysis from 44 studies reported a positive relationship between physical activity and academic performance on standardized tests (effect sizes on math: 0.20, verbal: 0.17, academic readiness: 0.39) in school-age children (Sibley & Etnier, 2003). Another meta-analysis that aggregated results across 18 intervention studies reported that fit, older adults outperformed unfit older counterparts in every category of cognitive function, including visuospatial processing and executive control tasks (Colcombe & Kramer, 2003).

Physical Fitness Affects Brain Regions Involved in Memory

Very few studies have examined fitness and neuroimaging data in young adults, but more studies have done this in children or the elderly.

A structural imaging study by Chaddock and colleagues (2010) reported that higher-fit children show greater bilateral hippocampal volumes than do lower-fit children, and the hippocampal volume differences were associated with better memory (Chaddock et al., 2010). Similarly, higher-fit children showed greater activation in the prefrontal cortex and parietal cortex than lower-fit children (Chaddock et al., 2012).

The hippocampus shrinks in late adulthood, which is thought to contribute to aging-related memory impairment (Raz et al., 2005). However, Erickson and colleagues (2011) reported that 1 year of exercise training increased hippocampal volume by 2%, effectively reversing age-related loss in volume. This change in hippocampal volume was associated with an increase in serum levels of Brain-Derived Neurotrophic Factor (BDNF) and improved spatial memory function (Erickson et al., 2011). Physical fitness prevents age-related loss of PFC and hippocampal volume, and cognitive impairment (Gordon et al., 2008; Weinstein et al., 2012).

Goal and Hypotheses of Experiment #2: Physical Fitness and Memory in College Students

Even though exercise is considered to be physically stressful, the studies reviewed above suggest that it may still facilitate cognition function. Since participations in exercise have been shown to counteract the deleterious effects of aging on cognition function (Erickson et al., 2011), it also may be helpful in counteracting the deleterious effects of stress on brain structure and function. Thus, exercise possibly can be used as an effective mechanism to develop stress resilience.

Due to the small sample size in the Experiment #2 pilot study, it was not possible to perform statistical analysis to assess the effects of physical fitness in counteracting the effects of stress. Rather, the goals of Experiment #2 were to independently investigate the

effects of physical fitness, self-reported stress, and self-reported depression/anxiety on cognitive function in a group of young adults ages 18-25. More subjects will be added to this pilot study in the future in order to perform other statistical analyses.

Experiment #2 was performed on a group of college freshmen taking HLED 120, Fit for Life, at Andrews University during the spring 2014 semester. The students' fitness levels were measured using MicroFit[®], an FDA-approved medical device. The students performed two memory tasks: a spatial working memory task thought to be dependent on the prefrontal cortex, and a spatial reference memory task thought to be dependent on the hippocampus. It was hypothesized that higher physical fitness would be associated with better memory performance on both of these tasks. The students' chronic stress levels were assessed using Kohn Hassles Scale (Inventory of College Student Recent Life Experiences, ICSRLE), and their depression and anxiety levels were assessed using the Depression Anxiety Stress Scale (DASS 21).

It was hypothesized that higher levels of self-reported stress, depression and anxiety would be associated with impaired memory performance on both hippocampal- and PFC-dependent memory tasks. The students' acute stress response was assessed by measuring heart rate as well as salivary cortisol response to the intrinsic stress of performing the PFC memory task. Similar to what has been previously reported in trained sportsmen (Rimmele et al., 2007), it was hypothesized that higher physical fitness levels would be associated with lower heart rate and cortisol responses following an acute, cognitive stressor. On the other hand, it was hypothesized that higher self-reports of stress, depression, and anxiety would be associated with a decreased cortisol response, and an increased cardiovascular response.

All hypotheses for Experiment #2 are summarized in Table 5. This table also summarizes the results of this pilot study, which will be further described in chapters 5 (result 2) and 7 (discussion 2) of this thesis.

CHAPTER 2

METHODS 1

Experiment #1: Testing for Stress Inoculation in a New Animal Model of Unpredictable/Controllable Stress

Subjects and Description of Housing Condition

Thirty-three male Sprague-Dawley rats (Charles River, Wilmington, MA) were housed in an animal colony at constant temperature and humidity on the campus of Stony Brook University, with food and water provided ad libitum. Because rodents are nocturnal, the housing rooms were kept on a reverse light/dark cycle (lights off at 10:00 AM and on at 10:00 PM) in order to conduct behavioral testing during the animals' active period. The rats were tested in three cohorts over a 6-month period, with approximately 10-12 rats per cohort and 4-8 rats per group.

The housing condition and stress manipulation were identical to those previously used (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015; D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). Each rat was housed individually in a platform (Figure 6) made of two standard tub cages connected by a 3-foot tunnel. One cage had food and the other cage had water, therefore the rats had to traverse the tunnel in order to access both. Infrared LED detectors, located at both ends of the tunnel, were connected to a computer to record the subjects' traversals and to control presentation of stress stimuli. A break in the LED light at one end and then the other one was recorded as a tunnel

traversal when a rat poked its head out and passed through the tunnel. Rats in both the unpredictable/controllable stress (UST) and control (CT) groups were allowed to habituate to the housing condition for 3 days prior to starting the experiment. Then for 21 days, the UST rats ($n=15$) were exposed to stressful stimuli while traversing the tunnel, presented simultaneously: flash of light, an abrupt sound, and a puff of air containing ferret dander odor. Ferret odor was obtained by placing a cloth in a ferret cage for at least 2 weeks. Then the cloth was transferred to a small bottle (500 mL plastic bottle) and air from the bottle was discharged into the tunnel with a pressurized air pump. Ferrets are a natural predator of rodents, and previous studies have reported that rats exposed to ferret odor (fur/skin) showed elevated HPA axis activation and higher plasma corticosterone and adrenocorticotropin hormone levels than did control rats (Masini, Sauer, & Campeau, 2005; Masini, Sauer, White, Day, & Campeau, 2006). The stressful stimuli were unpredictable because they were presented randomly and on average only once out of every four tunnel traversals. However, the stimuli were highly controllable because the rats always reached the other side safely (the stress condition never causes physical harm to the rats), and they always successfully obtained food and water. UST rats were housed in this treatment condition for 21 days based on previous reports of impaired spatial memory (Conrad, 2010) and altered hippocampal morphology (Conrad, 2006) following this duration.

Control rats (CT; $n=18$) were housed in the same type of platforms for 21 days, but were not exposed to stressful stimuli. UST and CT rats were housed in separate rooms to avoid any stress to CT rats. After 21 days, the rats were removed from the platforms and housed individually in new, standard tub cages in a new room.

Spatial Memory Testing in the Barnes Maze

The Barnes maze apparatus and procedures were the same as those previously used (Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson, 2003; D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015), except that testing was done under high stress conditions, described below. The Barnes Maze (Figure 7) is a white, circular platform (1.22 m in diameter), with 12 evenly spaced holes (10 cm in diameter) around the perimeter. A black, plexiglass escape chamber (hereafter called the “goal box”) was placed under one of the holes, and five more identical boxes without bottoms were placed under random holes to prevent visual discrimination of the goal box position. Constant visuo-spatial cues (e.g., a file cabinet, storage cupboard, poster) were located around the maze to act as extramaze cues.

Rats were habituated to the maze in a series of three trials on the day after the stress manipulation ended. For the first trial, each rat was placed in the goal box under one of the holes for 2 minutes, and then returned to its home cage. For the second trial, the rat was placed next to the hole above the goal box and encouraged to enter. It was allowed to stay in the goal box for 2 minutes and then returned to the home cage. For the third trial, it was placed in the center of the maze in a four-walled alleyway leading to the hole with the goal box. Once it found and entered the goal box, it was allowed to stay there for 2 minutes, and then returned to the home cage. The rats were placed in a dark, adjacent room between trials, and the maze and the goal box were cleaned with a 30% ethanol solution between rats to prevent the next rat from using any possible odor cues.

Over the next 4 days, spatial memory testing was conducted under high stress conditions: bright light, loud noise (forest sound and fan sound), and wind from a fan

above the center of the maze. Each rat was tested four trials per day with a 15-20 minute inter-trial interval, for a total of 16 trials. The position of the goal box was chosen randomly, but was different from the habituation day. Each rat had the same goal box position on the 1st and 2nd days (trials 1-8) and a new goal box position on the 3rd and 4th days (trials 9-16). At the start of each trial, the rat was placed inside a start box in the center of the maze. This box gives the animal a random orientation relative to the top of the maze and the goal box. The start box was lifted after 30 seconds and the rat was allowed to explore the maze and seek the goal box, to escape from the high stress conditions. If the rat did not find and enter the goal box after 3 minutes, it was gently picked up by one of the experimenters and placed near the hole with the goal box. Once the rat entered the goal box, it was allowed to remain there for 2 minutes, and then returned to its home cage in a dark adjacent room. Like habituation, the surface of the maze and the interior of the goal box were cleaned with a 30% ethanol solution between rats. Additionally, the maze was randomly rotated to prevent rats from using an odor “trail” to find the goal box.

Two or three experimenters, blind to the treatment condition, recorded the observed behavior of the animals on data record sheets. Video recordings were also taken of all trials for two of the three cohorts to facilitate data collection and analysis.

Spatial memory measurements included “latency,” “errors,” and “repeat errors.” “Latency” was the time in seconds to find the hole with the goal box. “Errors” were the number of holes visited before the goal box for the first time, and “repeat errors” were the number of holes re-visited before approaching the goal box. The patterns of errors and repeat errors on the record sheets were used to analyze possible navigation strategies.

Three navigation strategies were possible: random, serial, and spatial. Rats using the random strategy exhibited no apparent pattern in visiting holes, but rather moved randomly on the maze and crossed the center multiple times (Figure 8). Rats using the serial strategy visited holes in consecutive order around the periphery of the maze until they found the goal box (Figure 8). Rats using the spatial strategy visited a cluster of holes near the correct goal box position (Figure 8). The first two navigation strategies were considered a stimulus-response strategy, controlled by the caudate nucleus, while the last strategy was considered a spatial navigation strategy controlled by the hippocampus and prefrontal cortex.

Statistical Analysis

Spatial memory data were analyzed with SPSS statistics software (V.21.0.0, IBM Corporation, New York, NY). Barnes maze latency, errors and repeat errors to goal box were analyzed with a repeated measures ANOVA with group as an independent factor and trials as the within subjects factor. Errors and latency on individual trials were analyzed with a one-way ANOVA. On the first testing day, a new goal box position different from habituation was introduced. Therefore, trial 1 was analyzed separately since it would not have been an accurate measure of spatial memory. Spatial working memory is thought to be dependent on both the prefrontal cortex and the hippocampus, while spatial reference memory is thought to be dependent on the hippocampus only (Kesner & Churchwell, 2011; Squire & Cave, 1991; Van Asselen et al., 2006). Therefore, trials 2-4, 6-8, 10-12, and 14-16 were analyzed as working memory separately from reference memory trials 5, 9, and 13.

An alpha level of 0.05 was used. When the assumption of sphericity was violated, the degrees of freedom were adjusted by the Greenhouse-Geisser estimate of epsilon. Sphericity is the condition in which the variances of the differences between all possible pairs of related groups are equal (Lund & Lund, 2013). In the results section, the epsilon (ϵ) value was reported when the assumption of sphericity was violated, but the unadjusted degrees of freedom were reported for simplicity.

CHAPTER 3

METHODS 2

Experiment #2: Testing the Effects of Exercise and Physical Fitness on Spatial Memory in College Freshmen

Subject Recruitment

Thirty subjects (15 females, 15 males) were recruited among the freshmen taking HLED 120, Fit for Life, at Andrews University during the spring 2014 semester. With the prior approval of the HLED120 instructors, the study investigators and research assistants took 3-5 minutes of class time to announce the research purpose and procedure, and to distribute consent forms to interested students. The consent forms were then collected, and interested students were later contacted by email. The consent form gave the investigators approval to use the students' physical fitness data for this research project only.

From the 30 subjects, six subjects were excluded because their physical fitness data were missing or incomplete. One more subject was excluded because his age (45 years old) was out of the correct age range (18-25). Therefore, data from a total of 22 subjects (12 females, 10 males) were analyzed for this pilot study. For the n-back test, only 20 subjects (10 males, 10 females) were analyzed because two subjects didn't understand the directions.

Assessment of Physical Fitness

Physical fitness was assessed with the MicroFit[®] FAS-2 system, a FDA-registered medical device that provides standardized measurements of body weight, body fat, blood pressure, heart rate, arm strength, back flexibility and cardiovascular fitness. MicroFit[®] has been used in research by other Andrews University faculty (Pribis, Burtnack, McKenzie, & Thayer, 2010), and it follows physical fitness guidelines established by the American College of Sports Medicine (ACSM, 2013).

The MicroFit[®] data for each subject were collected by trained technicians in the department of Public Health and Wellness. Figure 9 shows a sample fitness profile for a research subject. Each fitness measurement, including a total fitness score, aerobic fitness score, systolic blood pressure, diastolic blood pressure, resting heart rate and percentage of body fat, was scored on a separate scale that was normalized for the subjects' age and sex. Based on that scale, each fitness measurement was classified into one of four categories (in order from lowest to highest): "Needs Work," "Fair," "Fit," and "Excellent."

Means for each fitness variable are summarized in Table 1. Independent samples *t*-tests were used to determine if there were sex-related differences for these variables (females: *n*=12; males: *n*=10). Two variables were significantly different: resting heart rate ($t_{(20)}=2.09, p<0.05$) and percentage of body fat ($t_{(20)}=5.68, p<0.01$). There were no significant differences between males and females on the total fitness score, aerobic fitness score, and the systolic/diastolic blood pressure scores.

Test of Spatial Memory Thought to Be Dependent on the Hippocampus (Object Location Task)

The object location task assesses memory to recall the spatial locations of individual objects (Choi & L'Hirondelle, 2005). This task has been used to assess hippocampus-dependent memory (Assini, Duzzioni, & Takahashi, 2009). This testing was conducted in a small room (240 cm x 413 cm) with several fixed cues, including a door, a sink, a desk and large plants. The objective of the task was explained to each subject before he/she entered the testing room: to learn the position of four objects (a blue rock, a basket, a notebook, and a pen). Each subject entered and stayed in the room for 10 seconds to learn the position of objects. After 10 seconds, the subject left the room, and the experimenter randomly changed the position of one object, switched the positions of two other objects, but kept the fourth object in its original location. The subject again entered and stayed in the room for 10 seconds. The experimenter then asked subjects the following questions:

1. Was there a change in the position of objects, yes or no? If yes:
 - a. Did object #1 change position, yes or no?
 - b. Did object #2 change position, yes or no?
 - c. Did object #3 change position, yes or no?
 - d. Did object #4 change position, yes or no?
2. Was there a switch in the position of two of the objects, yes or no? If yes:
 - a. Which two items were switched?

Test of Spatial Working Memory Thought to Be Dependent on the PFC (N-Back Test)

In neuroimaging studies, the dorsolateral PFC region was activated when primate and human subjects carried out spatial working memory tasks (Goldman-Rakic, 1995; Owen, Downes, Sahakian, Polkey, & Robbins, 1990). This indicates that spatial working memory is localized in the dorsolateral PFC. In the current study, the subjects' spatial working memory was tested with the spatial 3-back test, a specific version of the n-back test. In this test, a 3x3 grid of black squares appeared on a computer screen. After 2 seconds, one of the squares turned red. The square remained red for 2 seconds, and then turned back to black. After 1 second of all the squares being black, another square turned red. Subjects were to touch the screen every time the current red square's position was the same as the one presented 3 positions back in the sequence (24-26 trials out of 100, "n-back same"); otherwise, they were not supposed to respond (74-76 trials out of 100, "n-back different"). Two separate memory scores were collected with the n-back test: "n-back same" and "n-back different."

Two additional female subjects were excluded from data analysis for the n-back test because they didn't understand the directions. Means for each fitness variable with the new sample sizes are summarized in Table 2. Independent *t*-tests were used again to determine if there were gender-related differences for fitness variables with the modified sample sizes (females: $n=10$; males: $n=10$). Only one measurement from the previous analysis still remained significantly different: percentage of body fat ($t_{(18)}=5.56, p<0.01$). There was no significant difference between males and females on the total fitness score, aerobic fitness score, the systolic/diastolic blood pressure scores, and heart rate. The total

fitness score was chosen as the representative variable to assess the effects of fitness on spatial memory.

Acute Stress Response to a Cognitive Stressor

Cortisol is a steroid hormone released from the adrenal gland upon activation of the hypothalamic-pituitary-adrenal (HPA) axis as part of the stress response (Gaab, Rohleder, Nater, & Ehlert, 2005). Since the level of saliva cortisol is almost identical to the cortisol level in blood serum, salivary cortisol can be used to assess cortisol level in serum (Aardal & Holm, 1995; Teruhisa et al., 1981). Saliva samples were collected from each subject both before and after performing the n-back test, a cognitively stressful test. The samples were kept on ice during testing, then stored at -70°C . The frozen samples were sent to Salimetrics (Carlsbad, CA) for analysis of cortisol levels. Pre- vs post-measures of cortisol were thus used to assess HPA axis activation to the n-back test. Since the HPA axis is influenced by circadian rhythmicity (Van Cauter, 1990), all subjects were tested and saliva samples were collected at approximately the same time (usually afternoon).

Heart rate and blood pressure normally increase as part of the stress response (Chrousos, 2009). Each subject's heart rate and blood pressure were measured both before and after the n-back test with a digital sphygmomanometer (Omron Health Care, Inc.; Lake Forest, IL). Pre- vs. post-measures of heart rate and blood pressure were thus used to assess the subject's acute cardiovascular response to the n-back test.

Table 3 compares the cardiovascular measurements collected in the MicroFit[®] fitness profile with the pre- n-back test measurement collected by the experimenters. Paired *t*-tests were performed to verify that there were no serious discrepancies between

the two sets of measurements. No significant differences were found between pre- and post- measurements on the systolic blood pressure ($t_{(21)}=1.137, p=0.27$), the diastolic blood pressure ($t_{(21)}=1.581, p=0.13$), and the resting heart rate ($t_{(21)}=0.037, p=0.97$).

Self-reports of Chronic Stress Levels, Depression and Anxiety

The Kohn Hassles Scale (Inventory of College Student Recent Life Experiences, ICSRLE) was used to assess the stress level of the research subjects over the past month. The survey form used in this experiment is called the Inventory of College Student Recent Life Experiences (hereafter called the ICSRLE). The ICSRLE contains 49 items and is designed especially for college students (Kohn, Lafreniere, & Gurevich, 1990). ICSRLE scores can range from 49 to 196. A higher score indicates a higher level of exposure to stressful life events.

The Depression Anxiety Stress Scale (DASS 21) is a 42-item, self-report instrument designed to measure the three 14-item related negative emotional states of depression, anxiety and tension/stress (Lovibond & Lovibond, 1995). The current pilot study used a short-form, 21-item version (called the DASS 21) that consists of three 7-item self-report scales to measure depression, anxiety and stress. Subjects are asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each state over the past week. Scores for depression, anxiety and stress are calculated by summing the scores for the relevant items. The DASS was developed in non-clinical populations, therefore is suitable for screening normal adolescents and adults.

Mean scores for the ICSRLE and the DASS 21 are summarized in Table 4. Independent t -tests were used to determine if there were sex-related differences in these self-reports. No significant differences were found between males and females on the

ICSRLE ($t_{(20)}=0.397, p=0.70$), and DASS 21 ($t_{(20)}=1.547, p=0.14$). Since there were no sex-related differences, male and female data were grouped together for further analysis.

Statistical Analysis

Human fitness, memory, and stress data were analyzed with SPSS statistics software (V.21.0.0, IBM Corporation, New York, NY).

The total fitness score was used as the representative variable to assess the effects of fitness. First, the data were dichotomized into two main groups. Subjects with a total fitness score within the “Needs Work” or “Fair” range (Figure 9) were assigned to the lower fitness group (hereafter called the “Fair” group). Subjects with a total fitness score within the “Fit” or “Excellent” range were assigned to the higher fitness group (hereafter called the “Fit” group). It was not possible to assign the fitness data into quartiles, because there was only one subject in the “Excellent” fitness category.

The data record sheet for the object location test had eight questions about object location. The experimenter recorded each subject’s answers onto the sheet. The number of correct answers was counted, giving each subject a memory score between 0 and 8. A higher score indicated a higher level of hippocampus-dependent memory capacity. The difference in object location memory between “Fair” and “Fit” groups was analyzed with independent samples *t*-tests.

The n-back test data were analyzed using statistical models developed with Dr. Karl Bailey (co-investigator on this project). There were two components to the n-back test data for each subject: “n-back different” and “n-back same.” The “n-back different” memory score refers to the subjects’ memory on trials in which the red square was in a different location from three trials earlier, thus they were not supposed to respond.

Therefore a higher “n-back different” memory score means that the subjects correctly inhibited a wrong response. The “n-back same” score refers to the subjects’ memory on trials in which the red square was in the same location as three trials earlier, where they were supposed to respond. Therefore a higher “n-back same” memory score means that the subjects correctly initiated a correct response. Since higher fitness has been reported to be beneficial to executive functions (Themanson & Hillman, 2006), the difference in working memory between the “Fair” and “Fit” groups was analyzed with independent samples *t*-tests.

Changes in salivary cortisol, heart rate and systolic/diastolic blood pressure were used to assess each subject’s acute stress response to the n-back test, a cognitive stressor. Fitness levels might affect the stress response to acute stressors (Doornen & Geus, 1989). Therefore, differences in the acute stress response between the “Fair” and the “Fit” groups were analyzed with independent samples *t*-tests.

Individuals with high levels of stress seem to be more susceptible to emotional mood disorders such as depression and anxiety (Kessler, 1997; Tennant, 2002). Therefore, a Pearson product-moment correlation coefficient was used to assess the relationship between the subject’s self-reports of stressful life events (ICSRLE score) and their self-reports of depression and anxiety (DASS 21 score).

Median splits were done on data for both the ICSRLE scores and DASS 21 scores. Subjects below the median with low ICSRLE and DASS 21 scores are hereafter called the “Bottom 50%” group, while subjects above the median with high ICSRLE and DASS 21 scores are hereafter called the “Top 50%” group. Stress has been reported to affect memory negatively (Liston et al., 2009; Qin et al., 2009) or positively (Barha et al., 2007;

Yuen et al., 2009; Yuen et al., 2011). Therefore, the difference in hippocampus-dependent memory on the object location task between the “Bottom 50%” and “Top 50%” groups was analyzed with independent *t*-tests. Likewise, the difference in prefrontal cortex-based memory on the n-back task between the “Bottom 50%” and “Top 50%” groups was analyzed with independent *t*-tests.

Finally, the effects of chronic stress (ICSRLE scores) and feelings of depression and anxiety (DASS 21 scores) on the acute stress response (changes in salivary cortisol, heart rate, and systolic/diastolic blood pressure) to the n-back test were analyzed with independent samples *t*-tests.

As will be reported in Chapter 5, some of the results reported for this pilot study did not reach statistical significance, but may represent a statistical trend. In cases of statistical trends (with *p* values between 0.06 and 0.075), the Cohen’s effect size (Cohen’s *d*) was calculated to express the mean difference between groups in standard deviation units. An online Cohen’s effect size calculator from Colorado Springs University was used (Lee, 2000). The value of *d* is categorized as a small, medium, or large effect: *d*=0.2 to 0.5 is considered a small effect; *d*=0.5 to 0.8 is considered a medium effect, and *d*=0.8 and higher is considered a large effect. Additionally, a power analysis was also calculated to see how many more subjects per group would be needed to get a significant finding. An online power analysis calculator was used (Rollin, 2015).

CHAPTER 4

RESULTS 1

Spatial memory on the Barnes maze was evaluated with three parameters: (a) time in seconds to find the hole with the goal box (*latency*), (b) number of holes visited before the goal box for the first time (*errors*), and (c) number of holes re-visited before approaching the goal box (*repeat errors*). Since the rats were exposed to a new goal box position on the first testing day, trial 1 would not have been an accurate measure of spatial memory. Trial 1 was therefore analyzed separately.

For latency to reach the goal box, there was no significant group effect for trial 1 ($F(1,31)=2.004, p=0.167$), but there was a significant effect between groups on the subsequent trials 2-16 (Figure 10 A). UST rats showed a significantly lower latency to find the goal box on trials 2-16 ($F(1,31)=5.024, p=0.032$). There was also a significant trial effect, with a decrease in latency in both groups over trials 2-16 ($F(14,434)=5.953, p<0.0001, \epsilon=0.416$), but no interaction ($F(14,434)=0.744, p=0.611, \epsilon=0.416$).

Each rat had the same goal box position on days 1 and 2 and a new goal box position on days 3 and 4. Latencies on the first trials of days 2 and 4 (trials 5 and 13) were analyzed to assess reference (long-term) memory from the previous testing day. There was a significant group effect ($F(1,31)=4.525, p=0.041$), with UST rats taking less time to find the goal box on reference memory trials than did CT rats. Latencies on trials 2-4, 6-8, 10-12, and 14-16 were analyzed to assess working (short-term) memory. There

was a significant group effect ($F(1,31)=3.938, p=0.05$) with UST rats taking less time to find the goal box on working memory trials than did CT rats.

For errors, there was no significant group effect ($F(1,31)=1.494, p=0.231$) for trial 1, but there was a significant group effect over trials 2-16 ($F(1,31)=5.953, p=0.032$), with UST rats making significantly less number of errors compared to the CT rats (Figure 10 B). There was a trial effect for errors over trials 2-16 ($F(14,434)=3.904, p=0.001, \epsilon=0.482$), with both groups showing fewer errors over subsequent trials, but there was no interaction ($F(14,434)=0.374, p=0.982, \epsilon=0.482$).

For repeated errors, there was no significant group effect for trial 1 ($F(1,31)=0.464, p=0.501$), but there was a significant group effect for trials 2-16 ($F(1,31)=15.988, p=0.0001$), with UST rats making fewer repeat errors than did CT rats (Figure 10 C). There was also a trial effect ($F(14,434)=2.926, p=0.019, \epsilon=0.323$) with both groups showing fewer repeat errors over subsequent trials, but no interaction ($F(14,434)=0.523, p=0.741, \epsilon=0.323$).

A new goal position was introduced to the rats on day 3. Therefore, the percentage of errors on the first trial of day 3 in which the rats first visited the goal position previously used on days 1 and 2 was assessed. UST rats visited the previous goal position as their first error at a rate of 46.67%, while CT rats visited the previous goal position at a rate of 27.78%, suggesting that UST rats had better spatial reference memory than did CT rats (Figure 11).

The patterns of errors were analyzed in order to assess three possible navigation strategies: random, serial, and spatial. The frequency of each navigation strategy was assessed across the four trials each day, so that the repeated measures variables were:

group (between subjects) and day (within subjects). For random strategy, there was a significant group effect ($F(1,31)=19.882, p=0.0001$), with CT rats using random strategy more frequently than UST rats (Figure 12 A). A day effect was also observed, as both groups decreased their use of this strategy over subsequent days ($F(3,93)=12.501, p=0.0001$). There was no interaction ($F(3,93)=0.698, p=0.556, \epsilon=0.875$). For spatial strategy, there was a significant group effect ($F(1,31)=17.117, p=0.0001$), with UST tending to use spatial strategies more frequently than did CT rats (Figure 12 B). A day effect was also observed, as both groups increased their use of this strategy over subsequent days ($F(3,93)=10.436, p=0.0001$). There was no interaction ($F(3,93)=0.136, p=0.938, \epsilon=0.962$). Interestingly, there was no significant group effect ($F(1,31)=0.112, p=0.74, \epsilon=0.945$) or day effect ($F(3,93)=0.134, p=0.939, \epsilon=0.945$) for serial strategy (Figure 12 C).

CHAPTER 5

RESULT 2

The Effects of Physical Fitness on Memory in College Freshmen

The effect of fitness on hippocampus-dependent spatial memory is shown in Figure 13. In this pilot study, a non-significant, statistical trend was found between object location memory scores of the “Fair” (subjects with total fitness scores in the “needs work” to “fair” range) and “Fit” (subjects with total fitness scores in the “fit” to “excellent” range) groups ($t_{(20)}=1.49$, $p=0.075$, Cohen’s $d=0.7$) (Figure 13). The Cohen’s effect size value ($d=0.7$; $M_{\text{Fair}}=6.07$, $SD_{\text{Fair}}=1.55$; $M_{\text{Fit}}=7.5$, $SD_{\text{Fit}}=1.07$) suggested a moderate to high practical significance. A power analysis indicated that this effect could reach statistical significance with 38 subjects per group, or 76 subjects total. This would require recruiting an additional 54 subjects to the 22 subjects in this pilot study. Twenty-seven subjects would therefore need to be added to each of the “Fair” and “Fit” groups.

The effect of fitness on prefrontal cortex-based memory is shown in Figure 14. Results of n-back test were analyzed in two ways: “n-back different” and “n-back same.” A high “n-back different” memory score means that the subject correctly inhibited a wrong response on trials where the location of the red square was different from three trials back, whereas a high “n-back same” memory score means that the subject correctly initiated a correct response on trials where the location of the red square was the same as three trials back. No significant difference was found between the “n-back different”

memory scores of the “Fair” and “Fit” groups ($t_{(18)}=1.10, p=0.286$) (Figure 14 A).

Likewise, no significant difference was found between the “n-back same” memory scores between those two groups ($t_{(18)}=0.841, p=0.411$) (Figure 14 B).

The effects of fitness on the acute stress response to the n-back test, a cognitive stressor, are shown in Figure 15. The acute stress response was assessed with changes in salivary cortisol levels, heart rate, and systolic/diastolic blood pressure before and after performing the n-back test. A significant effect was found for the salivary cortisol response ($t_{(20)}=2.275, p=0.034$), with the “Fair” group having decreased cortisol levels than before the n-back test, while the “Fit” group had increased cortisol levels (Figure 15 A). No significant difference was found for the heart rate response between the “Fair” and “Fit” groups ($t_{(20)}=0.19, p=0.851$) (Figure 15 B). A significant effect was found for the systolic blood pressure response ($t_{(20)}=2.38, p=0.028$). While systolic blood pressure dropped in both groups after the n-back test, the drop was more pronounced in the “Fair” group than in the “Fit” group (Figure 15 C). The change in systolic blood pressure in the “Fit” group was not significantly different from zero. A statistical trend was seen for the diastolic blood pressure response between the “Fair” and “Fit” groups ($t_{(20)}=1.71, p=0.10$, Cohen’s $d=0.57$) (Figure 15 D). The Cohen’s effect size value ($d=0.57; M_{\text{Fair}}=1.07, SD_{\text{Fair}}=4.03; M_{\text{Fit}}=1.5, SD_{\text{Fit}}=6.59$) suggested a moderate, practical significance. A power analysis indicated that this effect could reach statistical significance with 30 subjects per group, or 60 subjects total. This would require recruiting an additional 38 subjects to the 22 subjects in this pilot study.

The Effects of Self-Reports of Chronic Stress, Depression and Anxiety on Spatial Memory in College Freshmen

Students self-reported their stress levels using the Inventory of College Student Recent Life Experiences (ICSRLE), and self-reported levels of depression and anxiety using the DASS 21 survey. A significant, positive correlation was seen between ICSRLE and DASS 21 ($r_{(22)}=0.552, p=0.008$) (Figure 16).

The effects of self-reported stress levels (ICSRLE) and levels of depression/anxiety (DASS 21) on hippocampus-dependent memory are shown in Figure 17. No significant difference was seen on object location memory scores between the “Top 50%” and “Bottom 50%” ICSRLE groups, based on a median split with higher scores representing higher stress levels ($t_{(20)}=0.881, p=0.389$) (Figure 17 A). However, a non-significant, statistical trend was found in this pilot study for object location memory scores between “Top 50%” and “Bottom 50%” DASS 21 groups. Those with higher self-reports of depression/anxiety tended to have higher object location memory scores ($t_{(20)}=1.87, p=0.075$, Cohen’s $d=0.8$) (Figure 17 B). The Cohen’s effect size value ($d=0.8$; $M_{\text{Bottom 50\%}}=6.36, SD_{\text{Bottom 50\%}}=1.63$; $M_{\text{Top 50\%}}=7.45, SD_{\text{Top 50\%}}=1.04$) suggested a high practical significance. A power analysis indicated that this effect could reach statistical significance with 28 subjects per group, or 56 subjects total. This would require recruiting an additional 34 subjects to the 22 subjects in this pilot study.

The effects of self-reported stress levels (ICSRLE) and depression/anxiety (DASS 21) on prefrontal cortex-dependent memory are shown in Figure 18. A significant difference was found on “n-back different” memory scores between the “Top 50%” and “Bottom 50%” ICSRLE groups ($t_{(18)}=2.74, p=0.013$). Subjects with lower self-reported stress levels in the bottom 50% group got higher “n-back different” scores than the top 50%

group (Figure 18 A). Therefore, subjects with lower self-reported stress levels were better at correctly inhibiting an incorrect response on trials where the location of the red square was different from three trials back. No significant difference was found on “n-back same” memory scores between the “Top 50%” and “Bottom 50%” ICSRLE groups ($t_{(18)}=0.130$, $p=0.898$) (Figure 18 B). Therefore, the “Bottom 50%” group was no better than the “Top 50%” ICSRLE group at initiating a correct response on trials where the location of the red square was the same as three trials back.

A non-significant, statistical trend was found in this pilot study between “n-back different” memory scores between the “Top 50%” and “Bottom 50%” DASS 21 groups ($t_{(18)}=1.95$, $p=0.066$, Cohen’s $d=0.87$) (Figure 18 C). Subjects with lower self-reports of depression and anxiety in the “Bottom 50%” group tended to get higher “n-back different” scores than did the “Top 50%” group. Therefore, the “Bottom 50%” DASS 21 group tended to be better at correctly inhibiting their response on trials where the location of the red square was different from three trials back. The Cohen’s effect size value ($d=0.87$; $M_{\text{Bottom 50\%}}=0.95$, $SD_{\text{Bottom 50\%}}=0.05$; $M_{\text{Top 50\%}}=0.89$, $SD_{\text{Top 50\%}}=0.08$) suggested a high practical significance. A power analysis indicated that this effect could reach statistical significance with 22 subjects per group, or 44 subjects total. This would require recruiting an additional 24 subjects to the 20 subjects in this pilot study. No significant difference was found between “n-back same” memory scores between the “Top 50%” and “Bottom 50%” DASS 21 groups ($t_{(18)}=0.446$, $p=0.661$) (Figure 18 D). Therefore, the “Bottom 50%” group was no better than the “Top 50%” DASS 21 group at initiating a correct response on trials where the location of the red square was the same as three trials back.

The effects of self-reported stress levels (ICSRLE) on the acute stress response to the n-back test, a cognitive stressor, are shown in Figure 19. A significant effect was found for the salivary cortisol response ($t_{(20)}=2.18, p=0.041$). Cortisol levels in the “Top 50%” ICSRLE group dropped after performing the n-back test, while cortisol levels in the “Bottom 50%” increased slightly (Figure 19 A). No significant difference was found for the heart rate response between the “Top 50%” and “Bottom 50%” ICSRLE groups ($t_{(20)}=0.044, p=0.964$) (Figure 19 B). No significant difference was found for the systolic blood pressure response ($t_{(20)}=0.063, p=0.953$) (Figure 19C). However, a significant difference was found for the diastolic blood pressure between the “Top 50%” and “Bottom 50%” groups ($t_{(20)}=2.08, p=0.05$). Diastolic pressure dropped in the “Bottom 50%” ICSRLE group after performing the n-back test, while the diastolic pressure change in “Top 50%” group was not different from zero (Figure 19 D).

The effects of self-reported depression and anxiety (DASS 21) on the acute stress response to the n-back test are shown in Figure 20. A significant effect was found for the salivary cortisol response between the “Top 50%” and the “Bottom 50%” DASS 21 groups ($t_{(20)}=2.39, p=0.027$). The pattern of results was consistent with the ICSRLE data: Cortisol levels in the “Top 50%” DASS 21 group dropped after performing the n-back test, while the change in cortisol in the “Bottom 50%” group increased slightly (Figure 20 A). A non-significant trend was found in this pilot study for the heart rate response ($t_{(20)}=1.66, p=0.113, \text{Cohen's } d=0.71$) (Figure 20 B). The “Top 50%” DASS 21 group tended to have an increased heart rate response to acute, cognitive stress. The Cohen's effect size value ($d=0.71; M_{\text{Bottom 50\%}}=-0.45, SD_{\text{Bottom 50\%}}=5.7; M_{\text{Top 50\%}}=2.9, SD_{\text{Top 50\%}}=3.59$) suggested a moderate to high practical significance. A power analysis indicated

that this effect could reach statistical significance with 35 subjects per group, or 70 subjects total. This would require recruiting an additional 48 subjects to the 22 subjects in this pilot study. Similar to the ICSRLE results, no significant effect was seen on the systolic blood pressure response between the “Top 50%” and “Bottom 50%” DASS 21 groups ($t_{(20)}=0.358, p=0.725$) (Figure 20 C). In contrast to the ICSRLE results, there was no significant effect on the diastolic blood pressure response between the “Top 50%” and “Bottom 50%” groups ($t_{(20)}=0.99, p=0.334$) (Figure 20 D).

Summary of Results

Table 5 summarizes all of the research findings and indicates whether or not the findings support the original hypotheses of this pilot study.

CHAPTER 6

DISCUSSION 1

Stress Inoculation in a New Animal Model

Results of Previous Study and Current Study Support the Stress Inoculation Hypothesis

Collaborators at Stony Brook University were the first to develop and use the new animal model of psychological stress illustrated in Figure 6 (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015; D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). Rats were exposed to unpredictable stressors (random presentations of ferret dander odor, light, and an abrupt sound) for 21 days. The stressors were controllable in the sense that the rats never experienced real predatory harm, and were always successful at obtaining food resources. Unpredictable but controllable stress (UST) has been reported to result in increased resilience-related behaviors (D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015). But when tested on the Barnes maze under low stress conditions, UST rats were slower to find the goal box and made more errors and repeat errors compared to control (CT) rats (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015). The current study built on these findings, with the goal of determining which of two possible hypotheses explains the observed increase in resilience but impaired spatial working memory after UST. The two possible hypotheses were: (H1) the variable adaptation hypothesis (Figure 3), or (H2) the stress inoculation hypothesis (Figure 5). To do this, all

procedures were the same as the previous studies (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015; D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015) except that spatial memory testing in the Barnes maze was conducted under high stress conditions (bright light, loud noise, fan blowing on the maze surface).

In contrast to the memory impairments reported under low stress conditions, UST rats in the current study showed better spatial memory than did CT rats under high stress conditions (Figure 10). These results are consistent with the stress inoculation hypothesis, which predicts that UST rats would show better spatial memory than CT rats under high stress conditions because of a rightward shift of the inverted u-shaped stress curve (Figure 5). Exposure to unpredictable but controllable stressors may have “inoculated” the rats, causing their stress curve to shift rightward. According to this hypothesis, UST rats showed impaired memory under low stress conditions because they were on the downward slope of their shifted stress curve. Under high stress conditions, UST rats showed better memory than did CT rats because they were at the peak of their shifted stress curve (Figure 5). This is consistent with other animal studies. Akirav and colleagues (2011, 2014) reported that rats with relatively high corticosterone levels showed optimal spatial memory performance in the Morris water maze when the training was done under higher stress conditions, that is, extreme water temperatures (Akirav et al., 2004; Akirav, Sandi, & Richter-Levin, 2001). The authors reported that this result might have been due to a rightward shift in the corticosterone response curve (Akirav et al., 2004). Another animal study also reported that rats exposed to predictable, chronic mild stress showed better memory on a water maze (Parihar et al., 2011).

Strategies That UST and CT Rats Used on the Barnes Maze Support the Stress Inoculation Hypothesis (H2)

Another set of findings in the current study also supports the stress inoculation hypothesis: Under high stress conditions, UST rats used spatial strategies more frequently than did CT rats (Figure 12 B), while CT rats used random strategies more frequently than did UST rats (Figure 12 A). There was no significant difference between UST and CT rats in their use of serial strategies under high stress conditions (Figure 12 C). These results do not support the variable adaptation hypothesis, which would predict that, regardless of stress levels during memory testing, UST rats would use caudate nucleus-dependent stimulus-response strategies (random or serial strategies) at a cost of hippocampus-dependent strategies (spatial strategies) (Schwabe et al., 2007).

The hippocampus plays an important role in spatial memory (Squire & Cave, 1991). The fact that UST rats used spatial strategies more frequently under high stress conditions suggests that their memory was based on the hippocampus rather than the caudate nucleus. According to the stress inoculation hypothesis (H2), rats exposed to unpredictable/controllable stress were “inoculated” against further stressors. The rightward shift in the stress curve placed them at optimum performance zone at relatively higher stress levels (Figure 5). Thus, the “inoculated” rats were able to use the most appropriate strategies to find the goal box when tested under high stress conditions. It should be noted, however, that not all animal and human studies have reported results consistent with this hypothesis. Only half of stressed rats used spatial strategies to find a platform in a Morris water maze, while all control rats used spatial strategies (J. Kim, Lee, Han, & Packard, 2001). Humans with higher stress levels were shown to use more caudate nucleus-dependent memory strategies than were those with lower stress levels

(Schwabe et al., 2008; Schwabe et al., 2007). The discrepancy of results between the current study and the other studies may be due to the difference in controllability of stress, which could have caused the difference in strategy: The current study used controllable stress, while the other studies used uncontrollable stress. In order to investigate this further, more studies concerning the strategies used on the Barnes maze should be conducted in the future.

UST Rats Exhibited Better Spatial Memory and Reference Memory Than Did CT Rats Under High Stress Conditions

Spatial working memory is the ability to remember spatial information for a short period of time. It is thought that both the prefrontal cortex and hippocampus are involved in spatial working memory (Kesner & Churchwell, 2011; Van Asselen et al., 2006). In contrast, the hippocampus alone seems to be more important for spatial reference memory (Squire & Cave, 1991; Squire & Schacter, 2002; Yoon et al., 2008), which is the consolidation of short-term spatial information into long-term (reference) memories that can be recalled later. The Barnes maze testing methods used in this study, and in previous works by collaborators at Stony Brook University, were employed to assess the effects of unpredictable/controllable stress on the respective memory functions of these brain regions. Four trials were run per day for 4 days, for a total of 16 trials. Working memory can be assessed over the three consecutive trials after the first trial each day (e.g., trials 2-4, 6-8, 10-12, and 14-16), in which each rat had a 15-minute interval to remember spatial information to find the goal box. Reference memory can be assessed on the first trial on days 2 and 4 (trial 5 and 13) since each rat had 24 hours to consolidate short-term memory into long-term memory (reference memory).

On working memory trials, UST rats found the goal box significantly faster and made fewer errors and repeat errors than did CT rats (Figure 10). Therefore, unpredictable/controllable stress seemed to improve spatial working memory when tested under high stress conditions.

On the reference memory trial on day 2 (trial 5; see Figure 10), UST rats took less time to find the goal box and made fewer errors than did CT rats. CT rats performed worse than the last trial of the previous day, while UST rats performed similarly. Therefore, reference memory was enhanced in UST rats under high stress conditions, while CT rats showed impaired reference memory. This result suggests that UST rats consolidated spatial information about the goal box overnight for future trials. When the new goal box position was introduced on day 3, the UST rats visited the previous goal box position more frequently than did CT rats (Figure 11). This again suggests that UST rats consolidated memory of their first goal box position more efficiently than did CT rats. Therefore, unpredictable/controllable stress also seemed to improve spatial reference memory when tested under high stress conditions. “Control” seems to be a key component of this stress condition, since rats exposed to chronic unpredictable and uncontrollable stress showed impaired spatial memory (Hill et al., 2004).

In previous studies conducted by collaborators at Stony Brook University, unpredictable/controllable stress selectively impaired spatial working memory in UST rats when tested under low stress conditions (D. Kim, Hudson, Molaro, Chorley, & Anderson, 2015). There was no significant group effect between UST and CT rats under low stress conditions for reference memory. The stress inoculation hypothesis seems to resolve these findings with those of the current study. A rightward shift of the stress

curve can explain why UST rats showed impaired spatial memory when tested under low stress conditions, but better memory under high stress conditions. UST rats were on the downward slope of their shifted curve under low stress conditions (Figure 4). They were at the peak of their shifted curve under high stress conditions (Figure 5).

Stress Inoculation as an Active Resilience Mechanism

In summary, the current study found that rats exposed to 21 days of unpredictable/controllable stress showed better spatial memory than did control rats when tested under high stress conditions. It is therefore concluded that exposure to unpredictable/controllable stress for this duration causes “stress inoculation,” causing neural and behavioral adaptations that may represent a rightward shift of the stress curve. Therefore, optimal performance of UST rats can be maintained with new environmental stressors on the Barnes maze.

Other researches have suggested that exposure to stress early in life prepares individuals more effectively to deal with novel stressors later in life (Buwalda et al., 2013; Champagne et al., 2008; Laban et al., 1995; Levine et al., 1956). The current study confirms but also extends these findings, since the stress exposure used in this study occurred later in the relative lifespan of the rats. The results suggest that exposure to a certain type of stress—specifically, what the subject feels is controllable even though it may not always be predictable—may play an important role as an active resilience mechanism to avoid deleterious changes in response to increased stress.

CHAPTER 7

DISCUSSION 2

The Effects of Fitness on Hippocampus-Dependent Memory

The hippocampus plays an important role in spatial learning and memory performance (Squire & Cave, 1991). In cross-sectional studies, higher levels of fitness have been associated with better memory and larger hippocampal volumes in both children (Chaddock et al., 2010) and the elderly (Erickson et al., 2009). Exercise intervention studies also have shown positive effects on hippocampal structure and function. In children, participation in aerobic exercise for 9 months enhanced relational memory that is dependent on the hippocampus (Monti, Hillman, & Cohen, 2012). In the elderly, participation in aerobic exercise for 6 months improved spatial memory and also increased hippocampal volume compared to non-exercisers (Erickson et al., 2011). This increased hippocampal volume was significant because it effectively reversed the loss in volume seen in the normal aging process. Consistent with these human studies, animal studies also report that voluntary participation in exercise improved spatial learning in mice (van Praag, Christie, Sejnowski, & Gage, 1999).

Reports of the effects of exercise on hippocampus-dependent memory in young adults have been mixed. Higher fitness levels in adolescents ages 15 to 18 were associated with better spatial learning in a Morris water maze-like task, and increased hippocampal volumes (Herting & Nagel, 2012). However, another recent study in college

students failed to show exercise-related improvements in a spatial learning task (Déry et al., 2013). Déry and colleagues (2013) noted that the students with lower levels of fitness showed the greatest improvements in cognitive performance; however, the overall effect was not statistically significant. As others have surmised (Voss et al., 2011), Déry and colleagues (2013) felt that cognitive improvements were not found because these college-aged young adults were likely “already near or beyond the threshold for optimal [cognitive] performance at the onset of [the] training regime,” thus leaving little room for improvement.

The current pilot study assessed the effects of fitness on spatial memory using the object location memory task. It is believed that this specific type of spatial memory is dependent on the hippocampus because individuals with a pathological reduction in hippocampal volume show impaired object location memory performance (Holdstock et al., 2002). In the current pilot study, a statistical trend was found ($p=0.075$, Cohen’s $d=0.7$) for object location memory between the “Fair” and “Fit” groups. The “Fit” group tended to get higher scores on the object location memory task than the “Fair” group. The Cohen’s effect size value ($d=0.7$) suggested a moderate to high practical significance. This result partially supports the original hypothesis that higher fitness levels would be associated with better hippocampal-dependent spatial memory (Table 5: Hypothesis #1). The investigators plan to add more subjects (approximately 34 according to the power analysis) in order to verify this preliminary finding.

To my knowledge, this is the first study to use the object location memory task to assess the effects of physical fitness on spatial memory. But the preliminary findings reported here are consistent with animal studies showing that exercise improved object

location memory. Four weeks of swimming exercise improved object location memory in old rats (Cechella, Leite, Rosario, Sampaio, & Zeni, 2014) and in middle-aged rats (Cechella, Leite, Gai, & Zeni, 2014). One week of running exercise improved object location memory and increased Brain-Derived Neurotrophic Factor (BDNF) expression in the hippocampus of mice (Ferreira-Vieira, Bastos, Pereira, Moreira, & Massensini, 2014). Another study in mice reported facilitated learning in an object location task after 3 weeks of running exercise (Intlekofer et al., 2013). This learning was thought to be dependent on expression of an important growth factor in the brain: Brain-Derived Neurotrophic Factor (BDNF), since blocking BDNF expression also blocked the learning effect.

Several neurobiological mechanisms have been suggested to determine the effects of physical fitness on memory. BDNF is a likely candidate, as it is up-regulated by exercise (Berchtold, Kessler, & Cotman, 2002), and has been shown to induce neurogenesis in the hippocampus (Scharfman et al., 2005). This might explain why exercise has been reported to up-regulate neurogenesis in the hippocampus (Olson, Eadie, Ernst, & Christie, 2006; Pereira et al., 2007; van Praag, Christie, et al., 1999; van Praag, Kempermann, & Gage, 1999). At the sub-cellular level, BDNF increases the outgrowth of dendrites and the expression of presynaptic/postsynaptic proteins in hippocampal neurons (Jovanovic, Czernik, Fienberg, Greengard, & Sihra, 2000; Kumamaru et al., 2008). BDNF also activates the signal transduction pathways used for long-term potentiation (LTP), which is a form of synaptic plasticity (Cunha, Brambilla, & Thomas, 2010). LTP is widely considered to be a neurophysiological mechanism for memory and learning (Bliss & Collingridge, 1993). Exercise is also reported to increase regional

microvascular density, which improves delivery of oxygen and nutrients to multiple brain areas, including the hippocampus in mice and in humans (Pereira et al., 2007). And in support of the hypothesis that exercise may be an active resilience mechanism to counteract the deleterious effects of stress, exercise-induced increases in neurogenesis and blood vessel density may reverse reductions in these measures observed in chronically stressed mice (Kiuchi, Lee, & Mikami, 2012).

The Effects of Fitness on Prefrontal Cortex-Dependent Memory

There is general agreement that participation in exercise is beneficial to the executive functions controlled by the PFC. Particularly relevant to this pilot study, regular physical activity improved cognitive processes on executive control in young adults (mean age: 21.1 years) (Kamijo & Takeda, 2009; Themanson & Hillman, 2006). Similar findings have been reported in children (van der Niet et al., 2014) and older adults (Colcombe & Kramer, 2003). Studies in both animals (Goldman-Rakic, 1995) and humans (Owen et al., 1990) have reported that the dorsolateral prefrontal cortex (DLPFC) is important for a specific type of executive function: working memory. The results of exercise and/or physical fitness on working memory have been mixed. A recent meta-analysis reported no significant relationship between fitness and working memory in older adults (Smith et al., 2010). More time spent in physical activity was associated with better planning ability and shorter total execution time in 80 school-aged children (8-12 years old), but did not significantly affect working memory (van der Niet et al., 2014). In contrast to these studies, high levels of physical fitness were associated with better verbal working memory in 58 young adults ages 18-30 (Padilla, Pérez, & Andrés, 2014). Higher levels of self-reported physical exercise also improved working memory capacity in 90

young adults ages 16-34 (Pluncevic-Gligoroska, Manchevska, Sivevska-Smilevska, & Bozhinovska, 2010).

The current pilot study assessed the effects of fitness on PFC-dependent working memory, specifically in the n-back test. No significant effect of fitness was found for this type of memory between the “Fair” and “Fit” groups. This result does not support the original hypothesis that higher fitness levels would be associated with better PFC-dependent spatial memory (Table 5: Hypothesis #2). This result is inconsistent with studies in other age groups (Erickson et al., 2013; Scudder et al., 2014). In a study of almost 400 children in the second and third grade, higher fitness levels were associated with better working memory in the n-back test (Scudder et al., 2014). Similarly, greater levels of physical activity were associated with better working memory on n-back tasks in middle-aged adults (Erickson et al., 2013). However, the results of this pilot study are consistent with those of another recent study on 75 college adults (mean age: 20.2) (Pontifex et al., 2014). Pontifex and colleagues (2014) reported no significant relationship between aerobic fitness and n-back test performance. Similar to what has been surmised for hippocampal function, these researchers concluded that college-aged students are near the peak of their cognitive function. In the current pilot study, the n-back test was sensitive enough to detect spatial working memory impairments associated with higher self-reports of stress and depression (described below), but not sensitive enough to detect fitness-related improvements. Thus a “ceiling effect” may have been reached in this particular memory task, and a more rigorous test may be needed to pick up fitness-related improvements. Another difficulty in interpreting the current research literature in this

area is the wide array of working memory tasks used, each of which likely uses a slightly different brain region.

The Effects of Fitness on Stress Response Measures to Acute Stress

In the current pilot study, the “Fair” group showed an attenuated cortisol response after acute, cognitive stress (i.e., performing the n-back test). This result does not support the original hypothesis that higher levels of fitness would be associated with a lower cortisol response (Table 5: Hypothesis #3), as has been reported elsewhere. For example, trained and elite sportsmen in their early 20s showed lower cortisol levels to psychological stress than did untrained men (Rimmele et al., 2009; Rimmele et al., 2007). Additionally, individuals with below-average levels of fitness showed a greater cortisol response to mental stress, while individuals with above-average fitness levels showed no difference (Webb et al., 2013).

However, other studies report no effect of fitness on cortisol response to cognitive stressors such as mental arithmetic and Stroop tests (Moyna et al., 1999; Sinyor, Schwartz, Peronnet, Brisson, & Seraganian, 1983). As others have suggested (Rimmele et al., 2007), the study’s discrepancies may be due to several factors. Cortisol responses are influenced not only by the level of physical fitness, but also by the age and gender of subjects, method of cortisol measurement, time of day of stress induction, and type of stressor. In addition, the studies summarized above used several different forms of psychological stress, and some of them may have been more stressful than others. Stressors with a social impact are considered to be among the most stressful. It has previously been shown that stressors that are uncontrollable or characterized by social-

evaluative threat (like being watched by an audience) are more stressful and may evoke a stronger physiological response (Dickerson & Kemeny, 2004).

In the current pilot study, no significant effect was found for heart rate to acute, cognitive stress between the “Fair” and “Fit” groups. The “Fit” group showed a smaller systolic blood pressure response to acute stress, though no significant effect was found for diastolic blood pressure. These results only partially support the original hypothesis that higher fitness would be associated with a lower heart rate and blood pressure response (Table 5: Hypothesis #4). Other studies have reported that higher fit individuals showed lower heart rate and blood pressure responses to psychological stress (Boutcher & Nugent, 1993; Claytor, 1991; Rimmele et al., 2007). One recent meta-analysis reported that fit individuals showed a lower heart rate and systolic blood pressure response, and a trend toward a lower diastolic blood pressure response to psychological stressors (Forcier et al., 2006).

However, these findings have not been found in all studies. Another recent meta-analysis reported that fitness increased integrated stress responses such as heart rate and blood pressure to an acute stressor, even though high fit individuals showed shorter recovery times (Jackson & Dishman, 2006). Similarly, 12 weeks of aerobic training increased aerobic capacity, but did not change heart rate or blood pressure responses to psychological stressors in 149 young adults aged 18-45 (Sloan et al., 2011). Researchers have suggested several reasons for the inconsistent findings. This may be due partly to the variation in laboratory stressors used, because it is known that different stressors will produce different physiological effects (Allen & Crowell, 1989). Other reasons may include differences in the dependent variables measured and the types of individuals

studied: Some studies use healthy subjects, while others use hypertensive subjects; some studies use just men or women, while others use both. The novelty of the stressor could have affected the results: Some studies report that fit and unfit subjects exhibit similar responses to novel stressors (Blumenthal et al., 1990; Claytor, 1991), while fit subjects show lower responses to familiar stressors (Claytor, 1991). The perceived level of control over the stressor can also impact stress responses: It is known that when subjects feel more in control, fewer catecholamines are released, and the stress responses such as heart rate and blood pressure are attenuated (Frankenhaeuser, 1991). Finally, the definitions of physical fitness used in the studies were varied. A recent meta-analysis pointed out that studies that measured fitness directly as peak oxygen uptake (i.e., with a VO₂ max score) reported the smallest effects of fitness on cardiovascular reactivity (Jackson & Dishman, 2006).

The Effects of Stress on Hippocampus-Dependent Memory

In the current pilot study, there was no significant effect of self-reported stress levels on hippocampus-dependent memory. This finding does not support the original hypothesis that higher levels of stress would be associated with impairments in hippocampal memory (Table 5: Hypothesis #5). This finding is also inconsistent with reports in both animals and humans of stress-induced impairments (Bremner, 1999; J. Kim & Diamond, 2002; McEwen, 2000b). In animals, chronic, uncontrollable stress causes spatial learning and memory deficits (Conrad, 2010; Hoffman et al., 2011; Luine et al., 1994; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). These functional impairments are thought to result from structural changes in the hippocampus with chronic stress, such as decreased length and branching of dendrites in hippocampal

neurons (Magarinos & McEwen, 1995; Vyas, Mitra, Rao, & Chattarji, 2002; Watanabe, Gould, & McEwen, 1992) and/or decreased neurogenesis (Warner-Schmidt & Duman, 2006). Humans with stress-related disorders such as PTSD exhibit impairment of hippocampus-dependent memory tasks. This has been found in war veterans (Vasterling, Brailey, Constans, & Sutker, 1998; Yehuda, Golier, Tischler, Stavitsky, & Harvey, 2005), rape victims (Jenkins, Langlais, Delis, & Cohen, 1998) and childhood abuse victims (Bremner, Vermetten, Afzal, & Vythilingam, 2004). Smaller hippocampal volumes have been reported in patients with PTSD as a result of combat (Bremner et al., 1995) and childhood abuse (Bremner et al., 1997). Only a few studies have used the object location task to assess hippocampal dependent memory with stress, but their results are consistent with the other memory tasks reported above.

For example, exposure to stress resulted in memory impairments in monkeys (Antunes & Biala, 2012) and in rats (Baker & Kim, 2002). The results of this pilot study are puzzling, given the body of data that suggests chronic stress impairs hippocampus-dependent memory. This non-significant result may be partially due to the small sample size and research methods. However, the sample was large enough, and the object location task was sensitive enough, to detect a putative effect of depression and anxiety on hippocampus-dependent memory (Figure 17). There was also a strong positive correlation between self-reported stress levels and self-reported depression and anxiety levels (Figure 16), so it is puzzling that one of these factors had an effect on hippocampus-dependent memory while the other did not. It is possible that the stress levels reported by the subjects had not yet reached an intensity or duration to reduce hippocampal memory. It is also possible that the subjects felt that at least part of their

reported stress was manageable or controllable, which may have decreased its deleterious effects (D. Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2015; Maier & Watkins, 2010; Russo et al., 2012). More data will be collected based on the power analyses in this pilot study to determine a final result.

The Effects of Stress on Prefrontal Cortex-Dependent Memory

In the current pilot study, individuals with lower levels of self-reported stress showed better prefrontal cortex-dependent memory than those with higher levels of self-reported stress. This result supports the original hypothesis that higher levels of stress would be associated with impairments in PFC-dependent spatial memory (Table 5: Hypothesis #6). This result is consistent with reports in both animals and humans that chronic stress reduced PFC volume and impaired PFC-dependent memory (Arnsten, 2009; Liston et al., 2009).

For example, in a study of almost 200 young adults (mean age: 17 years), those with higher reports of chronic stress during childhood exhibited greater working memory impairment (Evans & Schamberg, 2009). Cumulative life stress was associated with working memory impairment and smaller PFC volume in 62 children (mean age: 11 years) (Hanson et al., 2012). Adults (ages 36-55) with higher levels of chronic work-related stress exhibited lower PFC volumes (Blix, Perski, Berglund, & Savic, 2013). The functional impairments and lower volumes in PFC are thought to result from structural changes: in neuroanatomical studies in animal models, chronic stress has been shown to reduce dendrite length, branching and spine density in PFC neurons (Holmes & Wellman, 2009; McEwen & Morrison, 2013; Radley et al., 2006). Chronic stress-induced dendritic changes in PFC are associated with PFC dysfunction in rats (Liston et al., 2006), and the

degree of working memory impairment correlated with the extent of spine loss (Hains et al., 2009). Consistent with the study above, exposure to acute psychosocial stress impaired working memory in the 3-back version of the n-back test in college students (mean age: 24.53 years) (Schoofs et al., 2008).

The Effects of Depression on Hippocampus-dependent Memory

In the current pilot study, a statistical trend ($p=0.075$, Cohen's $d=0.8$) was found for the effects of self-reported depression and anxiety levels on hippocampus-dependent memory. Those with higher self-reports of depression tended to perform better on the object location task than those with lower self-reported depression levels. The Cohen's effect size value ($d=0.8$) suggested a high practical significance. This surprising result does not support the original hypothesis that higher levels of depression and anxiety would be associated with impaired hippocampus-dependent spatial memory (Table 5: Hypothesis #7). Other studies in this age group have reported mixed results. One study reported that college students with higher depression levels performed the same as non-depressed on the paired associate learning task, a visuospatial associative learning task thought to be hippocampus-dependent (Becker, MacQueen, & Wojtowicz, 2009). In another study, college students with higher levels of depression showed poorer memory on a task thought to be dependent on neurogenesis in the hippocampus (Déry et al., 2013). MacQueen and colleagues (2003) reported that young adults with depression showed impairment in hippocampus-dependent recollection memory, and intensity of depression relates with reduction of hippocampal volumes (MacQueen et al., 2003).

Stress is thought to trigger depression (Brown, Ruch, & McEwen, 1999; Pittenger & Duman, 2007), and like chronic stress, depression has generally been found to impair

hippocampal memory (Hickie et al., 2005; MacQueen et al., 2003; Sheline, Wang, Gado, Csernansky, & Vannier, 1996; Vythilingam et al., 2004). Stress and depression also affect hippocampal structure in similar ways. Just as chronic stress induces hippocampal atrophy (Sapolsky, 2000), recent meta-analyses show that depression is associated with hippocampal atrophy in humans (Bremner et al., 2000; Campbell, Marriott, Nahmias, & MacQueen, 2004; MacQueen et al., 2003). Additionally, BDNF serum levels are reduced in patients with depression (Shimizu et al., 2003; Ventriglia et al., 2013). Depression has also been reported to reduce hippocampal neurogenesis (Becker & Wojtowicz, 2007; Santarelli et al., 2003).

Similar to the effects of self-reported stress on hippocampal memory, the results of this pilot study regarding the effects of self-reported depression and anxiety are likewise puzzling. The results are in the opposite direction than might be expected. The current statistical trend does not support the original hypothesis that higher levels of stress, or stress-induced depression and anxiety, would be associated with impairments in hippocampus-dependent memory. Interestingly, the self-reports of depression and anxiety seem to have a negative effect on prefrontal cortex-dependent memory (see next section), but a positive effect on hippocampal memory. The small sample size and research methods could have influenced this result. More data will be collected based on the power analyses in this pilot study to determine a final result.

The Effects of Depression on Prefrontal Cortex-Dependent Memory

In the current pilot study, a statistical trend was found for the effects of self-reported depression on prefrontal cortex-dependent memory. Individuals with higher levels of self-reported depression tended to have worse working memory on the “n-back

different” test ($p=0.066$, Cohen’s $d=0.87$) than those with lower self-reported depression levels. The Cohen’s effect size value ($d=0.87$) suggested a high practical significance. These results support the original hypothesis that higher levels of depression and anxiety would be associated with impaired PFC-dependent spatial memory (Table 5: Hypothesis #8). This statistical trend is consistent with reports that patients with depression exhibited working memory deficits (Landrø, Stiles, & Sletvold, 2001; Pelosi, Slade, Blumhardt, & Sharma, 2000; Sweeney, Strojwas, Mann, & Thase, 1998). Seventy-nine depressed patients showed more deficits on the Wisconsin card sorting test, thought to be dependent on the prefrontal cortex, than did healthy individuals (Merriam, Thase, Haas, Keshavan, & Sweeney, 1999). Another study replicated the working memory impairment on the Wisconsin card sorting test, and additionally reported impaired memory on the n-back test in depressed patients compared to healthy individuals (Borkowska, Drozd, Jurkowski, & Rybakowski, 2009). In contrast, another study found no significant difference between patients with major depression and healthy controls on performance of the n-back test (Harvey et al., 2005). Researchers suspect that the discrepancy among the studies may be due to small sample sizes, inter-subject variability of age, phases of depression, and important cognitive measures such as IQ (Grant, Thase, & Sweeney, 2001; Merriam et al., 1999).

The Effects of Stress on Stress Response Measures to Acute Stress

In the current pilot study, individuals with higher levels of self-reported stress experienced a drop in cortisol levels after an acute, cognitive stressor, while those with lower levels of self-reported stress experienced an increase in cortisol levels. This result

supports the original hypothesis that higher levels of stress would be associated with a decreased cortisol response (Table 5: Hypothesis #9), and is consistent with other studies.

For example, medical students (mean age: 21 years) with higher stress scores showed lower cortisol levels to an acute physiological stressor (Loft et al., 2007). In a group of 60 college students, those who self-reported higher levels of stress for 4 weeks before an exam period showed significantly decreased cortisol levels during the exam period (Vedhara, Hyde, Gilchrist, Tytherleigh, & Plummer, 2000). Firefighters (ages 19-31) with higher levels of self-reported stress had reduced cortisol levels to psychological stressors (e.g., speech tasks) (Roy, 2004). Likewise, middle-aged adults (ages 24-36) with higher levels of self-reported chronic stress showed lower cortisol levels to acute stressors (e.g., arithmetic and public speaking) (Matthews, Gump, & Owens, 2001).

Consistent with these human studies, exposure to chronic stress decreased corticosterone levels to acute stressors in birds (Rich & Romero, 2005). To explain the pattern of results described above, one researcher (Roy, 2004) suggested “allostatic load,” because subjects with chronic stress showed inadequate responses to stressors. The term “allostatic load” was coined by McEwen and Stellar (1993) to explain the body’s stress responses to acute threats during chronic stress (McEwen & Stellar, 1993). It refers to “wear and tear on the body” that results from chronic stress (McEwen, 2006). Due to overexposure of stress hormones during chronic stress, there has been a larger “allostatic load” placed on the body that causes malfunction of the hypothalamic-pituitary-adrenal (HPA) axis and inadequate responses to acute stress (McEwen, 1998).

In the current pilot study, self-reported stress levels did not significantly affect heart rate and systolic blood pressure responses to acute, cognitive stress, although

individuals with higher levels of stress showed smaller changes in diastolic blood pressure compared to those with lower levels of stress. These results partially support the original hypothesis that higher levels of stress would be associated with smaller changes in cardiovascular response (Table 5: Hypothesis #10). The results are partially consistent with an animal study reporting that chronically stressed animals showed smaller changes in blood pressure and heart rate to acute restraint stress (Bhatnagar, Dallman, Roderick, Basbaum, & Taylor, 1998). However, results of stress on cardiovascular responses to an acute stressor have been mixed. For example, in a study of 129 medical students (mean age: 21 years), those with higher self-reported stress scores showed a higher heart rate response to an acute physiological stressor, but no change in systolic/diastolic blood pressure (Loft et al., 2007). In 62 middle-aged adults (ages 24-36), those with higher levels of self-reported chronic stress experienced lower systolic blood pressure and suppressed cardiovascular stress responses during acute stress (e.g., arithmetic and public speaking) (Matthews et al., 2001). Matthews and colleagues (2001) suspected that a few factors would affect the results of cardiovascular stress responses to acute stress: gender, health conditions such as hypertension, and consumption of caffeine, food, or medicine before the experiments.

The Effects of Depression on Stress Response Measures to Acute Stress

In the current pilot study, high levels of self-reported depression decreased cortisol levels after acute, cognitive stress, while low levels of depression tended to increase cortisol levels. These results support the original hypothesis that higher levels of depression and anxiety would be associated with a decreased cortisol response (Table 5:

Hypothesis #9). This pattern of results is consistent with the effects of self-reported stress levels, and it is also consistent with other studies. For example, a meta-analysis reported that depressed individuals had blunted cortisol responses to stress (Burke, Davis, Otte, & Mohr, 2005). Cortisol levels decreased after controllable or uncontrollable stress in depressed individuals, while cortisol levels increased after uncontrollable stress and decreased after controllable stress in healthy individuals (Croes, Merz, & Netter, 1993). Women (mean age: 29 years) with high levels of depressive symptoms exhibited blunted cortisol responses to a psychological stressor, while women with lower levels of depressive symptoms showed increase of cortisol levels (Burke, Fernald, Gertler, & Adler, 2005).

In the current pilot study, self-reported depression levels did not significantly affect heart rate or blood pressure responses to acute, cognitive stress, although a statistical trend was found for heart rate ($p=0.11$, Cohen's $d=0.71$). The Cohen's effect size value ($d=0.71$) suggested a moderate practical significance. These results only partially support the original hypothesis that higher levels of depression and anxiety would be associated with an increased cardiovascular response (Table 5: Hypothesis #10). Reports of the effects of depression on cardiovascular responses to an acute stressor have been mixed. For example, one study reported that stress and depression interaction didn't affect heart rate (Lin, Lin, Lin, & Huang, 2011). The University of Maryland Medical Center (UMMC) reported that patients with anxiety disorders showed no difference in their cardiovascular responses to acute stress compared to healthy people (UMMC, 2013). However, young adults (mean age: 23.4 years) with higher levels of self-reported depression showed a significant increase in heart rate and blood pressure after acute

stress (speech tasks) (Hamer, Tanaka, Okamura, Tsuda, & Steptoe, 2007). This discrepancy may be due to a few factors. First, self-reported higher levels of anxiety and depression were associated with low blood pressure in over 60,000 general adults (age 20-89) (Hildrum, Mykletun, Holmen, & Dahl, 2008; Hildrum et al., 2007). Other studies also reported an association between low diastolic blood pressure and depression in general (Barrett-Connor & Palinkas, 1994; Jorm, 2001). Practically, in the current study, individuals with higher levels of self-reported depression and anxiety had lower diastolic blood pressure before facing acute, cognitive stress than those with lower levels of depression and anxiety ($p=0.03$, data not shown). The usual decreased diastolic blood pressure in depressed individuals might be due to “allostatic load,” which would affect cardiovascular responses to acute stress (McEwen, 2000a). In addition, cardiovascular responses would be influenced by gender and pre-existing health conditions such as hypertension and hypotension.

Physical Fitness as an Active Resilience Mechanism

The goal of the current pilot study was to assess the effects of physical fitness on hippocampus- and prefrontal cortex-dependent memory, and to determine if fitness influenced the physiological stress response (measured in cortisol levels and cardiovascular changes) to an acute, psychological stressor. Despite reports of improved cognitive function in the hippocampus and PFC in other age groups, physical fitness did not influence prefrontal cortex memory in this study. Physical fitness improved hippocampal memory slightly, and this result may become statistically significant if more subjects are added in the future. In general, human studies seem to support the idea that fitness can result in improved cognitive function, but the fitness-related effects may

change across the lifespan. Studies generally show fitness-related improvements in memory in children and in the elderly. This pilot study was conducted in college-aged young adults who may be near the peak of their cognitive performance. This fact may be one of the reasons why this pilot study only partially supports the original hypothesis that higher fitness would be associated with better memory. Therefore, a more rigorous test than the one used in the current study may be needed for college-aged adults to find fitness-related improvements in prefrontal cortex memory, as others have reported (Padilla et al., 2014). Sample size was probably also a factor in the results. For example, only 22 subjects were assessed for hippocampal memory in this pilot study, but in accordance with power analysis calculators, at least 76 subjects are needed to make the results significantly different.

In addition, the current pilot study found that higher levels of self-reported chronic stress were associated with impairment of prefrontal cortex-dependent memory, and higher levels of self-reported depression and anxiety also impaired prefrontal cortex-dependent memory slightly. This effect may also become significant if more subjects are added in the future. These results support the original hypothesis that stress would be associated with impairment of hippocampus-dependent memory. The current pilot study also found that higher levels of chronic stress, depression and anxiety were not associated with impairment of hippocampus-dependent memory. Rather, higher levels of depression and anxiety improved hippocampus-dependent memory slightly, though it was not statistically significant. This may be due partially to it being a relatively easy test for college-aged adults. Another possibility is that like chronic stress, depression and anxiety

would facilitate memory in certain situations (Hamilton & Gotlib, 2008; Silva & Frussa-Filho, 2000).

The results of this pilot study indicate directions for future studies: First, more subjects should be added (at least 54) to validate the current statistical results. The low sample size precluded the investigators from running statistical tests to reveal the relative effects of fitness, stress, and depression/anxiety on memory performance and stress responsiveness to acute stressors. Second, more rigorous spatial memory tasks should be used in order to avoid “ceiling effects.” With these modifications, future studies would be able to more clearly elucidate the relationships between the effects of fitness as well as the effect of chronic stress, depression, and anxiety on cognitive function and stress responsiveness to acute stressors.

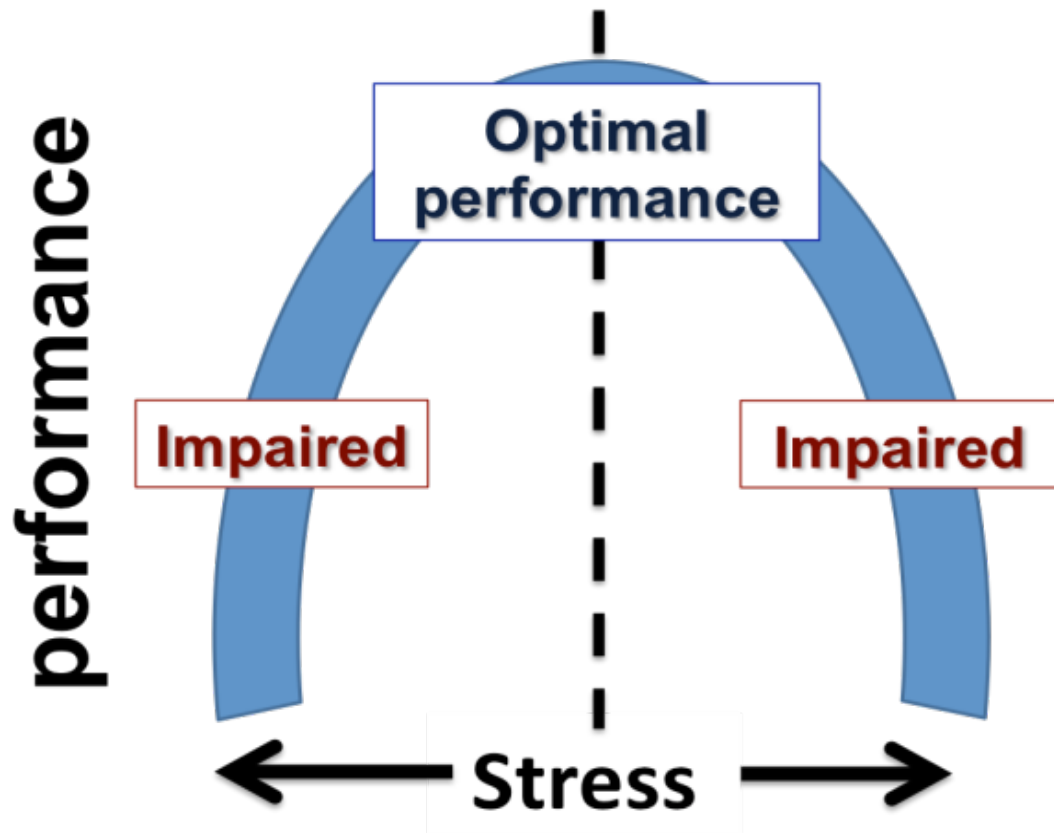


Figure 1. The inverted U-shaped curve and stress response. Stress response can be described with an inverted U-shaped curve. The highest level of performance was found when the animal was under optimal stress, but was impaired under conditions below or above optimal levels.

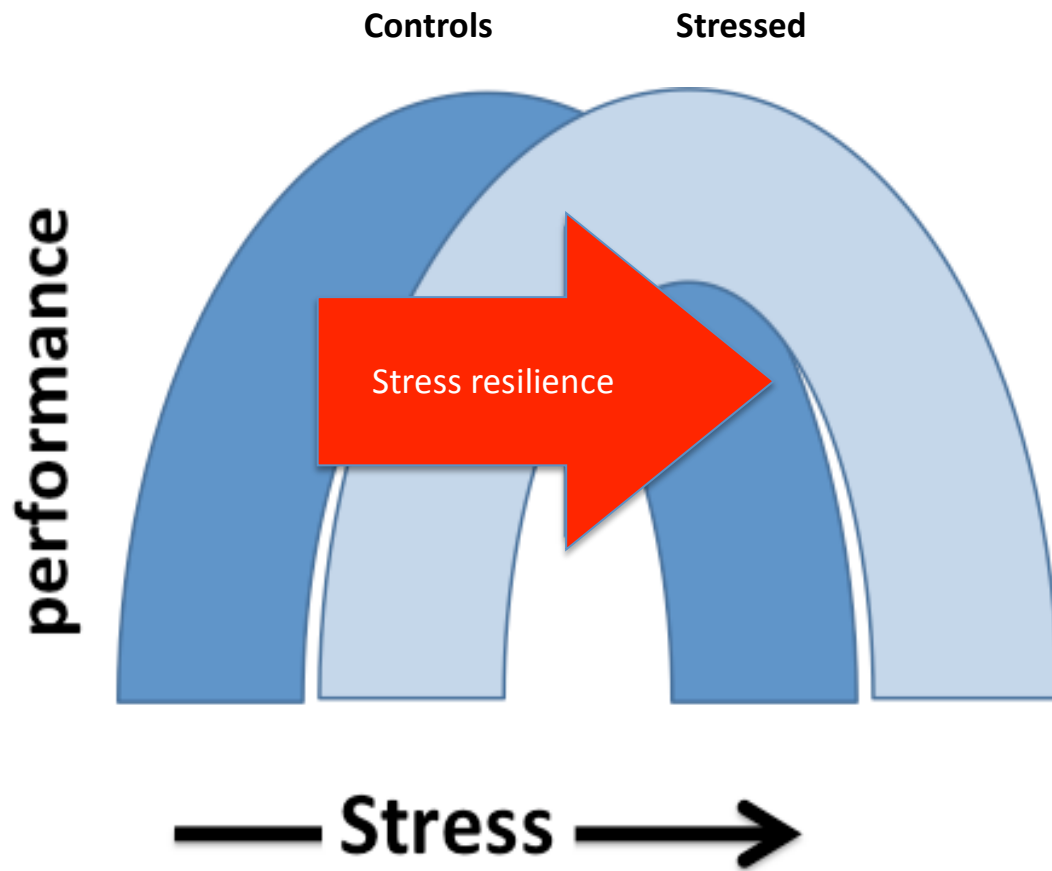


Figure 2. A shifting of the stress curve may promote resilience to stress. The inverted U-shaped stress curve can shift under certain conditions (e.g., exposure to moderate, controllable stress). A rightward shift of the stress curve would move the peak of the curve more to the right, and results in stress resilience. “Resilience” is the ability to avoid deleterious changes in response to chronic or increased stress.



Figure 3. Unpredictable/controllable stress causes a neural shift. The variable arbitration hypothesis suggests that unpredictable/controllable stress causes a neural shift in the control of behavior, away from the methodical and reflective control of the PFC, toward more rapid responses and reflexive actions associated with the caudate nucleus.

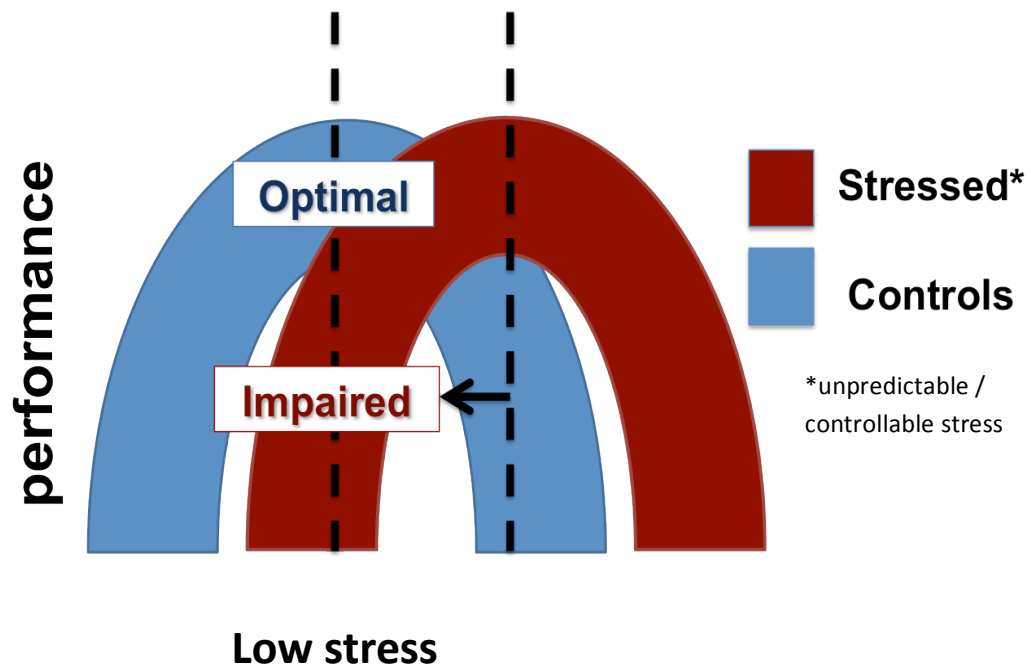


Figure 4. UST rats showed impaired spatial memory when tested under low or no stress conditions. Exposure to unpredictable/controllable stress may have caused a rightward shift of the stress curve. The UST rats showed impaired spatial memory compared to control rats when tested under low or no stress conditions. At low stress levels, the UST rats would be on the downward slope of the curve, while the control rats would be at the peak.

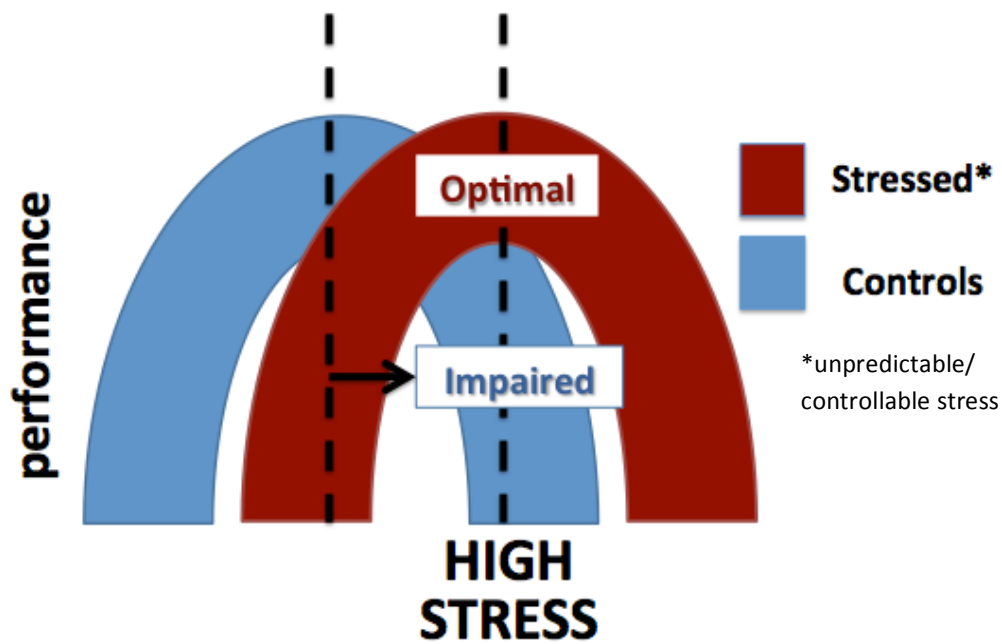


Figure 5. The stress inoculation hypothesis. The UST rats would exhibit better spatial memory than controls under high stress conditions because of a rightward shift of the inverted U-shaped stress curve. In contrast, control rats would be relatively impaired compared to the UST rats under high stress conditions, because they would be on the downward, maladaptive side of their stress curve.

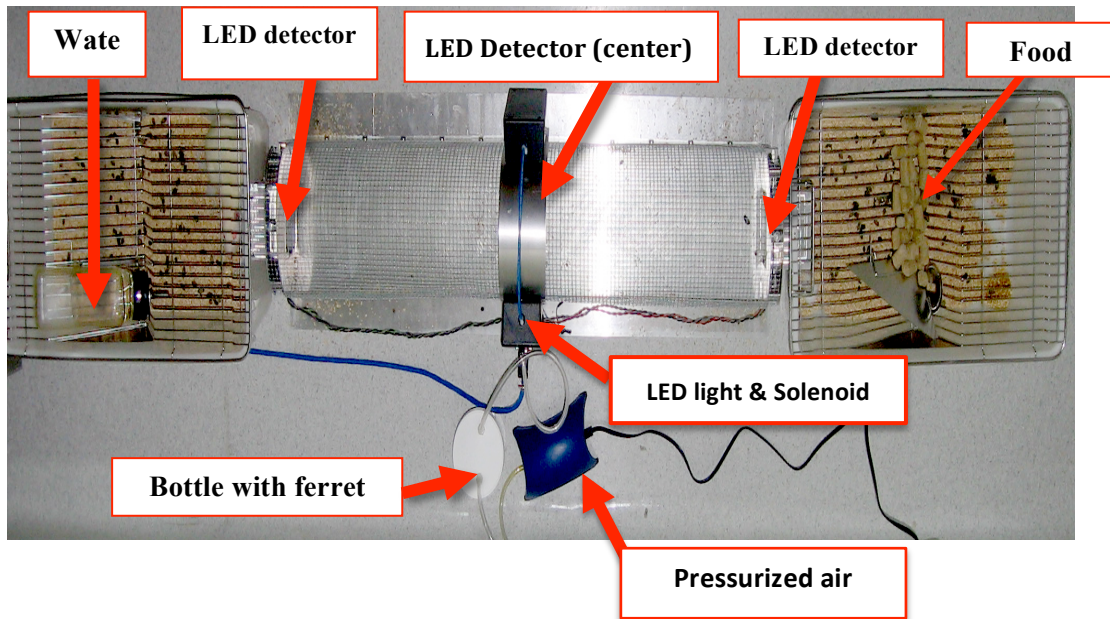


Figure 6. Each rat was housed individually in a platform for 21 days. This platform is designed to induce unpredictable/controllable stress in rats. The stimuli were presented randomly and on average only once out of every four-tunnel traversals. The rats always reached the other side safely and always successfully obtained food and water.



Figure 7. Spatial memory testing in the Barnes maze. Spatial memory testing started on the day after stress manipulation for 21 days. This memory testing was conducted under high stress conditions: bright light, loud noise, and wind.

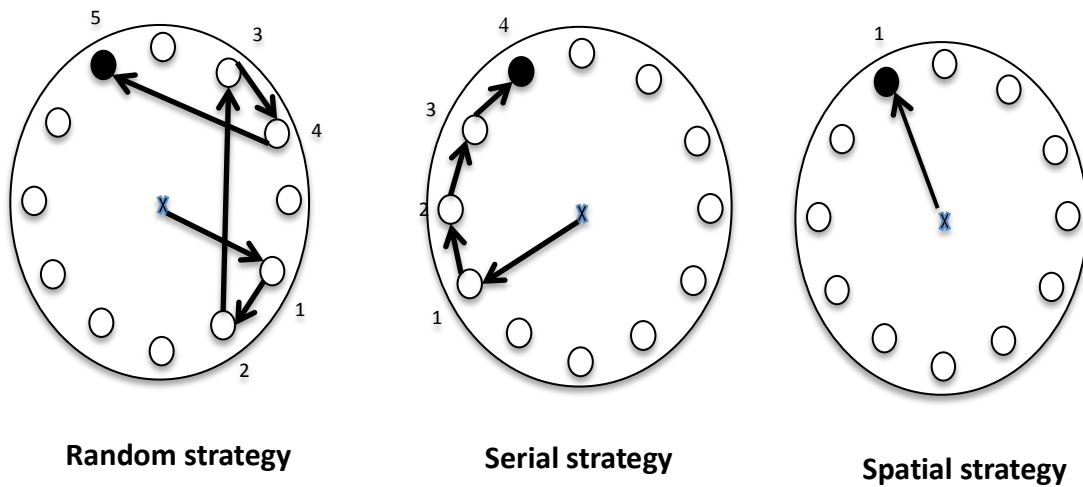


Figure 8. Examples of strategies. The patterns of errors and repeat errors on the record sheets were used to analyze possible navigation strategies. (1) Random strategy: no apparent pattern in visiting holes, but rather moved randomly on the maze and crossed the center multiple times. (2) Serial strategy: visited holes in consecutive order around the periphery of the maze until they found the goal box. (3) Spatial strategy: visited a cluster of holes (or directly) near the correct goal box position.

Example Fitness Profile

Name: Joe Smith
Age: 22

Standard: 20-29 years
Gender: Male

Date: April 25, 2014

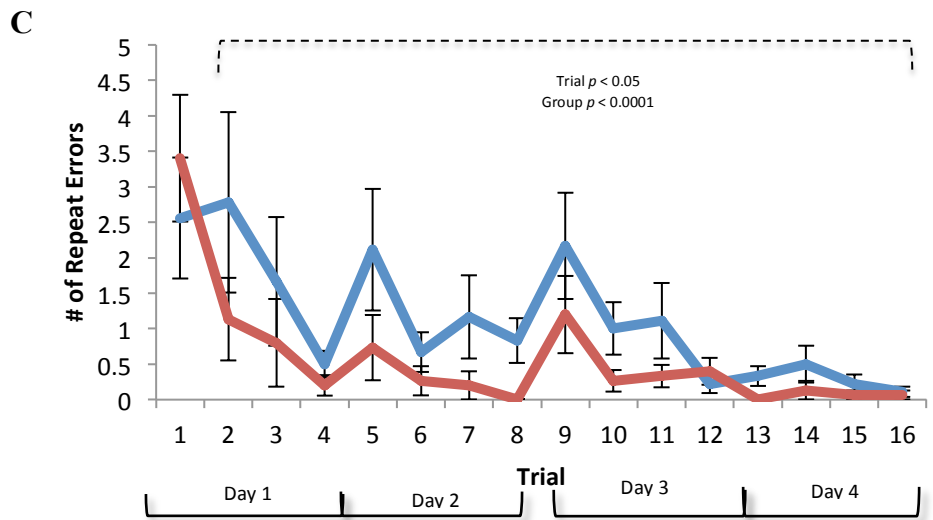
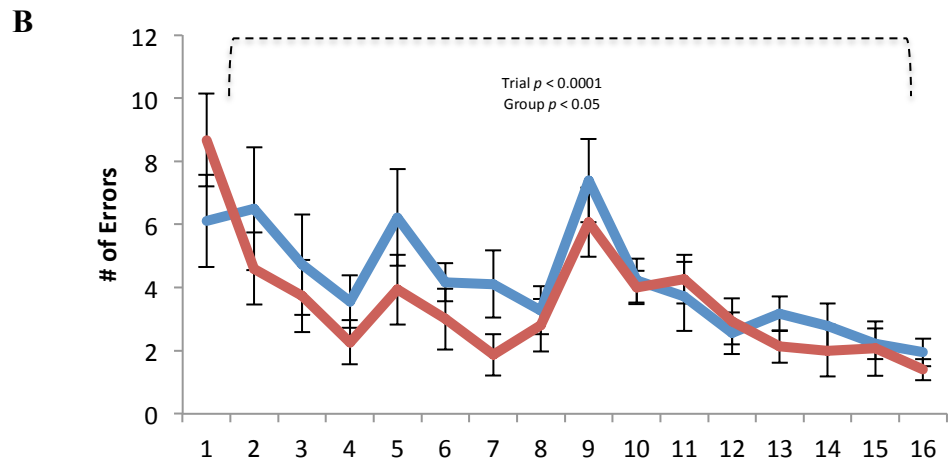
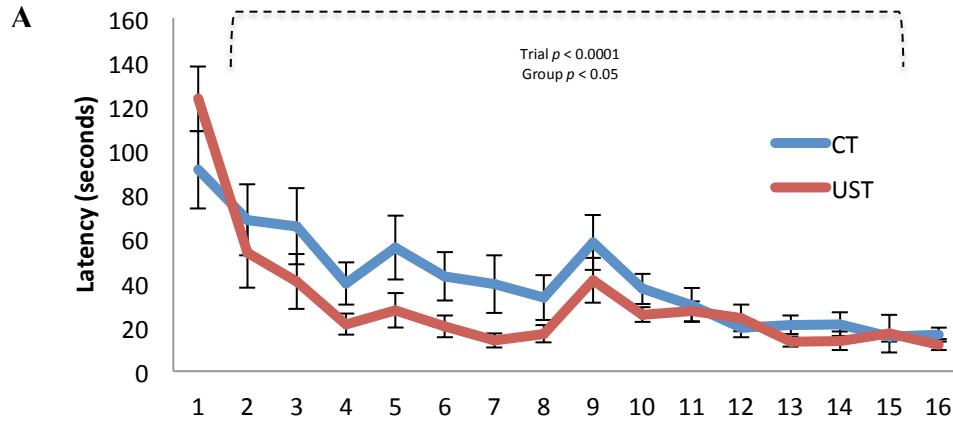
Description	Score	Needs Work	Fair	Fit	Excellent	
Total Fitness Score	65	0	40	60	80	100
Aerobic Fitness Score (ml/kg/min)	43	19	36	41	48	62
Systolic Blood Pressure (mm Hg)	122	180	139	119	109	100
Diastolic Blood Pressure (mm Hg)	81	110	89	79	69	50
Resting Heart Rate (beats per minute, bpm)	74	110	100	80	70	50
% Body Fat (% body wgt)	11	45	25	18	12	5

Figure 9. Example of a fitness profile collected with the Microfit[®] FAS-2 System. Each fitness score was rated on a separate scale, normalized for the subject's sex and age. Based on the scale, each fitness score was classified in one of 4 categories: "needs work," "fair," "fit," and "excellent."

Figure 10. UST Rats Showed Better Spatial Memory Than CT Rats.

- (A) Barnes Maze latency. UST rats took significantly less time to find the goal box than did CT rats over trials 2-16.
- (B) Barnes MAZE errors. UST rats made significantly fewer errors than did CT rats.
- (C) Barnes Maze repeat errors. UST rats made significantly fewer repeat errors than did CT rats.

The same pattern of results seems to appear in all three (latency, errors, and repeat errors): UST rats do significantly better in early trials, suggesting UST rats have better spatial memory. But both groups do better over time due to a significant trial effect, therefore the UST and CT groups do similarly well by trial 16.



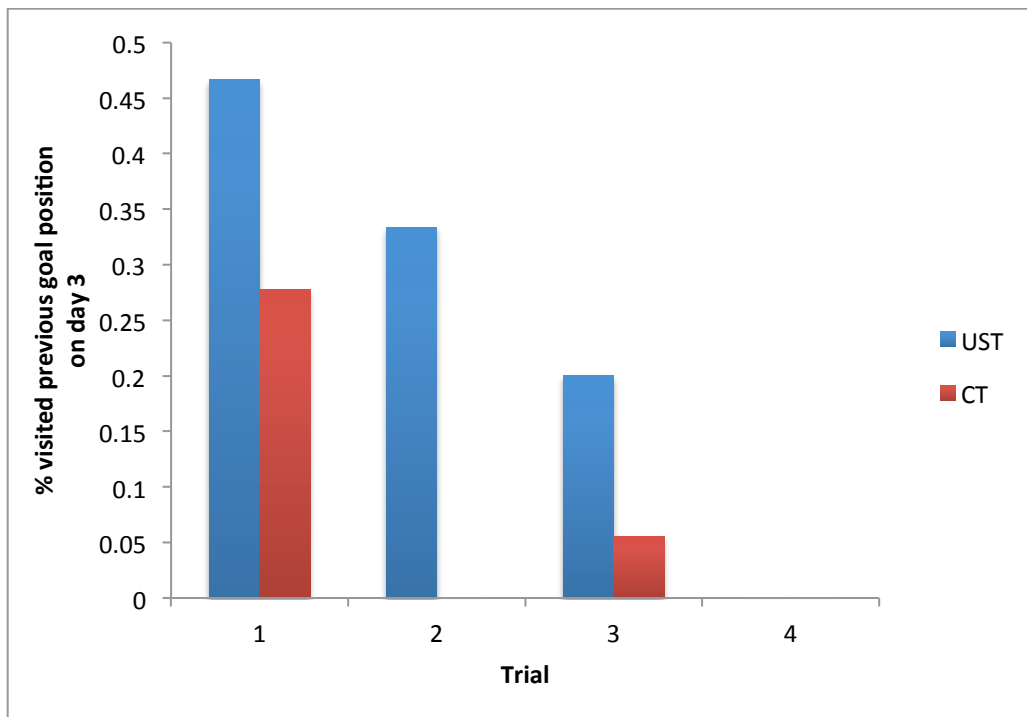
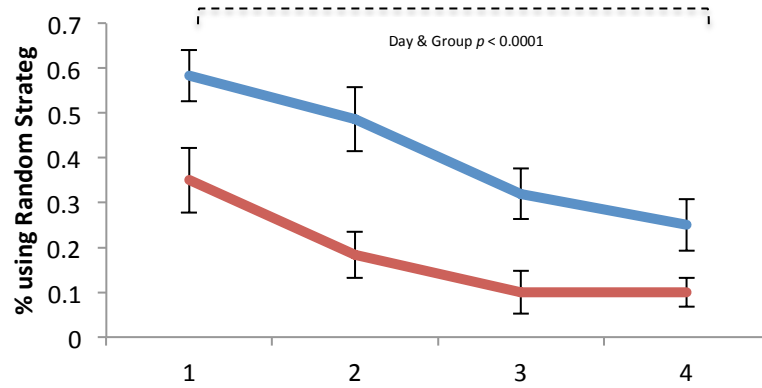


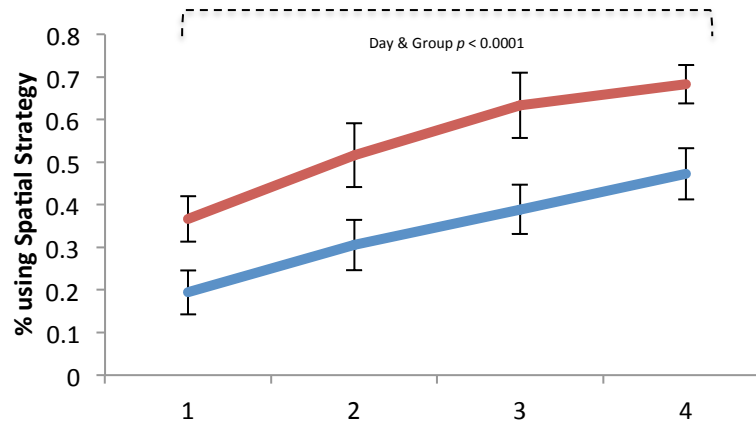
Figure 11. A higher percentage of UST rats visited the previous goal position as their first error on day 3. UST rats visited the previous goal position as their first error on trial 1 of day 3 at a rate of 46.67%, while CT rats showed 27.78%.

Figure 12. Random, spatial and serial strategy proportion. CT rats used higher random strategy proportion compared to UST rats, which used higher spatial strategy. Interestingly, there was no significant group effect for serial strategy.

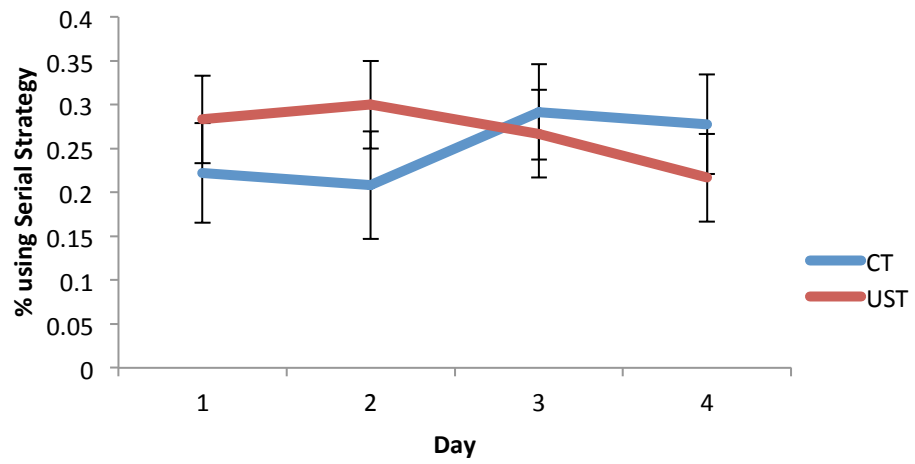
A



B



C



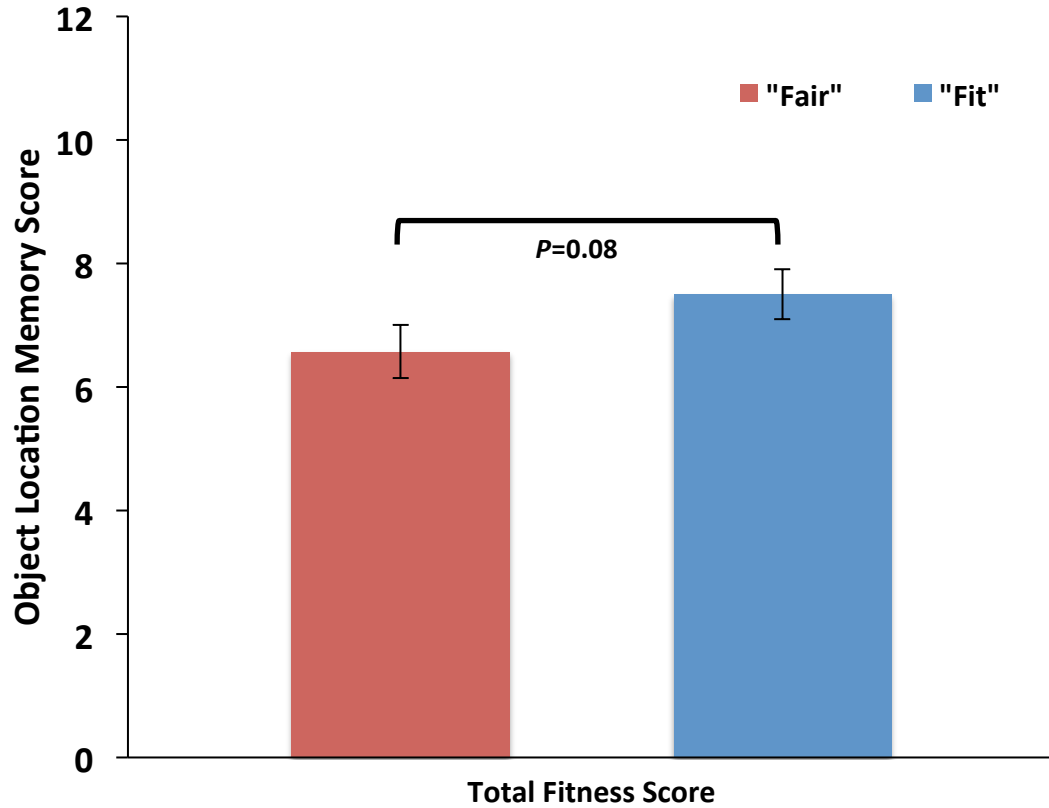


Figure 13. Effects of fitness on hippocampus-dependent memory. A non-significant, statistical trend was found between object recognition memory scores of the “Fair” and “Fit” groups ($t_{(20)}=1.49$, $p=0.075$, Cohen’s $d=0.7$). The Cohen’s effect size value ($d=0.7$) suggested a moderate to high practical significance.

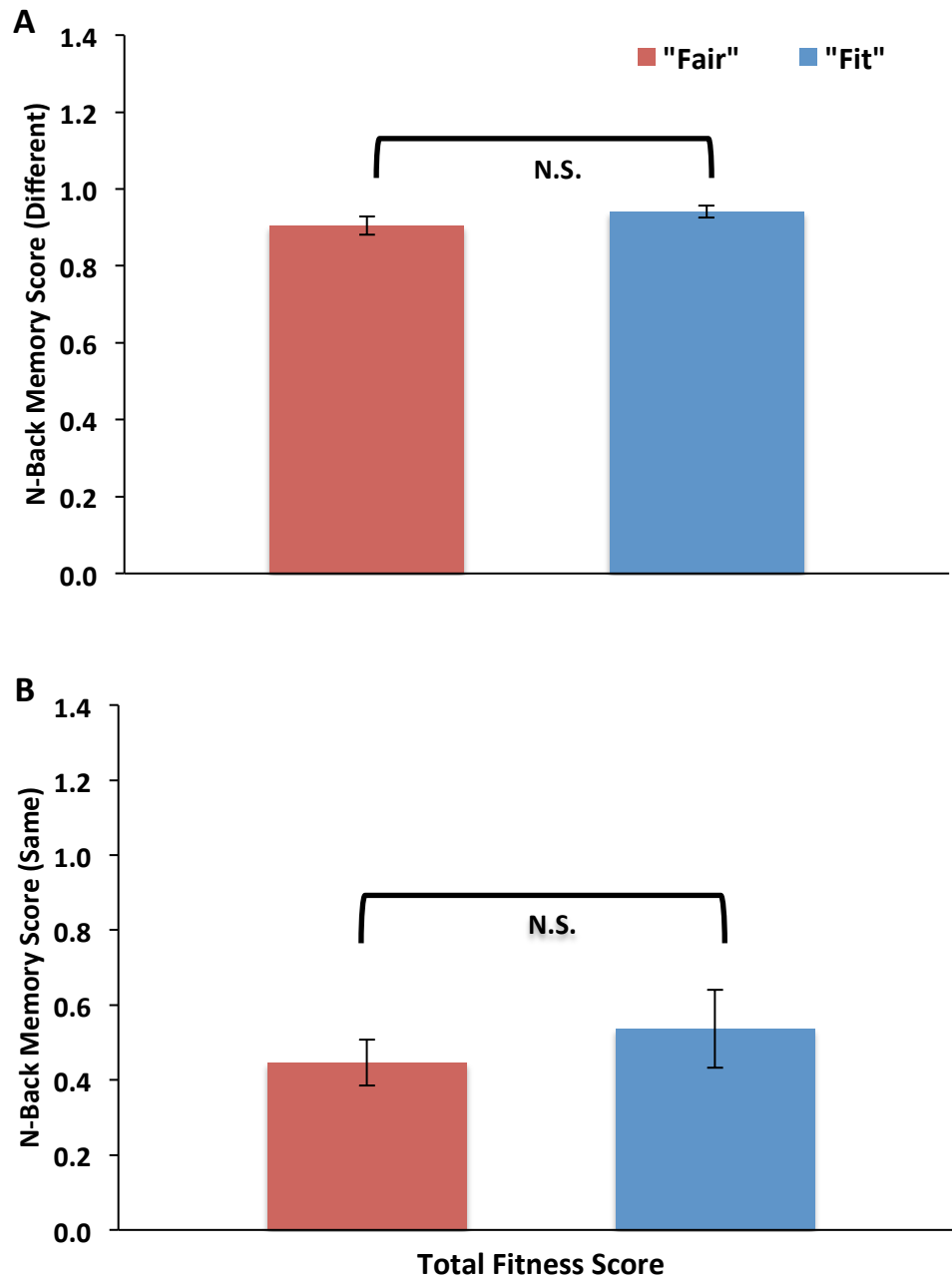
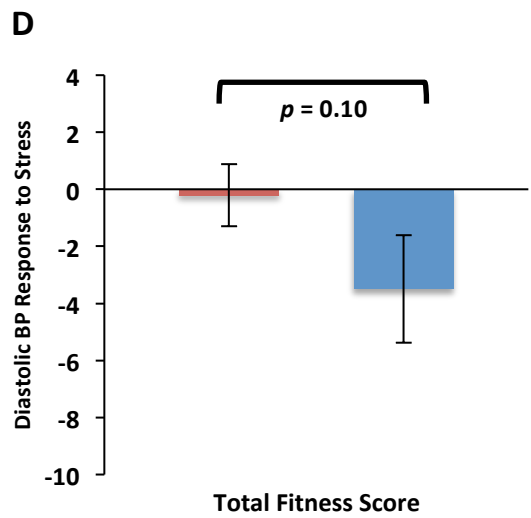
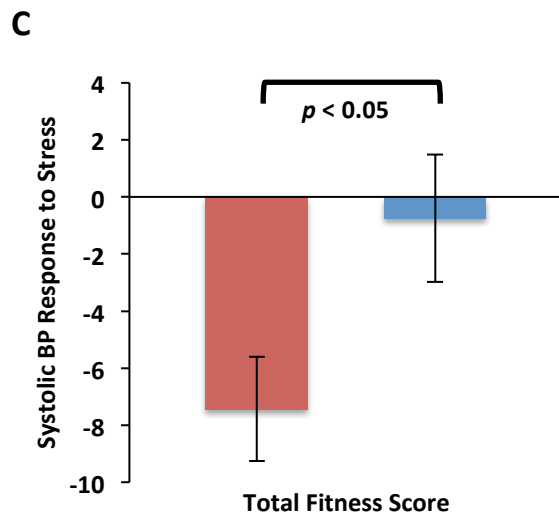
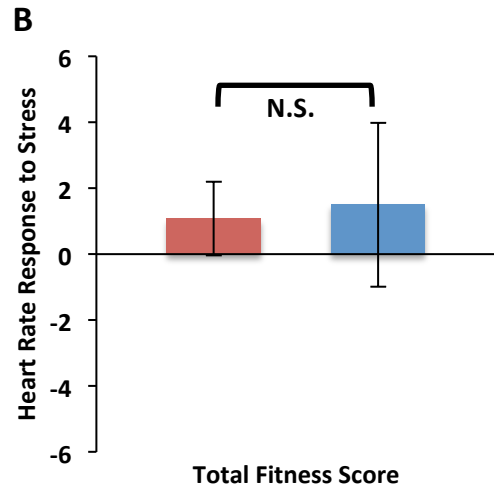
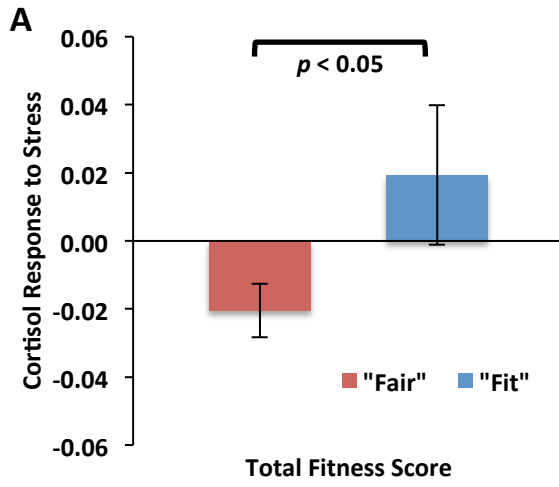


Figure 14. Effects of fitness on prefrontal cortex-dependent memory. No significant difference was found between the “Fair” and “Fit” groups for (A) the “n-back different” memory score ($t_{(18)}=1.10, p=0.286$) and (B) the “n-back same” memory score ($t_{(18)}=0.841, p=0.411$). The “n-back different” score refers to the subjects’ memory on trials in which the red square was in a different location as three trials earlier (requiring no response). The “n-back same” score refers to the subjects’ memory on trials in which the red square was in the same location as three trials earlier (requiring a response).

Figure 15. Effect of fitness on the acute stress response. (A) A significant difference was found between the “Fair” and “Fit” groups for the cortisol response to acute stress, n-back test ($t_{(20)}=2.275, p=0.034$). The “Fair” group had decreased cortisol levels than before the n-back test, while the “Fit” group had increased. (B) No significant difference was found for the heart rate response ($t_{(20)}=0.851, p=0.19$). (C) A significant difference was found for the systolic blood pressure response ($t_{(18)}=2.38, p=0.028$). While systolic blood pressure dropped in both groups after the n-back test, the drop was more pronounced in the “Fair” group than the “Fit” group. (D) No significant effect was found for the diastolic blood pressure response ($t_{(18)}=1.71, p=0.10$, Cohen’s $d=0.57$).



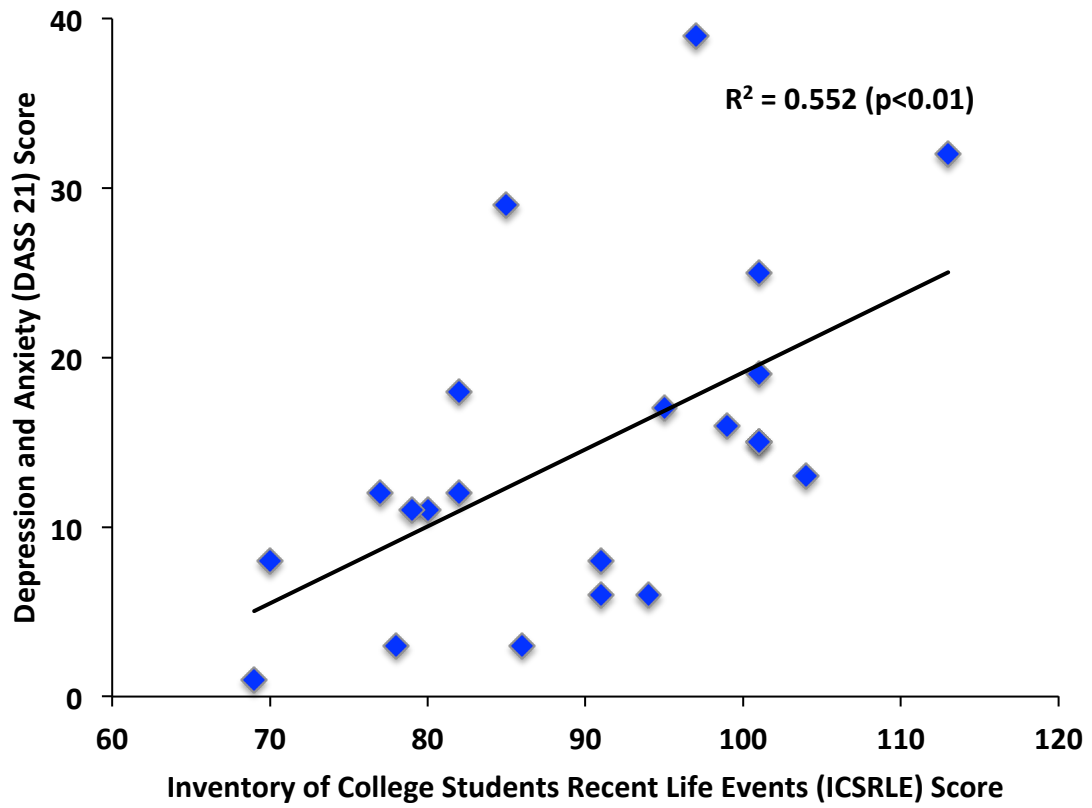


Figure 16. Relationship between self-reports of stressful life events and self-reports of depression and anxiety. A significant, positive correlation was seen between ICSRLE and DASS 21 scores ($r(22)=0.552, p=0.008$).

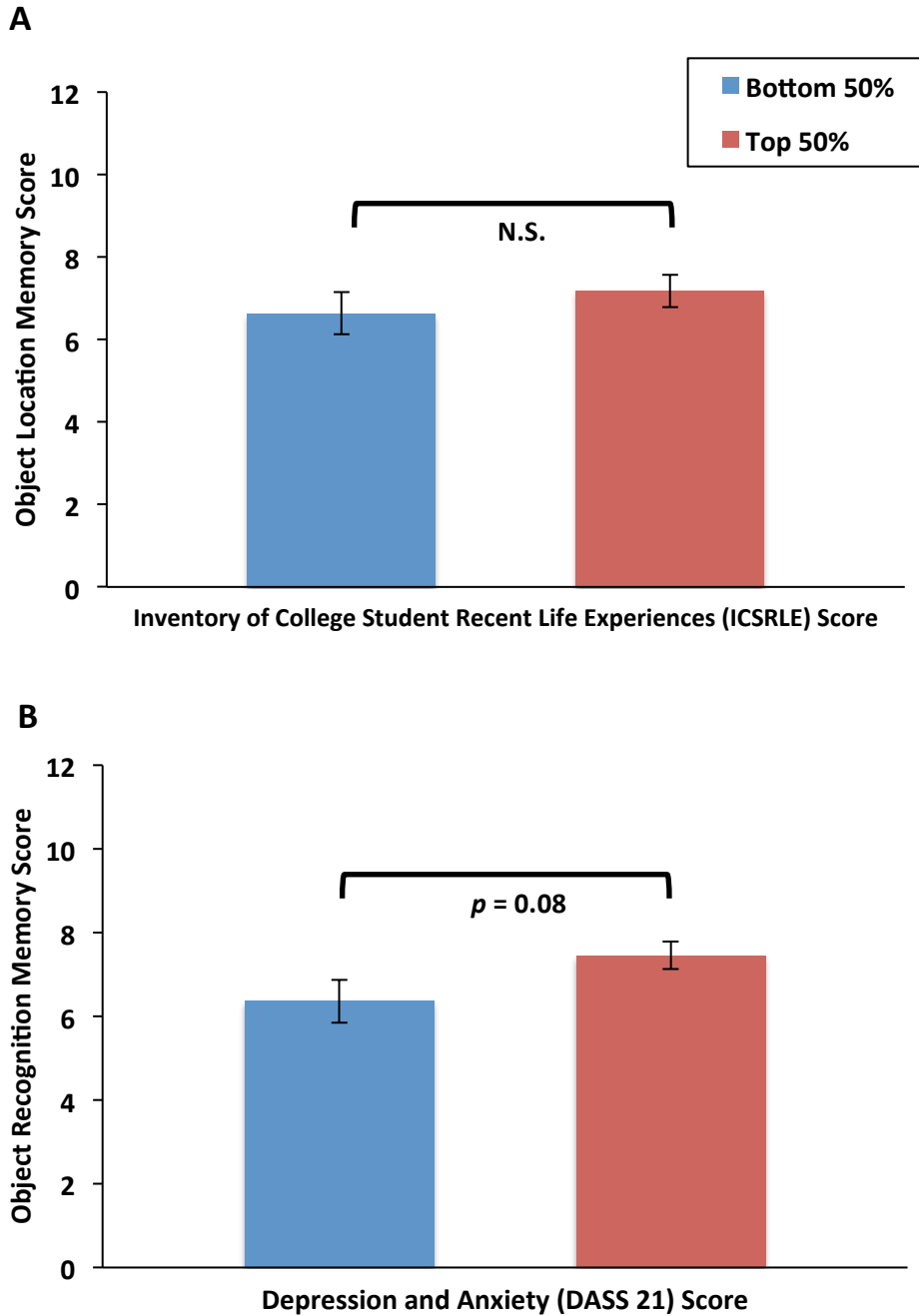
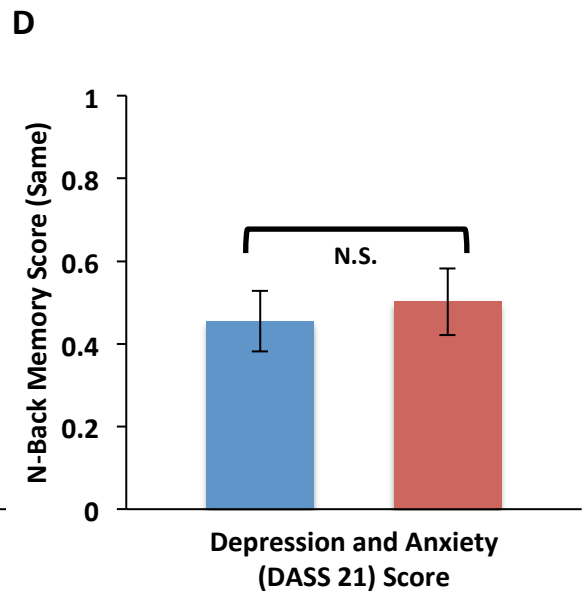
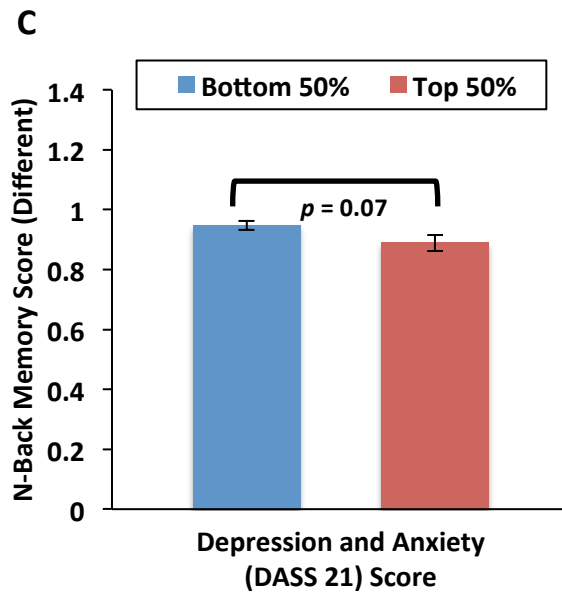
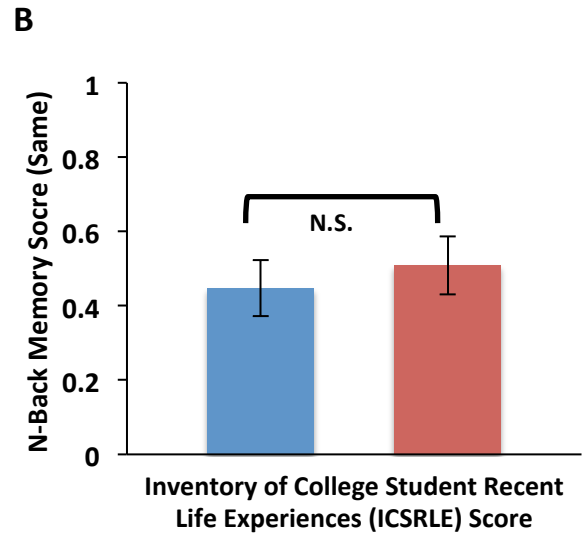
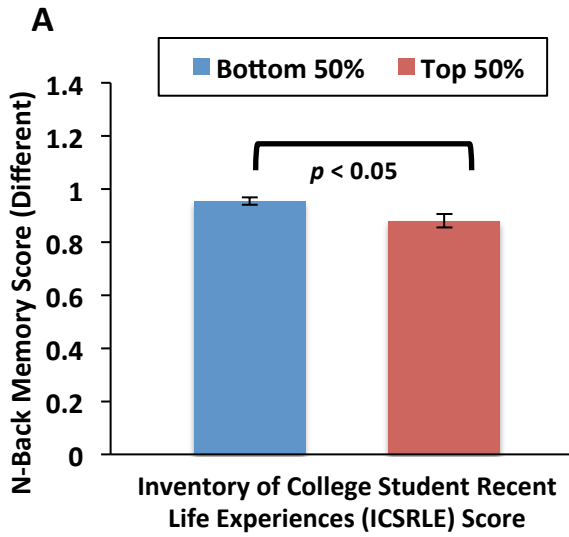


Figure 17. Effects of self-reports of stressful life event, depression and anxiety on hippocampus-dependent memory. (A) No significant difference was found for object location memory scores between the “Top 50%” and “Bottom 50%” ICSRLE groups ($t_{(20)}=0.881$, $p=0.389$). (B) A statistical trend was found object location memory scores between the “Top 50%” and “Bottom 50%” DASS 21 groups ($t_{(20)}=1.87$, $p=0.075$, Cohen’s $d=0.8$).

Figure 18. Effects of self-reports of stressful life events, depression and anxiety on prefrontal cortex-based memory. (A) A significant difference was found on “n-back different” memory scores between the “Top 50%” and “Bottom 50%” ICSRLE groups ($t_{(18)}=2.74, p=0.013$). The “Bottom 50%” group (meaning subjects in this group reported fewer stressful life events) got higher scores than the “Top 50%” group. (B) No significant difference was found on “n-back same” memory scores between the “Top 50%” and “Bottom 50%” ICSRLE groups ($t_{(18)}=0.13, p=0.898$). (C) A statistical trend was found for “n-back different” memory scores between the “Top 50%” and “Bottom 50%” DASS 21 groups ($t_{(18)}=1.95, p=0.066$, Cohen’s $d=0.87$). The “Bottom 50%” group (meaning subjects in this group reported lower levels of anxiety and depression) got higher scores than the “Top 50%”. (D) No significant difference was found on “n-back same” memory scores between the “Top 50%” and “Bottom 50%” DASS 21 groups ($t_{(18)}=0.446, p=0.661$).



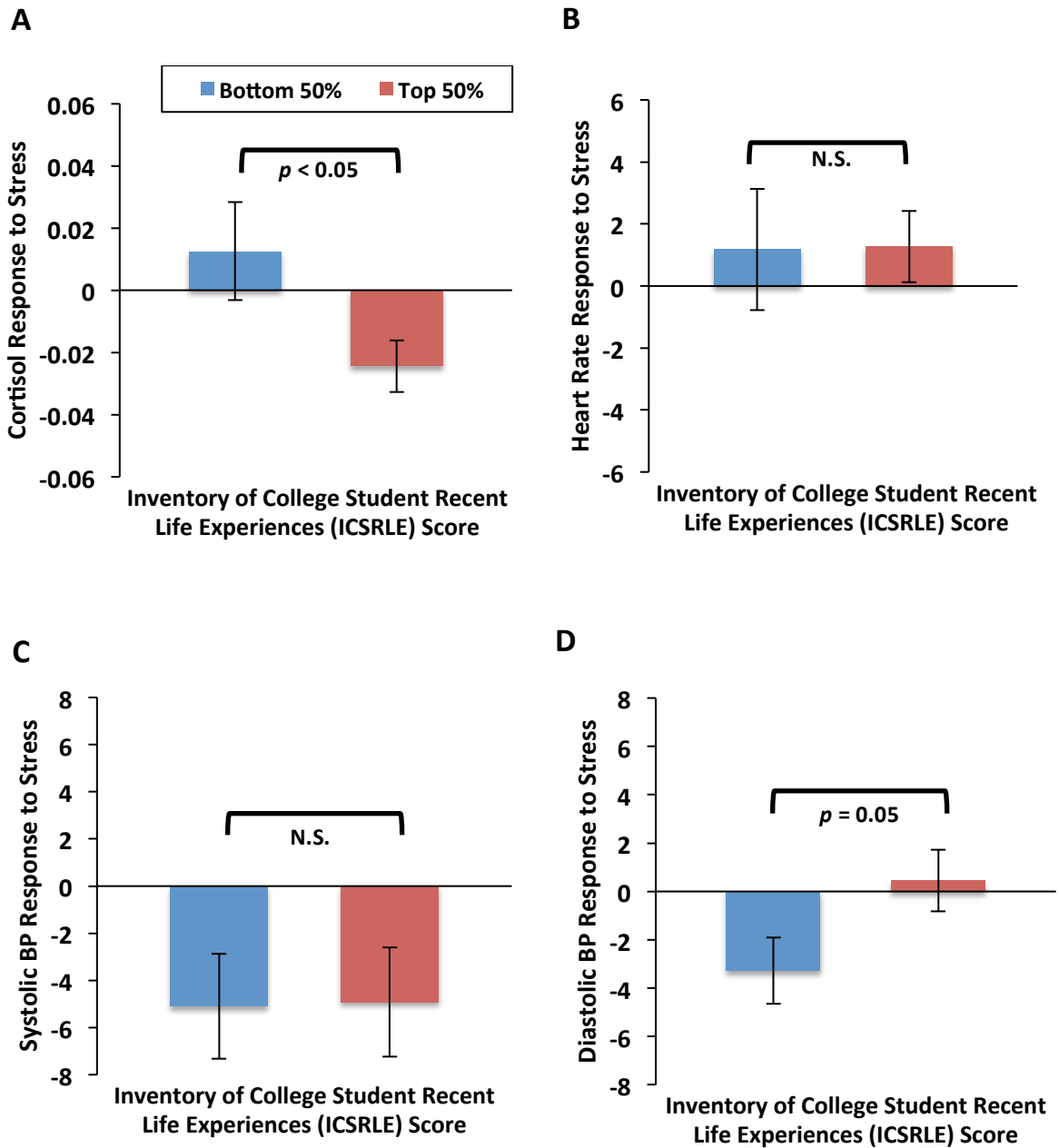


Figure 19. Effect of self-reports of stressful life event on the acute stress response.

(A) A significant difference was found between the “Top 50%” and “Bottom 50%” ICSRLE groups for the salivary cortisol response ($t_{(20)}=2.18, p=0.041$). Cortisol levels in the “Top 50%” ICSRLE group (meaning subjects in this group reported higher stressful life event) dropped after the n-back test. (B) No significant difference was found for the heart rate response ($t_{(20)}=0.044, p=0.964$). (C) No significant difference was found for the systolic blood pressure response ($t_{(20)}=0.063, p=0.953$). (D) A significant difference was found for the diastolic blood pressure response ($t_{(20)}=2.08, p=0.05$). Diastolic pressure dropped in the “Bottom 50%” ICSRLE group after n-back test.

Figure 20. Effects of self-reports of depression and anxiety on the acute stress response.

(A) A significant difference was found between the “Top 50%” and “Bottom 50%” DASS 21 groups for the salivary cortisol response ($t_{(20)}=2.30, p=0.027$). Cortisol levels in the “Top 50%” group (meaning subjects in this group reported higher levels of depression and anxiety) dropped after n-back test. (B) A non-significant trend was found on the heart rate response ($t_{(20)}=1.66, p=0.113$, Cohen’s $d=0.71$). (C) No significant difference was found on the systolic blood pressure response ($t_{(20)}=0.358, p=0.725$). (D) No significant difference was found on the diastolic blood pressure response between those two groups ($t_{(20)}=0.99, p=0.334$). The pattern of results was consistent with the ICSRLE data, but only the result of the diastolic blood pressure response was different.

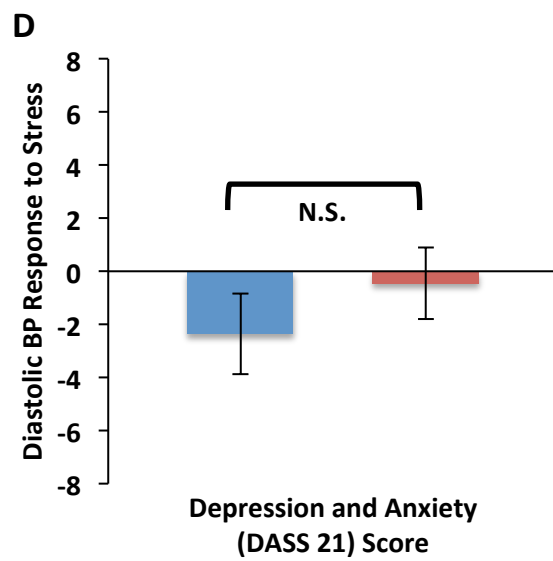
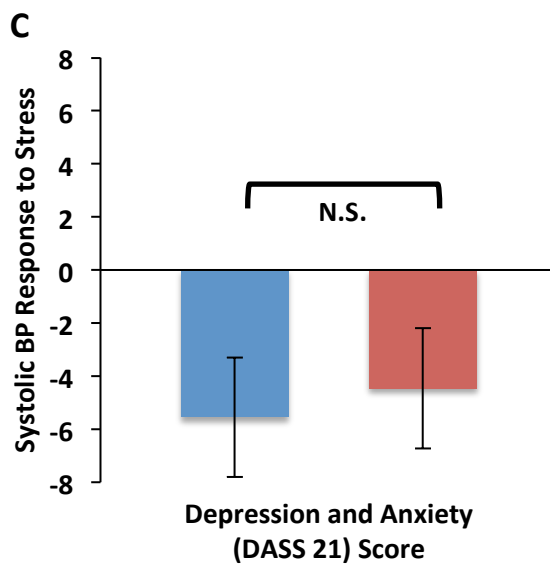
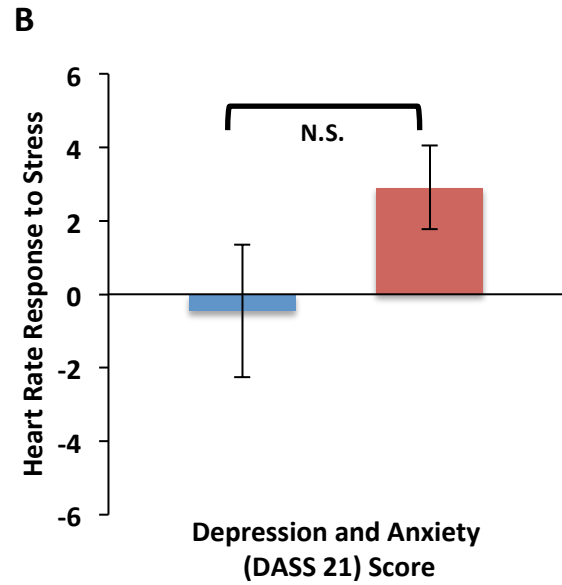
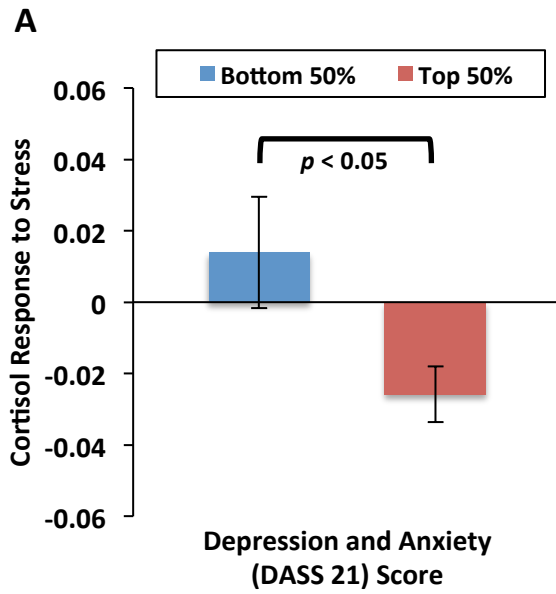


Table 1

Summary of Microfit[®] Fitness Variables

	All (n=22)	Males (n=10)	Females (n=12)
Age (years)	20.0 ± 0.5	20.2 ± 1.1	19.8 ± 0.4
Total Fitness Score	58.1 ± 2.70	58.4 ± 3.3	57.9 ± 4.4
Aerobic Fitness Score	41.6 ± 2.29	44.4 ± 4.5	39.3 ± 2.2
Systolic BP (mm Hg)	115 ± 2	120 ± 4	112 ± 3
Diastolic BP (mm Hg)	73 ± 2	75 ± 3	72 ± 2
Resting Deart Rate (bpm)	65 ± 5	55 ± 7	73 ± 5 *
% Body Fat	15.3 ± 1.5	9.5 ± 1.3	20.2 ± 1.5**

Note. Values = means ± S.E.M.

* $p < 0.05$. ** $p < 0.01$.

Table 2

Summary of Microfit[®] Fitness Variables for the N-back Test

	All (n=20)	Males (n=10)	Females (n=10)
Age (years)	20.0 ± 0.6	20.2 ± 1.1	19.8 ± 0.5
Total Fitness Score	57.7 ± 2.92	58.4 ± 3.3	57.0 ± 5.2
Aerobic Fitness Score	41.6 ± 2.53	44.4 ± 4.5	38.8 ± 2.6
Systolic BP (mm Hg)	116 ± 2	120 ± 4	112 ± 3
Diastolic BP (mm Hg)	73 ± 2	75 ± 3	71 ± 2
Resting Heart Rate (bpm)	63 ± 5	55 ± 7	72 ± 2
% Body Fat	15.1 ± 1.7	9.5 ± 1.3	20.8 ± 1.7*

Note. Values=means ± S.E.M.

* $p < 0.01$.

Table 3

Comparison of Cardiovascular Measures (Microfit[®] vs. N-back Test Day)

	All (n=20)		Males (n=10)		Females (n=10)	
	Microfit[®]	Test day	Microfit[®]	Test day	Microfit[®]	Test day
Systolic BP	115 ± 2	113 ± 2	120 ± 4	116 ± 3	112 ± 3	110 ± 2
Diastolic BP	73 ± 2	70 ± 2	75 ± 3	69 ± 2	72 ± 2	72 ± 2
Resting Heart Rate	65 ± 5	65 ± 2	55 ± 7	64 ± 2	73 ± 5	65 ± 3

Note. Values = means ± S.E.M.

Table 4

Summary of Self-reports of Stress, Depression and Anxiety and Stress Response

	Males (n=10)	Females (n=10)
Inventory of College Student Recent Life Experiences (ICSRL) Score	88.7 ± 4.3	90.8 ± 3.1
Depression Anxiety Stress Scale (DASS21) Score	11.1 ± 1.9	17.3 ± 3.3
Cortisol Response to Stress	-0.09 ± 0.02	-0.003 ± 0.01
Heart Rate Response to Stress (bpm)	1.2 ± 1.7	1.3 ± 1.4
Systolic BP Response to Stress (mmHg)	-3.2 ± 2.5	-6.5 ± 1.8
Diastolic BP Response to Stress (mmHg)	-0.1 ± 1.3	-2.5 ± 1.4

Note. Values = means ± S.E.M.

Table 5

Summary of the Original Hypotheses and the Results of the Current Pilot Study

	Original Hypotheses	Pilot Study Results	Cohen's <i>d</i>	#Subjects needed for significance
Effects of Fitness on Spatial Memory	Hypothesis 1: Higher fitness levels will be associated with better hippocampus-dependent spatial memory.	Partially supported	0.7	54
	Hypothesis 2: Higher fitness levels will be associated with better PFC-dependent spatial memory.	Not supported	--	--
Effects of Fitness on Physiological Response to Acute, Cognitive stress	Hypothesis 3: Higher fitness levels will be associated with a decreased cortisol response.	Not supported	--	--
	Hypothesis 4: Higher fitness levels will be associated with a decreased cardiovascular response (smaller heart rate & blood pressure changes).	Partially supported	0.57	38
Effects of Self-reports of Stress on Spatial Memory	Hypothesis 5: Higher levels of self-reported stress will be associated with impaired hippocampus-dependent spatial memory.	Not supported	--	--
	Hypothesis 6: Higher levels of self-reported stress will be associated with impaired PFC-dependent spatial memory.	Supported	--	--
Effects of Self-reports of Depression and Anxiety on Spatial Memory	Hypothesis 7: Higher levels of self-reported depression and anxiety will be associated with impaired hippocampal-dependent spatial memory.	Not supported	--	--
	Hypothesis 8: Higher levels of self-reported depression and anxiety will be associated with impaired PFC-dependent spatial memory.	Partially supported	0.9	24
Effects of Self-reports of Stress, Depression and Anxiety on Physiological Response to Acute, Cognitive Stress	Hypothesis 9: Higher self-reports of stress, depression and anxiety will be associated with a decreased cortisol response.	Supported	--	--
	Hypothesis 10: Higher self-reports of stress, depression and anxiety will be associated with an increased cardiovascular response (large heart rate and blood pressure changes).	Partially supported	0.7	48

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