

**ACUTE STRESS-INDUCED CORTISOL ELEVATION ENHANCES MEMORY  
CONSOLIDATION OF SIMILAR ITEMS**

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## **Abstract**

Though considerable evidence has indicated that stress-induced changes in cortisol crucially affect memory function, the precise relationship between cortisol and long-term memory remains poorly understood. Cortisol has been shown to enhance memory consolidation, the process of converting short-term memories into long-term memories, and impair memory retrieval, the process of accessing previously stored memories. However, very little is known about the way stress affects the hippocampus and its subregions, which have recently been shown to contribute critical functions to learning and memory. In the present study, we examined the effect of acute stress, induced by the Trier Social Stress Test, on a memory task designed to differentially tax the functioning of dentate gyrus and CA3 subregions of the hippocampus. The study aimed to determine whether stress affects hippocampal-dependent memory consolidation and retrieval processes in otherwise healthy young adults. It was observed that a stress-induced elevation of glucocorticoids during memory consolidation enhanced the ability to discriminate between highly similar stimuli while elevated glucocorticoids during memory retrieval had no significant effect on memory performance. Additional findings reported a dose-dependent effect of cortisol on memory function and negative correlations with self-report ratings of depression, anxiety, and perceived stress. Taken together, this study provides evidence that acute stress differentially affects the consolidation and retrieval stages of memory, and particularly enhances the encoding of highly similar items, a function thought to rely strongly on the dentate gyrus and CA3 subregions of the hippocampus.

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## **1. Introduction**

Exposure to stressful situations rapidly stimulates complex changes throughout the body and brain. Although stress is generally considered detrimental to the organism's well being, it induces changes that are evolutionary relevant for survival. In stressful situations, adrenal hormones are released to prepare the body to respond quickly to a perceived threat; for instance, blood flow is directed towards the muscles to prepare for action while accelerated breathing increases the availability of oxygen (for review, see Lupien et al., 2007). Stress also has prominent effects on cognition. During the initial stress response, rapidly unfolding neurochemical events exert immediate effects on attention, sensory, and mnemonic processes (de Kloet et al., 2005). When elevated during consolidation, the process of converting new memory traces into long-term storage, stress hormones have memory-enhancing effects; however when elevated during retrieval, the process of accessing previously stored memories, stress hormones may yield memory-impairing effects (for review, see Wolf et al., 2003). Stress-enhanced memory consolidation may have been evolutionarily adaptive to help adjust to a quickly changing environment while stress-impaired memory retrieval may be functionally relevant to prevent the interference of noise in the face of an immediate threat (de Kloet, 1998). Prolonged exposure to stress, known as chronic stress, is detrimental to health as it impairs growth and repair, suppresses immune function, and produces memory deficits by causing atrophy of the hippocampus (Herbert et al., 2006, McEwen 2000).

Though many studies have investigated the effects of acute and chronic stress on memory function broadly, there is limited research on the effect of stress on the different aspects of memory function and the contributions of the memory processes instantiated



by the different subregions of the hippocampus. Although it is well established that the hippocampus and the surrounding cortices in the medial temporal lobe are critically important for episodic memory function, recent studies have highlighted the unique contributions of the individual hippocampal subregions on the ability to learn and remember information. In particular, the dentate gyrus (DG) and CA3 subregions of the hippocampus contribute critically to every day memory function and appear to be sensitive to stressors and aging (for review, see Yassa & Stark, 2011; Fa et al., 2014). This study aims to investigate the effects of stress on hippocampal-dependent memory and hippocampal subregion-specific functions. Given the prevalence of high stress in many individuals today and the potentially lasting consequences of stress on the structure and function of the brain, it is important to further elucidate the relationship between stress and long-term memory function.

### *1.1. Neuroendocrinology of stress*

Exposure to a stressful, e.g. threatening or demanding, situation leads to the release of adrenal hormones that cause physiological and psychological changes to help cope with the stressor. The initial rapid response occurs through the sympathetic nervous system and results in the release of catecholamines. These hormones induce physical changes characteristic of the “fight or flight” response, which include increased heart rate and sweat production. They can also indirectly stimulate various regions of the brain, most notably the amygdala (Roozendaal, Okuda, de Quervain, & McGaugh, 2006). Catecholamines act through G-protein coupled receptors, producing fast-acting, transient signaling cascades in the cell.

The secondary response to stress is activation of the hypothalamic-pituitary-adrenal axis, named for the neuroendocrine organs that control this process. Through this pathway, the hypothalamus secretes corticotrophin-releasing hormone, which stimulates the pituitary gland to secrete adrenocorticotrophin, which in turn stimulates the adrenal cortex to secrete glucocorticoids (cortisol in humans, corticosterone in rodents). Glucocorticoids are steroid hormones that can pass through the blood brain barrier and bind to two different intracellular receptors: mineralocorticoid receptors and glucocorticoid receptors. These steroid receptors act as transcriptional regulators that change gene expression. Typically, mineralocorticoid receptors have a higher affinity for glucocorticoids, however, under times of stress, glucocorticoid receptors are overwhelmingly saturated instead (for review, see Harris et al., 2013). Thus, glucocorticoid receptors are believed to play a critical role in the stress response. Particularly high levels of glucocorticoid receptors are found in the hippocampus, which is known to be important for learning and memory. Glucocorticoids influence a range of cellular functions including dendritic remodeling, neurogenesis, and intracellular signaling that can be best described in terms of receptor activation (Herbert et al., 2006; Bisaz, Conboy & Sandi, 2009).

### *1.2. Animal studies: stress response and memory function*

Studies investigating the effects of stress on memory function in animals have used a variety of tasks to measure different aspects of memory. In rodents, the effects of glucocorticoids on hippocampal dependent spatial memory has been examined through water maze spatial and cued training, while general memory retention has been studied

with object recognition tasks and conditioned taste aversion tasks (de Quervain et al., 2009; Roozendaal et al., 2006). These studies show that memory processes depend on different brain systems and require specific learning tasks.

Studies have differentiated between acute and chronic, or prolonged, exposure to stressful conditions as they likely have differential effects on physiological and cognitive functioning. An acute stress response is important for a quick reaction to threatening situations and has been reported to have both positive and negative effects on cognition. Acute systemic administration of glucocorticoids during or immediately after a training experience enhanced memory consolidation, the process during which short-term memories are converted into long-term memories (McGaugh & Roozendaal, 2002). When conditioning paradigms were made more stressful for rats (e.g. by decreasing the temperature in a water maze to uncomfortable levels), there was an increased secretion of corticosterone and enhanced performance on memory tasks (Sandi, Loscertales & Guaza, 1997; Cordero, Merino & Sandi, 1998). The improvement presumably occurred as glucocorticoid levels, initially elevated from the training condition, were sustained and acted on the consolidation process, which takes place for an extended period of time after training. The enhancement in memory performance has been observed for simple conditioning paradigms, avoidance learning, and spatial tasks associated with hippocampal function (de Kloet, Vreugdenhil, Oitzl, & Joels, 1998; de Kloet, Oitzl, & Joels, 1999). McGaugh found that glucocorticoid effects were greatest when administered immediately after training and were generally ineffective when administered several hours after (1966, 1989). These results are consistent with the standard model of synaptic consolidation, which suggests there is a critical time frame following learning during

which the molecular mechanisms supporting memory function are susceptible to disruptions.

In addition to its enhancing effects on memory consolidation, elevated glucocorticoids have also been implicated for its impairing effects in the memory retrieval process. Animal studies in which testing occurred shortly after the administration of glucocorticoids pre- or post-training, i.e. when glucocorticoid levels were still elevated during memory retrieval, observed impaired performance on memory tasks. To distinguish between glucocorticoid effects on consolidation versus retrieval, it is necessary to maintain a long interval (i.e. 24 hours) between initial learning and retention testing. Rats that were exposed to foot shock stress 30 minutes before a water-maze task exhibited impaired retrieval of spatial memory from 24 hours prior (de Quervain, Roozendaal & McGaugh, 1998). Retention performance was not impaired in rats shocked two minutes or four hours before the water-maze task. These time-dependent effects correspond to the plasma corticosterone levels at the time of testing, suggesting a direct link between retrieval impairment and adrenocortical function.

Despite the varied effects of acute stress, chronic stress generally has detrimental effects on cognition and mood. Chronic stress occurs from the prolonged and repeated activation of the hypothalamic-pituitary-adrenal axis, causing a slow wear and tear on the mind and body. In addition to obesity, reduced immune system function, and synaptic deficits in the hippocampus (Kvarta et al., 2015), chronic exposure of rats to glucocorticoids disrupt concentration and decision making (Dias-Ferreira et al., 2009), induces depression-like and anxiety-like behaviors (Zhu et al., 2014), and impairs working memory and spatial memory (Mizoguchi et al., 2000). Notably, mild stress

induction without glucocorticoid elevation (inhibited by metyrapone) did not show depression-like behaviors in these animals, thus suggesting that the production and circulation of glucocorticoids play a key role in the pathology of depression (Zhu et al., 2014).

### *1.3. Human studies: stress response and memory function*

While memory-enhancing effects of glucocorticoids on consolidation have also been observed in humans (Buchanan & Lovallo, 2001; McCullough & Yonelinas, 2013), other studies have failed to find the same beneficial effects (Rimmele, Domes, Mathiak, Hautzinger, 2003). Furthermore, negative effects of cortisol on retrieval have been observed in humans on the delayed recall of words (de Quervain, Roozendaal & Nitsch, 2000; Smeets, Otgaar, Candel & Wolf, 2008) and pictures (McCullough, Ritchey, Ranganath & Yonelinas, 2015), but not in all studies (Boehringer et al., 2010; Rimmele et al., 2015). Though most studies induce a single surge in glucocorticoids via drug administration or through a stressful task, one study indicates that prolonged glucocorticoid treatment (e.g. at least several days) is needed before impairing effects on learning occur (Newcomer et al., 1999).

One explanation for the range of results observed might be due to the variety of memory tasks used in these types of studies. A meta-analysis by Het et al. found that eight out of 16 studies investigating acute effects of cortisol administration measured declarative (explicit) long-term memory performance by using simple word lists and two studies used word pairs (2005). Declarative memory, or memory for facts and events, can be separated into recognition memory, a sense of familiarity for previously encountered

stimuli, and recollection, the ability to clearly recall past events. Recognition memory was investigated in three studies while both recognition and recall performance was measured in six studies. Seven studies tested immediate recall while eleven tested delayed recall, and four studied both. Neuroimaging studies have observed differential neuronal activation in the hippocampus and perirhinal cortex during recall and familiarity of word and scene stimuli (Ryals et al., 2013) as well as variability in performance between word and picture versions of the Free and Cued Selective Reminding Test (Zimmerman et al., 2015). Moreover, there were limited correlations between three episodic memory tests despite their purpose to assess the same psychological process (Cheke & Clayton, 2013). Finally, some studies have used images (McCullough et al., 2015), false recall of word pairs (Smeets et al., 2008), and perceptual priming (Holz et al., 2014) while measuring memory performance. Overall, there are a plethora of tasks used to test declarative memory in humans that will likely yield varied results depending on the precise processes being investigated.

The heterogeneity in the findings of the various studies may also be attributed to design factors, including time of day (morning vs. afternoon) and timing of glucocorticoid treatment (before learning, after learning, or before retrieval) (for review, see Het et al., 2005). In humans, cortisol is secreted on a diurnal rhythm, with concentrations highest in the morning upon awakening and decreasing throughout the day; cortisol concentrations then rise again during sleep. There is a period of relative quiescence in the afternoon (around 2pm to 6pm) during which cortisol-related research commonly occurs, though other studies have been conducted in the morning instead (Kirschbaum et al., 1993, Hsu et al., 2003). A meta-analysis by Het et al. found

significant differences between studies that administered cortisol in the morning compared to the afternoon, potentially due to rhythmic changes in cortisol concentrations (2005). Furthermore, the secretion of glucocorticoids occurs with the secondary stress response that takes longer to act throughout the body. Upon stress induction, peaks in cortisol levels occur 10 min after cessation of stress exposure (Het et al., 2009; Petrowski et al., 2010; Smeets et al., 2008), although peaks have also been observed 20-25 minutes after the beginning of stress induction (Rohleder et al., 2001). The delayed peak response could have caused elevated glucocorticoids to act on multiple memory processes. For example, studies in which cortisol was administered before learning with recall being tested immediately afterwards would be difficult to interpret since it is unclear which memory phase(s) were affected (e.g. initial learning, consolidation, or retrieval).

Beyond the timing of the treatment, the levels of circulating glucocorticoids may also have an impact on memory performance. Glucocorticoids have been predicted to act on memory in an inverted U-shape dose response curve, where very high or very low glucocorticoid levels are associated with weaker memory performance compared to moderate glucocorticoid levels. This relationship has been reported in neuropsychopharmacology experiments in which memory impairment was observed for very high and very low stress responders, while memory enhancement was observed for moderate stress responders (Kovacs et al., 1976; Flood et al, 1978). In a more recent study, participants exposed to the cold-pressor stress test (a stress-inducing task that involves placing the participant's hand in cold water for up to three minutes) had overall better performance on the free recall test compared to the control group (Andreano & Cahill, 2006). Participants with a moderate glucocorticoid response from the cold-pressor

stress test had better recall performance compared to those with small or large stress responses (Andreano & Cahill, 2006).

The effects of glucocorticoids on different types of memories are also still not fully understood. Emotionally-charged memories have been reported to be better remembered than neutral memories (LaBar & Cabeza, 2006). Some studies suggest that emotional valence, the subjective evaluation of an experienced state, is less significant than emotional arousal, the physiological and psychological reaction to a stimulus through the sympathetic nervous system. Emotional valence is thought to be processed in the prefrontal cortex while emotional arousal is processed by the amygdala (Kensinger 2004). The amygdala is especially important for processing emotional memories and modulating the memory of arousing events. Lesions of the basolateral complex of the amygdala (BLA) blocked the memory-enhancing effects of post-training injections of glucocorticoids (McGaugh, 2004), but lesions of the adjacent central nucleus did not. This highlights the critical role of the BLA in facilitating the consolidation of emotional memories. Moreover, inhibition of beta-adrenergic receptors (thus preventing catecholamines from binding) in the BLA blocked the memory-enhancing effects of glucocorticoids (Roosendaal et al., 2006). These findings strongly suggest that modulatory effects of glucocorticoids are mediated only in part by direct binding of glucocorticoid receptors to the BLA and may require noradrenergic activation for emotional memory processing. While most studies indicate that memory was enhanced for emotional, but not neutral materials (Cahill et al., 2003; Smeets et al., 2008; Holz et al., 2014), others have found enhanced recall of both neutral and emotional memories (Nielson & Lorber, 2009) or even enhanced recall of neutral memories but not emotional



memories (Preuss & Wolf, 2009). Further analysis suggests that stress-related memory enhancements occurred in recognition but not recollection, though it remains unclear which memory processes (e.g. recollection or recognition) were influenced by stress (McCullough et al., 2013).

While acute stress can have enhancing as well as impairing effects, chronic stress has predominantly negative impacts on the human body and brain. The experimental induction of chronic stress in humans is obviously not feasible for ethical reasons, however prolonged activation of the hypothalamic-pituitary-adrenal axis has been linked to a range of health-damaging effects, including a weakened immune system (Sapolsky et al., 2000), obesity (de Vriendt, Moreno, and Henaarw, 2009), and poorer prognosis for cancer and heart disease (Maddock and Pariante, 2001). Chronic stress is associated with reduced hippocampal volume, a decrease in neurogenesis, and abnormal diurnal rhythms, which can all lead to long-term detrimental effects on memory (McEwen, 2001; Schulz, Kirschbaum, Pruner, & Hellhammer, 1998). In addition to inhibiting proliferation, excess glucocorticoids reduce the survival of immature neurons in the dentate gyrus subregion of the hippocampus (Wong & Herbert, 2015). Studies show that chronic stress results in dendritic atrophy in the CA3 region of the hippocampus, leading to spatial memory and declarative memory dysfunction (Herbert et al., 2006). Finally prolonged hypothalamic-pituitary-adrenal axis alterations have been implicated in many other neurological and psychiatric disorders including post-traumatic stress disorder (PTSD) and schizophrenia (for review, see Wolf, 2007; Holz et al., 2014).

#### *1.4. Pattern separation and pattern completion in long-term memory function*

It is well established that the structures of the medial temporal lobe, particularly the hippocampus and surrounding cortices, play a critical role in declarative memory, a type of long-term memory that allows the storage and retrieval of facts and events (Scoville & Milner, 1957). Computational studies of hippocampal memory function have suggested that declarative memory is supported by two distinct and complementary processes called pattern separation and pattern completion. Pattern separation, the ability to differentiate highly similar experiences into distinct non-overlapping representations, relies specifically on dentate gyrus (DG) and is crucial to reducing interference in the formation of new episodic memories. Balanced against pattern separation is the process of pattern completion, which allows for the re-instantiation of a memory based on partial or noisy degraded input. The significance of pattern separation and the DG has been previously examined in rodents exposed to environments of varying similarity (Leutgeb et al., 2004; Wilson et al., 2005). Computational models of the hippocampus suggest that the DG granule cells are especially well suited to facilitate pattern separation due to its anatomic and network properties, such as the high density of granule cells, sparse pattern of activation, and powerful projecting signals to CA3 pyramidal cells (for review, see Yassa et al., 2011).

Recently, behavioral tasks to tax principles of pattern separation in humans have been developed to parallel rodent studies in this area (Kirwan & Stark, 2007; Bakker et al., 2008; Stark et al., 2013). While only an inferential measure, these behavioral tasks can be used to assess neurobiological changes and indirectly measure DG and CA3-mediated memory function. High-resolution imaging studies in humans have shown that

brain activity specifically in the DG/CA3 subregion<sup>1</sup> of the hippocampus is consistent with pattern separation, whereas brain activity in other regions, such as the CA1, the subiculum, and the entorhinal and parahippocampal cortices, is consistent with pattern completion (Bakker et al., 2008). Pattern separation is a particularly sensitive measure to stressors and aging (for review, see Yassa & Stark, 2011; Fa et al., 2014). Impaired pattern separation is also observed in multiple neurological and psychiatric conditions. In patients with very early stages of Alzheimer's disease, impairments in pattern separation performance can be detected even before other symptoms of cognitive decline are manifested (Stark et al., 2013). Depression and anxiety disorders such as PTSD have been associated with pattern separation deficits that are attributable to dentate gyrus dysfunction (for review, see Kheirbek et al., 2012; Shelton & Kirwan, 2013). These findings emphasize the utility of using a pattern separation task as an indirect measures of DG/CA3 functioning, i.e. in situations where imaging may not be possible or practical.

### *1.5. Overview of the present study*

The present study aimed to investigate the effects of acute stress on DG/CA3-dependent memory function, in particular pattern separation, to reflect an individual's ability to encode and retrieve novel information. Although many studies have focused on the effects of stress on memory processes and some research has been done on other factors influencing episodic memory, there are, to our knowledge, no reports that have investigated the role of stress on pattern separation and completion. In this study, we used

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<sup>1</sup> In human neuroimaging studies, the spatial resolution of fMRI data remains insufficient to reliably separate the dentate gyrus and CA3 subregions of the hippocampus. In human fMRI studies, the DG and CA3 subregions are thus considered as a single locus of activation.

the Trier Social Stress Test (TSST), a laboratory protocol that combines public speaking, mental arithmetic, threat anticipation, and social evaluation, to produce a robust and reliable response in the hypothalamic-pituitary-adrenal axis in humans (Kirschbaum et al., 1993). We also used an incidental encoding task designed to tax pattern separation processes of memory (Kirwan & Stark, 2007; Bakker et al., 2008; Stark et al., 2013). Together, these procedures assessed whether acute stress affects the discrimination accuracy of highly overlapping stimuli. We hypothesized that acute stress would enhance memory consolidation and impair retrieval due to mechanistic differences in each of the processes, consistent with previous studies. Our results allowed us to infer behavioral changes in pattern separation performance to the role of stress on specific hippocampal subfields supporting pattern separation and pattern completion. Understanding acute stress on critical memory processes in otherwise healthy young adults may shed light on the how stress differently affects various memory processes and how dysfunctional regulation of the system may cause memory deficits seen in neurological diseases.

## **2. Methods**

### *2.1. Participants*

Eighty healthy young adult males participated in the study (mean age, 19.72 years; standard deviation, 1.47 years; range, 18-24 years). Females were excluded to avoid potential confounds due to menstrual cycle phases and use of hormonal contraceptives (Kirschbaum et al., 1999; Bouma et al., 2009). Inclusion criteria required all participants to be young adult males between the ages of 18 and 30 years old, native English speakers, and able to provide written informed consent. Exclusion criteria prevented individuals who met any of the following characteristics from participating in the study: major psychiatric or behavioral disorders (including current major depression, psychosis, bipolar disorder, post traumatic stress disorder/attention deficit disorder, attention-deficit/hyperactivity disorder, autism, schizophrenia), major neurological conditions (including multiple-sclerosis, intra-cerebral hemorrhage, embolic occlusion of major cortical vessels, seizure disorder, progressive tumors), or presence of clinically significant disease (cardiac disease, respiratory disease, primary or metastatic intracranial neoplasm, severe head trauma). These criteria were evaluated through interviews and self-report.

All participants provided written informed consent and were compensated with class credit. The study was conducted in accordance with guidelines of the Johns Hopkins Medical Institutions Institutional Review Board.

## *2.2. Study design and procedures*

Participants were randomly assigned to one of four groups: consolidation-stress, consolidation-control, retrieval-stress, and retrieval-control (the timeline of the experimental groups is illustrated in Figure 1A). In the consolidation-stress condition, exposure to stress was aimed to particularly influence the conversion of short-term memories into long-term memories. Participants in the consolidation-stress group first completed the encoding phase of the memory task, which presented images of objects for the participant to study. Immediately afterwards, they were exposed to a stressful situation through the Trier Social Stress Test (TSST). In the second visit that occurred 24 hours later, participants completed the recognition phase of the memory task, which tested the participant's memory for the images seen the day before. In the retrieval-stress group, exposure to stress was aimed to influence the process of retrieving long-term memories into working memory. Participants in the retrieval-stress group completed the encoding phase of the memory task during their first visit, and then were exposed to a stressful situation with the TSST and recognition phase of the memory task in the second visit. All control participants in the study completed a non-stressful, control version of the TSST (control-TSST). Of the control participants, half were exposed to the control-TSST after the encoding phase of the memory task (consolidation-control group) while the other half were exposed prior to the recognition phase of the memory task (retrieval-control group). All participants were asked to provide saliva samples immediately prior to and ten minutes following the TSST or control-TSST test. After all measures were completed, participants were debriefed and compensated for their time.

### *2.3. Saliva sampling and biochemical analysis*

In order to minimize differences in baseline cortisol levels and enable adequate saliva sampling for cortisol assessment, participants were asked to refrain from the consumption of alcohol and any recreational drugs 12 hours before each session; heavy physical activity and caffeinated drinks 3 hours before each session; and food, non-caffeinated drinks, tooth brushing/flossing, and smoking 1 hour before each session. To reduce the impact of diurnal variation in cortisol levels, all testing was performed in the afternoon between 3:00 P.M. and 6:00 P.M., when hormone levels are relatively stable. Upon arrival, participants were asked to rinse their mouths with water to prevent contamination of the saliva sample.

Saliva samples were collected to obtain free cortisol levels, a marker of hypothalamic-pituitary-adrenal axis activity. Saliva samples were collected by the passive drool method into 2mL cryovials. Samples were kept at -20°C until they were analyzed. Saliva was assayed for salivary cortisol concentrations using a commercially available immunoassay kit (Salimetrics). The minimal concentration of cortisol that could be distinguished from 0 was 0.007 µg/dL. The intra-assay coefficient of variation (n=38) was 3.7%, and the inter-assay coefficient of variation (n=5) was 10.2%.

### *2.4. Behavioral pattern separation task*

The Behavioral Pattern Separation Task-Object Version (BPS-O) is a computer based pattern separation task developed by Stark and colleagues that has been validated and extensively studied (Stark et al., 2013, Stark et al., 2015).

There were two phases in the BPS-O task: encoding and recognition (Figure 1B). In the incidental encoding phase, participants passively encoded pictures of everyday objects by determining whether they were indoor or outdoor objects via a button press (128 items total, 2.5 s each, with an interstimulus interval (ISI) of 0.5 s). In the recognition phase performed 24 hours later, participants were tested on whether images they saw were “old,” “similar,” or “new” to the images they saw in the study phase (192 items total, 2.5 s each, 0.5 s ISI). One-third of the images (64 images) were exactly the same from the study phase (repeats), one-third of the images were similar but not identical to those seen during the study phase (lures), and one-third of the images were novel images seen for the first time (firsts). The critical trials were the lure trials assessed by the rates at which the participant correctly identified lure items as “similar.” Incorrectly identifying lures as “old” is believed to reflect a bias towards pattern completion (e.g. overgeneralization of a memory) and an inability to utilize newly encoded differentiating information.

Images from the behavioral pattern separation task were previously normed by similarity (change in input) with a large, otherwise healthy, young adult population to generate mnemonic similarity ratings for each pair of lure and repeat items (Yassa et al., 2010; Lacy et al., 2011). Theoretically, repeat items should have no changes in input, first (novel) items should have large changes in input, and lure items should have variable changes in input depending on the degree of similarity between the lure item and its corresponding repeat item. Based on the probability of incorrectly calling lures “old,” the lure items were divided into five bins, with the most mnemonically similar lures in lure bin 1 (L1) and least mnemonically similar lure items in lure bin 5 (L5) (Figure 1C).



**Figure 1. (A)** Outline of study design. Participants in the consolidation-stress group completed the encoding phase of the memory task followed by the TSST in the first visit. In the second visit, 24 hours later, participants completed the recognition phase of the memory task. Participants in the retrieval-stress group completed the encoding phase of the memory task during their first visit, and then were exposed to the TSST and recognition phase of the memory task in the second session. Consolidation-control and retrieval-control groups followed the same sequence and timing of procedures as the corresponding experimental groups, but completed a non-stressful control-TSST instead. **(B)** Behavioral pattern separation task. On the first day, participants completed an incidental indoor-outdoor judgment task during the encoding phase. Twenty-four hours later, recognition was tested using an old-similar-new judgment task with repeats, firsts and lure images. **(C)** Sample stimuli and their lures, arranged in order of mnemonic similarity from most similar (L1) to least similar (L5).



### *2.5. Trier Social Stress Test*

The standardized stressful component of the TSST was divided into three 5-minute parts. In the first part, participants were asked to prepare a speech explaining why they were the best candidates for an ideal job. Participants were allowed to use pen and paper during the preparation, but were not allowed to use their notes during the presentation. In the second part, the participants presented their speech to a panel of two researchers, one male and one female, posing as expert judges. The judges maintained neutral expressions and observed the participant without comment. If the participants did not use the full 5 minutes allocated for the part, the judges asked the participants to continue until time was up. In the final part of the TSST, the participants performed a mental arithmetic task by serially subtracting the number 13 from 3087. If a mistake was made, the judges asked the participants to start again from the beginning.

Prior to and immediately following the stressful portion of the TSST, participants were given a 10-minute rest period and 10-minute recovery period. The rest period gave participants a chance to reach baseline hormone levels before beginning the stressful task. The recovery period served to coincide the post-TSST saliva sample with the delayed cortisol peak response.

The standardized control version of the TSST was designed to be as similar as possible to the TSST, but without the stressful components, e.g. specifically lacking the social evaluative threat (Het et al., 2009). The control-TSST was performed in the same room as the TSST. Instead of preparing a speech to give in front of a panel of judges, the control-TSST gave participants 5 minutes to prepare for a 5-minute talk about a movie, novel, or recent holiday trip. Participants were told that they would speak alone in an

empty room, and were given paper and pen to prepare and use throughout the task. The researcher only entered the room between each part to give instructions to the participants. After preparing and talking on the topic of their choice, participants were asked to begin serially adding by increments of 15, starting at 0. The researcher, once again, left the participants alone to complete the task, and controlled for the participants' compliance by asking them for the final number reached in the serial addition.

## *2.6. Self-report questionnaires*

During the 10-minute recovery period following the TSST, participants completed self-report questionnaires, including the Beck Anxiety Inventory, designed to assess severity of anxiety over the past week; the Beck Depression Inventory designed to assess severity of depression over the past week; the Pittsburgh Sleep Quality Inventory aimed to assess sleep quality over the past month; the Cohen Perceived Stress Scale to examine perceived stress over the past month, and an exercise questionnaire to assess total hours exercised over the past week. The State Trait Anxiety Inventory was also given to participants before and after the TSST as a subjective measure of pre and post-psychological stress.

### **3. Results**

#### *3.1. Parameters for analysis*

Accuracy for correctly identifying novel and repeated images was used to ensure that participants understood and completed the task as instructed. Eleven participants (2 from the consolidation-stress group, 2 from consolidation-control, 4 from retrieval-stress, and 3 from retrieval-control) were excluded from data analysis due to poor memory task performance, which was defined as scoring below a 33% (chance) correct response rate for first or repeat images. Seven participants (3 from the consolidation-stress group, 2 from consolidation-control, 1 from retrieval-stress, and 1 from retrieval-control) were further excluded from cortisol data analysis due to undetectable salivary cortisol levels in the samples collected either pre- or post-TSST administration. After removing these participants, the consolidation-stress group included 21 participants, the consolidation-control group included 10 participants, retrieval-stress group included 20 participants, and retrieval-control group included 11 participants.

We examined differences between the two control subgroups (consolidation-control and retrieval-control) and found no significant differences between the groups for all values measured (Table 1). Therefore we collapsed the data from these groups to form a single combined control group (n=24 for behavioral data, n=21 for cortisol analysis) for the rest of our analysis.

Characteristic	Consolidation-control		Retrieval-control		Statistics		
	Mean	SD	Mean	SD	p value	t ratio	df
Age	20.00	2.00	19.46	1.13	0.44	0.78	22
BAI	14.17	9.09	12.08	6.24	0.52	0.65	22
BDI	6.67	5.21	9.17	5.01	0.10	1.73	22
PSS	16.42	4.17	15.67	4.77	0.19	1.37	22
PSQI	6.00	2.70	6.67	3.14	0.58	0.56	22
Exercise (total h/wk)	4.04	3.99	5.25	4.16	0.48	0.73	22
Pre-STAI score	32.17	7.42	36.75	7.11	0.13	1.54	22
Post-STAI score	34.50	6.47	37.08	6.40	0.33	0.98	22
Pre-CORT (ug/dL)	0.16	0.24	0.16	0.11	0.90	0.13	19
Post-CORT (ug/dL)	0.18	0.24	0.18	0.11	0.98	0.02	19
Repeats called "old"	0.62	0.18	0.64	0.08	0.75	0.32	22
Lures called "similar"	0.33	0.11	0.33	0.11	0.90	0.13	22
Firsts called "new"	0.77	0.15	0.76	0.09	0.87	0.16	22

**Table 1.** Clinical data and analysis of control group participants

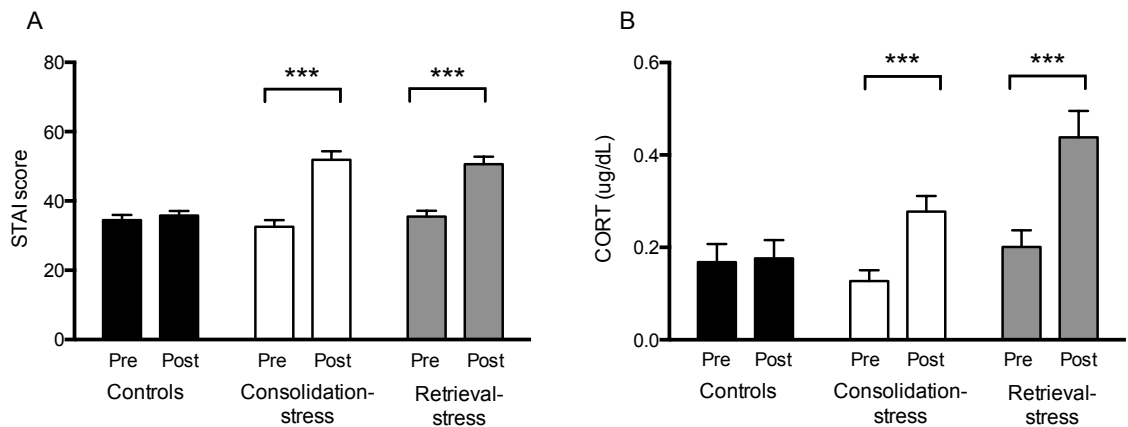
Note: BAI= Beck Anxiety Inventory, BDI= Beck Depression Inventory, PSS= Perceived Stress Scale, PSQI= Pittsburg Sleep Quality Index, STAI= State Trait Anxiety Inventory, CORT= salivary cortisol concentration.

### 3.2. Effectiveness of stress induction

Analysis of the self-report State Trait Anxiety Inventory (STAI) before and after administration of the TSST revealed that the TSST successfully induced subjective psychological stress (Figure 2A). Prior to the TSST, STAI scores did not differ between the control group, consolidation-stress group, and retrieval-stress group ( $F(2,66)=0.72$ ,  $p=0.49$ ). Ten minutes after the TSST, STAI scores were significantly higher in the consolidation-stress group ( $t(23)$ ,  $p<0.0001$ ) and retrieval-stress group ( $t(20)=6.37$ ,  $p<0.0001$ ) compared STAI scores within the same group before the administration of the

TSST. Participants in the control conditions did not exhibit an increase in subjective stress following the control-TSST ( $t(23)=1.18$ ,  $p=0.25$ ).

To verify that the TSST also induced a physiological stress response, we compared the salivary cortisol levels between stress and control groups (Figure 2B). Prior to the TSST, concentrations of salivary cortisol did not differ between the control group, consolidation-stress group, or retrieval-stress group ( $F(2,59)=1.218$ ,  $p=0.30$ ). Ten minutes after the TSST, concentrations of salivary cortisol were significantly higher in the consolidation stress group ( $t(20)=4.90$ ,  $p<0.0001$ ) and retrieval stress group ( $t(19)=5.26$ ,  $p<0.0001$ ) compared to the salivary concentrations within the same group before the administration of the TSST. Participants in the control conditions did not exhibit an increase in salivary cortisol following the control-TSST ( $t(20)=0.47$ ,  $p=0.65$ ).



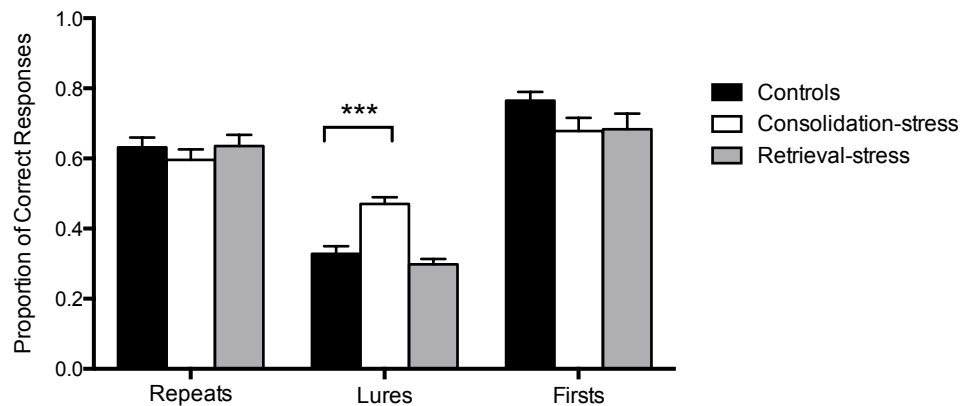
**Figure 2.** The TSST induces a psychological and physiological response to stress. Mean (A) STAI scores and (B) salivary cortisol concentrations are higher post-TSST for stress groups but not for control groups. Error bars represent standard error of the mean.

\*\*\*  
 $p<0.001$

### 3.3. Memory performance

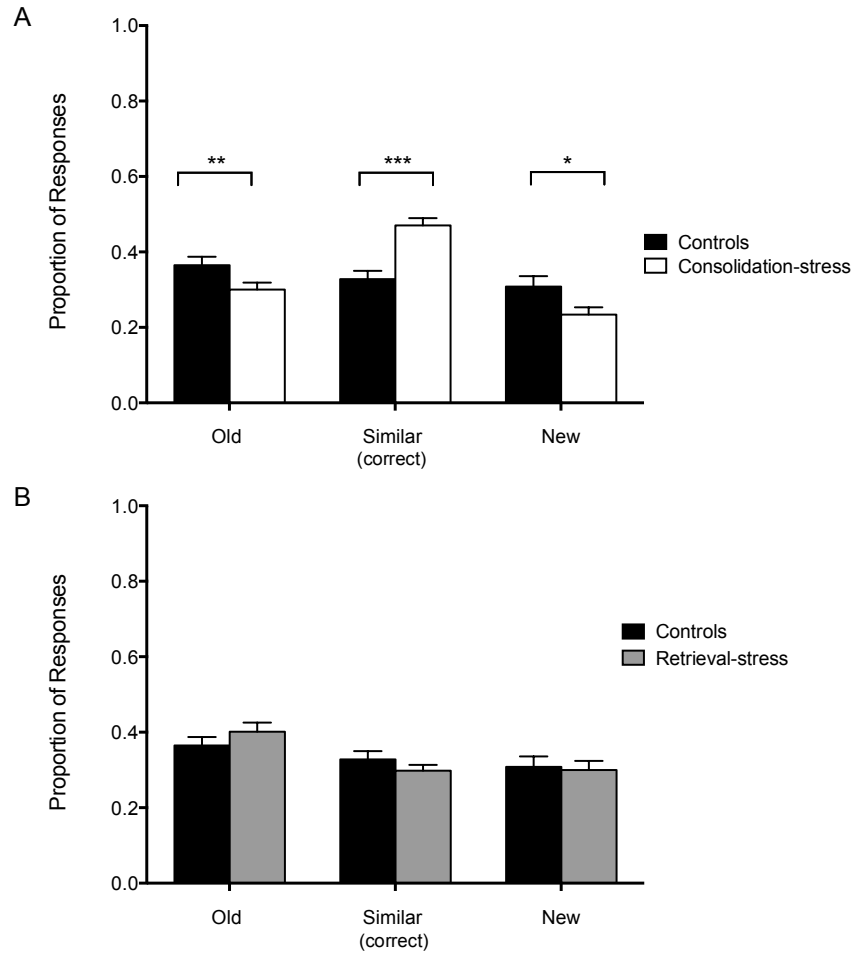
Participants identified repeats as “old” and firsts as “new” at similar rates across groups (Figure 3). This performance was consistent with a previous study using a similar population (Borota et al., 2013).

To determine whether the ability to differentiate the critical lure items was affected by stress, we examined the proportion of lure stimuli correctly identified as “similar.” Multiple paired t-tests were used to compare pattern separation performance between the consolidation-stress and control groups as well as the retrieval-stress and control groups. Pattern separation performance is defined as the proportion of correct responses to lure items. Participants in the consolidation-stress condition were significantly more likely to correctly call lure items “similar” than the participants in the control condition, who were more likely to call lure items “old” ( $F(2, 138)=15.90$ ,  $p<0.0001$ ) (Figure 4A). There was no significant difference in lure response between retrieval-stress and control groups ( $F(2,129)=1.055$ ,  $p=0.35$ ) (Figure 4B).



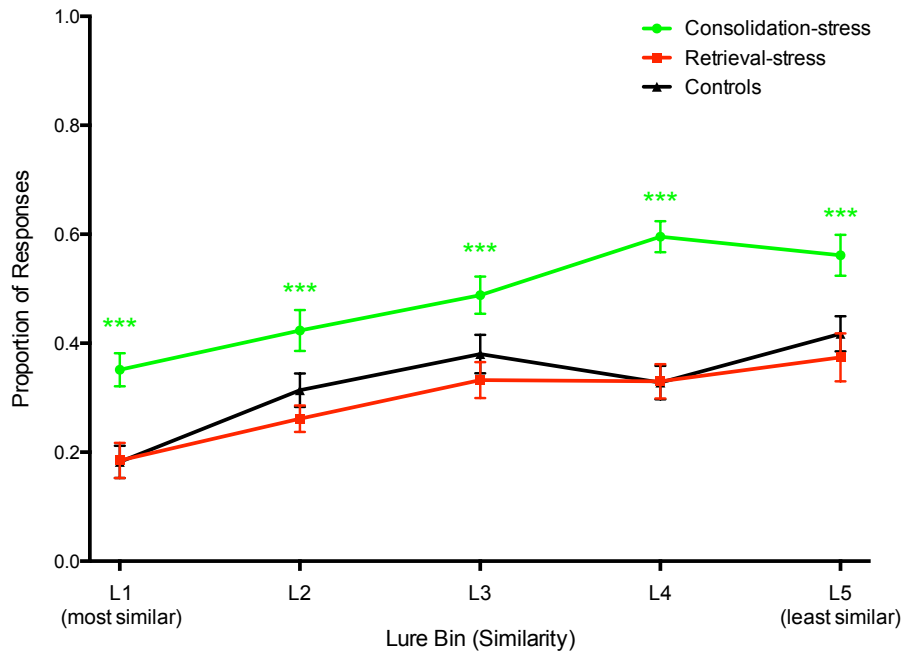
**Figure 3.** Stress during consolidation, but not retrieval, enhances the identification of lure items. Error bars represent standard error of the mean. \*\*\* $p<0.001$





**Figure 4.** Distribution of responses to lure items (**A**) The consolidation-stress group was more likely to call a lure item “similar” than “old.” (**B**) The retrieval-stress group identified lure items at similar rates as controls. Error bars represent standard error of the mean. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$

We next considered whether pattern separation performance was affected by varying changes in the input (degree of similarity). From our results, the consolidation-stress group correctly identified more lure items as “similar” compared to the control and retrieval-stress groups (Figure 5). This performance occurred across all lure bins, without any bias for high similarity or low similarity. The retrieval-stress group did not perform significantly different from the control group across similarity categories.



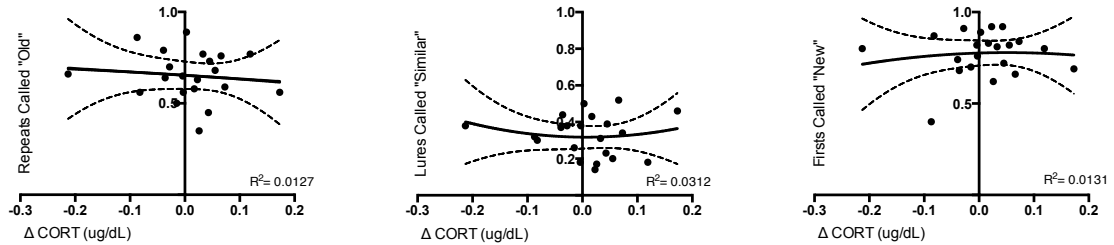
**Figure 5.** Proportion of correct responses by lure bin (similarity). Error bars represent standard error of the mean. \*\*\*  $p < 0.001$

### 3.4. Cortisol responses and memory

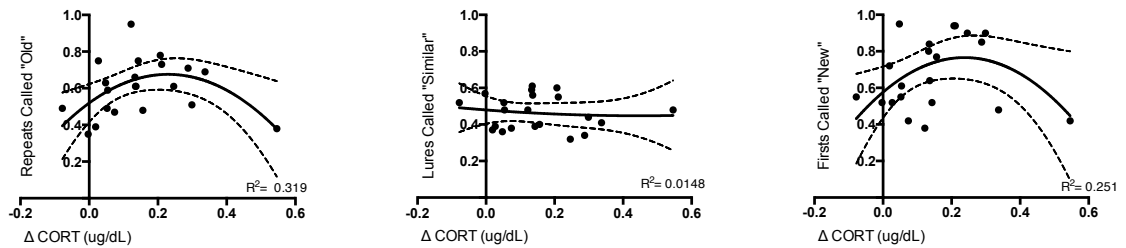
We subsequently examined the relationship between stress-related changes in cortisol and memory performance by plotting the change in cortisol concentration between the pre- and post- samples with the proportion of correct responses to items on the behavioral pattern separation task (Figure 6). The control group showed weak quadratic regressions between cortisol response and memory performance for all items (Figure 6A). The consolidation-stress group also showed a very weak quadratic regression between the change in cortisol concentration and identification of lure items, but a strong quadratic relationship between the change in cortisol concentration and identification of repeat and first items (Figure 6B). The quadratic plots reveal an inverted U-shaped relationship between changes in cortisol concentration and memory

performance, such that consolidation-stress group participants with moderate cortisol responses more often identified items correctly than participants with lower or higher cortisol responses. The retrieval-stress group showed a moderately weak quadratic relationship of cortisol responses and memory performance for all items (Figure 6C).

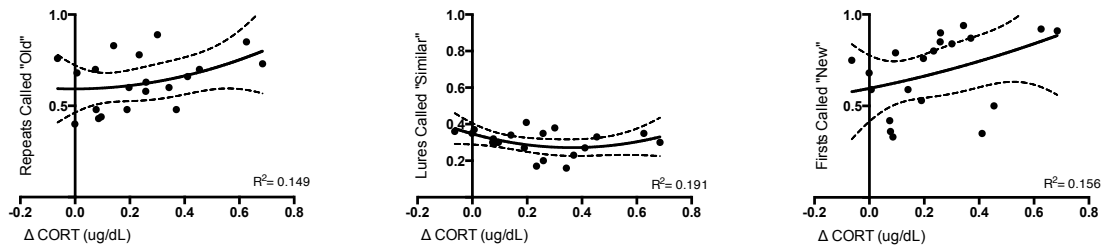
**A. Controls**



**B. Consolidation-stress**



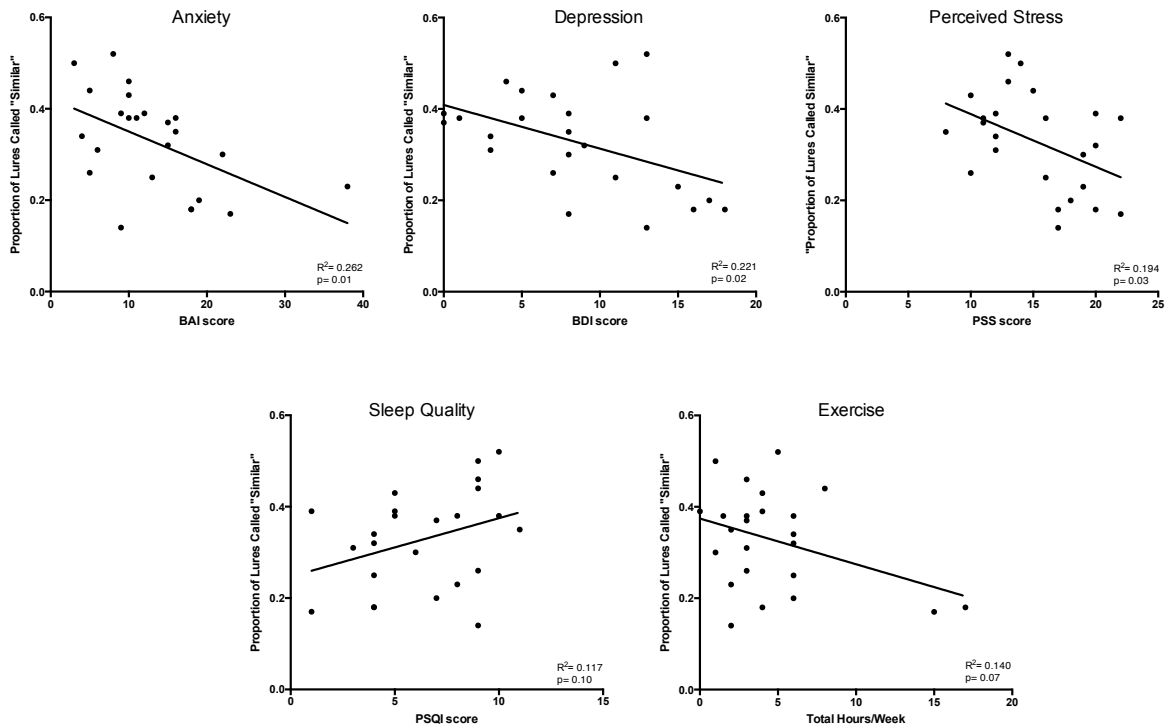
**C. Retrieval-stress**



**Figure 6.** Memory performance is correlated with change in cortisol concentration. **(A)** The control group follows a weak quadratic relationship for all items. **(B)** The consolidation-stress group follows a strong quadratic function for correctly identifying repeat and first items, but not lure items **(C)** The retrieval-stress group follows a weak quadratic function for all items. R-squared values are indicated on each graph. Dashed lines represent 95% confidence intervals.

### 3.5. Correlational analysis of self-report questionnaires

Self-report questionnaires were administered after the stressful portion of the TSST. In order to prevent the potential confound that the stressful portion of the TSST affected self-report questionnaire scores in the consolidation-stress and retrieval-stress groups, only the control groups were analyzed for correlations between pattern separation performance and self-report questionnaires. Anxiety ( $R^2=0.262$ ,  $p=0.01$ ), depression ( $R^2=0.221$ ,  $p=0.02$ ), and perceived stress ( $R^2=0.194$ ,  $p=0.03$ ) scores had a significant negative correlation with pattern separation performance (Figure 7, top row) while sleep ( $R^2=0.117$ ) and exercise ( $R^2=0.140$ ,  $p=0.07$ ) levels did not (Figure 7, bottom row).



**Figure 7.** Pattern separation performance is correlated with self-report symptoms of anxiety, depression, and perceived stress in control participants. Anxiety, depression, perceived stress have significant negative correlations with responses to lure items (top row). Sleep and exercise do not exhibit a significant correlation with the proportion correct responses to lure items (bottom row). R-squared and  $p$  values are indicated on each graph.

## 4. Discussion

The present study examined the relationship of acute stress on memory consolidation and retrieval in healthy young adults. We used a behavioral pattern separation task to assess memory processes thought to be supported by the DG/CA3 hippocampal subregions during stress. Our results show that acute stress induced during consolidation, the process of converting short-term memories into long-term memories, enhanced pattern separation performance, reflecting an increased ability to differentiate and recall highly similar items. Participants in the consolidation-stress group exhibited higher response accuracy in identifying lure items across all degrees of similarity. Stress-related changes in cortisol exhibited an inverted-U shaped relationship in identifying repeat and first items in the consolidation-stress group, suggesting that moderate amounts of stress improves the consolidation of this type of information while low and high amounts of stress had detrimental effects on consolidation. Our results additionally show that stress induced during retrieval does not significantly impact memory compared to controls. Finally, results from this study showed that pattern separation performance was negatively correlated with self-reported anxiety, depression, and perceived stress, such that the most severe symptoms were related to an impaired ability to correctly identify lure items.

### *4.1. Effects of stress on memory performance*

The findings reported here are consistent with previous studies that observed enhancing effects of cortisol on memory performance (de Kloet et al., 1999; McGaugh & Roozendaal, 2002). Studies of the stress response in animals have consistently

demonstrated the beneficial effects of glucocorticoids on memory consolidation. As described previously, human studies have reported much more varied effects of stress on memory consolidation. For instance, Cahill, Gorski, & Le (2003) found that the cold-pressor stress test enhanced memory of emotionally arousing images but not neutral ones. Yet in another study using the cold-pressor stress test, memory enhancement was observed for both neutral and negative images (McCullough & Yonelinas, 2013). Many other studies in humans suggest that cortisol only has an effect for emotionally arousing experiences and does not modulate neutral information (Kuhlmann & Wolf, 2006; Holz et al., 2014). Despite using emotionally neutral images in our memory task, our results show an enhancing effect of cortisol on memory consolidation, potentially supporting the notion that pattern separation is indeed a more sensitive measure to stress than other memory tasks. The behavioral pattern separation task has previously been used in research on age-related changes in cognitive function. In a study by Stark et al., performance on this task showed a linearly decreasing memory function with age when no changes in traditional measures of recognition memory were observed (2013). These findings support the sensitivity of pattern separation in detecting changes in memory function to various stimuli.

Our results show that acute stress during consolidation significantly enhances the ability to discriminate between similar stimuli. Participants in the consolidation-stress group were more likely to correctly respond “similar” to lure items, which is indicative of improved pattern separation abilities. This improved performance was observed for all lure items, regardless of mnemonic similarity, in a linear relationship such that there was higher response accuracy in identifying lure items of least similarity compared to lure

items of most similarity. For the lure items, the proportion of “new” responses shifted less than the proportion of “old” responses shifted towards “similar” responses, reflecting a change in memory accuracy rather than a complete failure of recall or recognition (misses). The observed lower frequency of “old” responses to lure items in the consolidation-stress group is also indicative of decreased pattern completion and further supports the effect of stress during consolidation on memory function.

Our finding that stress induced immediately before retrieval did not have significant effects on memory is inconsistent with some previous reports. In both animals and humans, most studies observed impairing effects of glucocorticoids on memory retrieval (for reviews, see Wolf, 2003; Het et al., 2004; de Quervain, 2009). However, some studies have found contradictory results. In one study, administration of a cortisol-suppressing drug led to significantly impaired free recall of emotional texts (Rimmele et al., 2015). In another study, highly stressed participants with large increases on measures of arousal were able to compensate for the impairing effects on retrieval and perform comparably to controls (Boehringer et al., 2010). It is possible that the participants in the present study also experienced both high stress and high arousal, and thus did not exhibit impaired memory retrieval. Yet, this is unlikely to be the sole reason given that the consolidation-stress group underwent the same stress induction as the retrieval-stress group. Recently there has been evidence, primarily from animals but also from human studies, that the balance between mineralocorticoid and glucocorticoid receptor activation of different brain regions determines glucocorticoid effects on memory retrieval (Rimmele et al., 2015; Harris et al., 2013). Though glucocorticoid receptors are believed to be the major effectors of the stress response pathway, mineralocorticoid receptor

activity and location may also mediate this response. Future experiments should consider the context of mineralocorticoids and glucocorticoid receptor activation when investigating the role of glucocorticoids in memory processes.

The observed quadratic relationship between stress-induced cortisol with memory performance is consistent with previous studies (Andreano & Cahill, 2006; McCullough et al., 2015). The consolidation-stress group exhibited the strongest inverted U-shaped relationship between cortisol concentrations and the proportion of correct responses to repeat and novel items on the behavioral pattern separation task. Notably, the responses to lure items did not exhibit a strong quadratic relationship for the consolidation-stress group. The observed dose-response curve may be a result of stress impacting various memory trade-offs, such as that between “gist,” the general theme, and “visual details, specific information, of an experience. In previous studies, arousal has enhanced “gist” memory but not memory for the visual details of studied images (Adolphs et al., 2001, 2001; Denburg et al., 2003). As the discrimination of lure stimuli requires memory for visual details of the studied images, a limited relationship between changes in cortisol concentration and response accuracy for lure items in the consolidation-stress group is consistent with reports of a trade-off between the strengthening of the gist but not an increase for the amount of detail for an event or stimulus. In the responses to repeats and firsts for the consolidation-stress group, stress led to an increase in memory performance up to a certain point, beyond which additional stress became detrimental.

The retrieval-stress group showed weakly positive (repeats and firsts) and weakly negative (lures) relationships between amounts of stress and memory performance, again supporting the idea that stress during retrieval did not have a significant effect. The weak



quadratic regression observed in controls between stress-induced cortisol changes and memory responses were expected. The control group did not show significant changes in cortisol concentrations before and after the control-TSST, and thus were not predicted to exhibit an inverted U-response curve or other type of change relationship.

#### *4.2. Correlates of pattern separation performance*

The negative relationship observed between depression and pattern separation performance is consistent with previous research. Studies have reliably demonstrated a reduction in hippocampal volume and impairment in memory performance in patients with depression (for review, see Campbell et al., 2004). These changes have been attributed to neuronal death and decreased adult neurogenesis necessary to support pattern separation processes. Depressed individuals treated with antidepressants showed larger dentate gyrus volume and more neural progenitor cells, suggesting that antidepressants may remediate memory deficits in depression through increased neurogenesis (Boldrini et al., 2009). Studies suggest that individuals with depression have a tendency to overgeneralize stimuli in the environment, a pathology that has been associated with functioning of the hippocampus and its subregions (Becker & Wojtowicz, 2007; Sahay & Hen, 2007). Accordingly, impairments in pattern separation may be an indicator for both the behavior and underlying pathology of depression and other mood disorders. Only a few other studies have correlated self-report depression symptoms with pattern separation performance (Dery et al., 2013; Shelton et al., 2013). Our results were highly consistent with these studies, suggesting that depression symptoms are closely

correlated with pattern separation performance, which may reflect a reduction in neurogenesis.

Anxiety-like behaviors have previously been tested in rats through observations on the open field test (measuring willingness to explore in an exposed space) and novelty-suppressed feeding test (measuring the latency of an animal to eat familiar food in a novel environment) (Sahay et al., 2011). Mice that were genetically manipulated for increased neurogenesis, the process thought to facilitate pattern separation, showed a decrease in anxiety-like behaviors (Sahay et al., 2011). In other tasks, mice have been tested on their ability to distinguish between an aversive context, in which they would receive a shock, and a similar but safe (no shock) context (McHugh et al., 2007; Kheirbek, Tannenholz, & Hen, 2012). High discrimination between contexts was observed by exploratory behavior, while low discrimination between contexts led to freezing behavior. In humans, however, no studies thus far have investigated the relationship between self-report anxiety and pattern separation performance. Our results are consistent with previous animal studies that suggest increased anxiety decreases discriminatory ability, and show this correlation in humans for the first time.

Sleep quality has also been not been well investigated in relation to pattern separation. We are only aware of one study, to date, that directly investigated the effect of sleep on the behavioral responses of pattern separation and pattern completion in humans. In this study, there was better memory discrimination in participants who slept (when testing occurred 12 hours later, on the following day) compared to those who did not (when testing occurred 12 hours later, on during the same day), consistent with a bias

toward pattern separation following sleep (James, 2014). However, our results did not find a significant correlation between sleep quality and pattern separation performance.

Although we did not find a significant correlation between total hours exercised per week and pattern separation performance, increasing evidence suggests that physical exercise promotes synaptic plasticity and hippocampal neurogenesis. Studies in mice found that running enhanced spatial pattern separation and increased the proliferation of young neurons (Creer et al., 2010; Snyder et al., 2009). The decline in neurogenesis in aged mice was reversed to 50% of the levels of young controls after a voluntary running wheel was introduced to sedentary mice (van Praag et al., 2005). A previous study reported that there was no effect of exercise on learning in rats, however in this study, forced treadmill training was used rather than voluntary wheel running, thus suggesting a difference in voluntary and forced exercise (Barnes et al., 1991). In humans, individuals who experienced the greatest improvement in aerobic fitness showed significant post-exercise enhancement in the ability to distinguish similar lures (Dery et al., 2013). A possible reason we did not observe this relationship was because the exercise questionnaire employed in this study did not ask about certain fitness criteria (e.g. changes in fitness level) and rather only asked about baseline exercise habits. A more extensive exercise questionnaire (e.g. including questions on exercise intensity, aerobic vs. nonaerobic exercise) may yield significant correlations to pattern separation performance.

#### *4.3. Possible mechanisms by which stress impacts memory*

Several influential models have attempted to explain the contradictory findings of facilitating and detrimental acute glucocorticoid effects on memory (de Quervain et al., 2009; Joels, 2006). Recently, a model has related the timing and dosage of glucocorticoids to the characteristics of neural activation (Sandi, 2011; McCullough & Yonelinas, 2013). In this model, moderate levels of post-encoding stress may be associated with better memory performance compared to that of lower or higher levels of stress. Our analysis of the salivary cortisol levels support the inverted U-dose response curve hypothesis in which moderate levels of glucocorticoids observes optimal memory performance.

Glucocorticoid-induced changes to synaptic strength, membrane excitability, or remodeling of neuronal structure may have mediated pattern separation performance (Herbert et al., 2006). Rapid processes can occur when glucocorticoids interact with membrane-bound receptors while delayed processes (protein-synthesis dependent) occur when glucocorticoids bind to intracellular receptors to alter gene expression in the nucleus. The glucocorticoid effects in the present study likely occurred through non-genomic mechanisms mediated by membrane-bound receptors, as behavioral measures were taken shortly following stress induction. Activation of the noradrenergic system through the TSST may also have induced a mechanism in which norepinephrine and glucocorticoids interacted to produce a G-protein mediated signaling cascade (Roozendaal, Williams, & McGaugh, 1999). Norepinephrine binding to beta-adrenoceptors, G-protein coupled receptors, in the BLA may enhance memory consolidation through downstream activation of cAMP, a signaling molecule (Ferry,

Roozendaal, & McGaugh, 1999). Overall, short-term and long-term activation of glucocorticoids may act through different mechanisms, and future studies should aim to uncover these processes by which the DG/CA3 is influenced by stress.

#### *4.4. The role of adult neurogenesis in pattern separation*

New adult-born neurons are believed to play a role in pattern separation (for review, see Aimone et al., 2011). Produced by adult neurogenesis, young neurons exhibit distinct physiological properties compared to mature neurons. For example, four-to-eight week-old granular cells show greater synaptic plasticity and increased excitability compared to the older granular cell population (Ming & Song, 2011). Studies that ablate neurogenesis in adult mice found impaired performance on tasks shown to engage pattern separation, such as contextual fear discrimination learning and radial maze delayed non-match to place (Clelland et al., 2009; McHugh et al., 2007). Furthermore, genetically improving the survival and proliferation of adult-born neurons enhanced rodents' abilities to distinguish between similar contexts (Sahay et al., 2011). The enhancement in pattern separation ability can be attributed to increased plasticity and hyperexcitability of young neurons relative to mature neurons. Young neurons have a lower threshold of activation, thus requiring weaker inputs to fire, which make them more readily available to respond to subtle changes in the environment (Markwardt, Wadiche, & Overstreet-Wadiche, 2009). Granule cells also have multiple place fields that remap quickly with small changes in environmental context (Leutgeb et al., 2007). Taken together, loss-of-function and gain-of-function studies support the role of these cells in discriminating between fine details and overlapping contextual representations.

Levels of adult neurogenesis may change as an adaptive response to different environments (for review, see Sahay et al., 2011). From the proliferation of neural precursors to the survival of newborn neurons, each phase of adult neurogenesis can be influenced by numerous physiological and environmental factors. Overall, neurogenesis-based circuit alterations are more relevant for reflecting longer-term changes in the environment. For instance, living in an enriching environment that promotes exploration and learning increases the survival of young adult-born neurons as they create a need for increased neurogenesis to maintain nonoverlapping representations (Kempermann et al., 1997). Learning is a major regulator of adult neurogenesis only if the tasks depend on the hippocampus, and specific learning paradigms can regulate the survival of new neurons in animals (for review, see Zhao et al., 2008). On the other hand, stressful environments may lead to decreased neurogenesis to enable organisms to generalize across similar situations and avoid potential danger in the future.

Disruptions in the normal regulation of neurogenesis may result in excessive or impaired pattern separation, either of which can be maladaptive for the individual. Excessive pattern separation may prevent the normal processing of information due to a fixation on fine details or preoccupation with minutiae; these behaviors can be seen in autism spectrum disorders and obsessive-compulsive personality disorder (Soulieres et al., 2009). In contrast, impaired pattern separation may lead to overgeneralization and a decreased ability to discriminate between multiple contexts. For individuals with panic disorder or PTSD, the reduced ability to distinguish between experiences may cause a neutral stimulus to trigger a traumatic memory. Impaired pattern separation and the tendency to overgeneralize are also characteristic of depression and aging (Yassa et al.,

2010; Shelton & Kirwan, 2013). While our results are cannot conclude a causal relationship between neurogenesis and pattern separation, they are consistent with the hypothesis that a reduction in neurogenesis is both related to impaired pattern separation performance and symptoms in anxiety and depression.

Overall, neurogenesis is implicated in a range of neurological disorders, and may be assessed behaviorally with pattern separation tasks. The identification of specific cognitive markers such as pattern separation deficits can allow for the development of approaches targeting underlying neural circuits. Recent improvements in behavioral assessments of human pattern separation and imaging methods of the hippocampal subregions has led to significant progress in understanding the relationship underlying pattern separation, neurogenesis, and neurological disorders.

#### *4.5. Limitations*

There are several limitations to the present study. First, with all behavioral tasks, there is a concern of whether the observed behavior is a true reflection the underlying neurobiological process. Here, pattern separation performance is used to assess function of the DG/CA3 subregion of the hippocampus. While the behavioral pattern separation task specifically taxes the DG/CA3 subregions, future studies will need to determine if the current findings can be replicated using more direct measures of hippocampal subregion function, such as functional magnetic resonance imaging studies. Nevertheless, behavioral assays have been critical to establishing much of the information known in the field, and pattern separation remains a useful indicator or for brain-specific measures (Deng et al., 2010).

Second, acute stress triggers multiple responses in the body beyond hypothalamic-pituitary-adrenal axis activation, and causes other hormones besides cortisol to be released. For example, norepinephrine, vasopressin, and pro-inflammatory cytokines, are also all secreted in response to stress. Since such changes occur in response to the same stimuli, there is a strong association between stress response systems. For example, peaks in salivary alpha-amylase, an indicator of the sympathetic-adrenal-medullary axis, and cortisol, an indicator of the hypothalamic-pituitary-adrenal axis, were closely correlated following a cold-pressor stress test (Smeets et al., 2008). Extensive evidence supports that salivary cortisol is a reliable and valid biomarker in stress research, and continues to be used as the biomarker of choice (Hellhammer, Wust, & Kudielka, 2009).

Third, the present study recruited an all-male sample, and thus the results may not be readily generalized to females. Females were excluded because they tend to exhibit smaller stress responses and more variable changes in salivary cortisol levels (Kajantie and Phillips, 2006). Stress effects are also less consistently observed in females depending on menstrual cycle phases and the use of oral contraceptives (Kirschbaum et al., 1999; Bouma et al., 2009). In this study, we chose the population with the most robust and stable stress response, as cortisol levels served as an independent variable for memory performance. Many studies measuring or manipulating the stress response limit their population to males (e.g. Andreano & Cahill, 2006; Henckens et al., 2009; McCullough et al., 2015). However, there is evidence suggesting that gender differences may moderate the link between stress and memory performance. Future research should consider a sample with both genders in order to better make comparisons.



Finally, only emotionally neutral images were shown in the present study. It would be important to assess the pattern separation performance of both emotionally neutral and emotionally charged images, and future studies should consider the effect of emotional valence and arousal on pattern separation behavior. Many neurological conditions that show impaired pattern separation performance are closely related to emotional responses. For example, in anxiety disorders, the inability to distinguish between neutral and emotional or fearful events may lead to intrusive memories and clinical symptoms.

#### *4.6. Conclusion*

The present study demonstrates the important role of acute stress in modulating hippocampal-dependent memory function. Specifically, an enhancement in the pattern separation of neutral objects was observed when stress was induced during memory consolidation. The improved ability to discriminate between stimuli occurred without bias across varying levels of similarity in lure items. No significant effect on pattern separation performance was observed when stress was induced on memory retrieval. This study is the first to focus on the effects of acute stress on pattern separation, a computational process of episodic memory that is specifically supported by the DG/CA3 subregions of the hippocampus. Our findings provide evidence that the memory consolidation process is associated with the DG/CA3 region and facilitated by acute stress hormones.

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## 6. Curriculum Vitae

### ALICE JIANG

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(248) 787-9510; ajiang3@jhu.edu

### EDUCATION

**Johns Hopkins University, Krieger School of Arts and Sciences** **Baltimore, MD**  
Master of Science in Molecular & Cellular Biology, *candidate* May 2016  
Bachelor of Science in Molecular & Cellular Biology Aug 2012- May 2015  
Minor in Psychology  
Dean's List: Fall 2012, Fall 2013, Spring 2014, Fall 2014, Spring 2015

### RESEARCH EXPERIENCE

**Johns Hopkins Medical Institute, Dept of Psychiatric Neuroimaging** **Baltimore, MD**  
*Research Assistant; Bakker Lab* Sept 2013-present

- Conducted independent research investigating the role of psychological and physiological factors on long-term memory function in young adults
- Performed detailed manual segmentations of the medial temporal lobe in fMRI scans with AFNI
- Aided in patient visits in a study on ApoE-4 allelic variations and memory impairment in the elderly

**Jiangsu Academy of Agricultural Services** **Nanjing, China**  
*Research Assistant; Yu Lab* Jan 2014, Nov 2014- Jan 2015

- Assisted in sample preparation, data collection, and manuscript writing
- Publication: Wang, T. T., Li, Y. S., Jiang, A. C., Lu, M. X., Liu, X. J., & Yu, X. Y. (2015). Suppression of Chlorantraniliprole Sorption on Biochar in Soil–Biochar Systems. *Bulletin of Environ Contamin and Toxicology*, 95(3), 401-406.

### TEACHING EXPERIENCE

**Johns Hopkins University, Krieger School of Arts and Sciences** **Baltimore, MD**  
*Teaching Assistant, General Biology I and II Lecture and Lab* Aug 2015- present

- Led a section of 20-24 students for three-hour laboratory periods every week
- Graded lab assignments and kept updated records of student grades
- Proctored and graded exams and quizzes for the lecture course

### WORK EXPERIENCE

**Desmond Tutu HIV Foundation Youth Centre** **Cape Town, South Africa**  
*Intern* June 2015-July 2015

- Conducted interviews to identify barriers in female adolescent transition from youth to adult clinics
- Developed and proposed a program to be implemented at the Youth Center to facilitate clinic transition



**Johns Hopkins University Office of Academic Support** **Baltimore, MD**  
*Study Consultant* Aug 2014-present

- Mentored undergraduate students on strengthening academic skills (e.g. time management, note taking)
- Tracked progress throughout the semester & tailored strategies to the student

### **VOLUNTEER EXPERIENCE**

**Hopkins Emergency Response Unit (HERU)** **Baltimore, MD**  
*Crew Member; Driver; Selections, Public Relations Committee* Sept 2012-May 2015

- Served as the first response for medical emergencies on the JHU Homewood campus (8 hours/week)
- Worked with JHU Security, Baltimore City Fire Department, and the Student Health and Wellness Center
- Engaged in the selections process by reading applications, leading group process, conducting interviews

**Advocates for Baltimore Community Health** **Baltimore, MD**  
*President (2014-2015); Secretary (2013-2014)* Sept 2013-present

- Established new clinic partnership after the previously-affiliated clinic closed
- Communicated between executive board, group members, and clinic coordinators
- Oversaw responsibilities of all board members; facilitates executive board and general body meetings, including initiating the meeting and preparing an agenda in advance

**Musicare** **Baltimore, MD**  
*Publicity and Programming Officer* Jan 2014-present

- Created all programs and flyers, scheduled performances at hospitals, played music for patients

**Shepherd's Clinic** **Baltimore, MD**  
*Joy Wellness Center Front Desk Volunteer* Sept 2014-Dec 2014

- Scheduled appointments, completed insurance forms, prepared patient charts

**Providence Park Hospital** **Novi, MI**  
*Medical Surgery/Oncology; Infusion Center Volunteer* May 2014-Aug 2014

- Worked with nurses and technicians to ensure patient comfort; transported patients, set up rooms, and delivered water, snacks, blankets

**People's Community Health Centers** **Baltimore, MD**  
*Student Medical Assistant* Sept 2013-May 2014

- Assisted patients in obtaining health insurance; triaged patients; completed medical history and risk assessment paperwork with patients, photocopied medical records