ABSTRACT

Title of Thesis: TIME-DEPENDENT EFFECTS OF ACUTE EXERCISE-INDUCED AROUSAL ON LONG-TERM MEMORY FOR EMOTIONAL AND NEUTRAL STIMULI

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Psychological research has strongly documented the memory-enhancing effects of emotional arousal, while the effects of acute aerobic exercise on memory are not well understood. Manipulation of arousal has been shown to enhance long-term memory for emotional stimuli in a time-dependent fashion. This presents an opportunity to investigate the role of acute exercise in memory modulation. The purpose of this study was to determine the time-dependent relationship between acute exercise-induced arousal and long-term emotional memory. Participants viewed pleasant, neutral, and unpleasant images before or after completing a high-intensity session of cycling exercise. Salivary alpha-amylase, a biomarker of central norepinephrine, was measured as an indicator of arousal. No effects of exercise on recognition memory were revealed, however; a single session of high-intensity cycling increased salivary alpha-amylase. Our results also indicate that the influence of exercise on emotional responsiveness should be considered in further exploration of the memory-enhancing potential of acute exercise.

TIME-DEPENDENT EFFECTS OF ACUTE EXERCISE-INDUCED AROUSAL ON LONG-TERM MEMORY FOR EMOTIONAL AND NEUTRAL STIMULI

By

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LIST OF ABBREVIATIONS

7-Day PAR – 7-Day Physical Activity Recall Interview Questionnaire

ANOVA – Analysis of variance

BDI-II – Beck Depression Inventory-II

CPS – Cold pressor stress

CV – Coefficient of variation

Ex-After – Experimental group assigned to exercise *after* encoding

Ex-Before – Experimental group assigned to exercise *before* encoding

HR – Heart rate

IAPS – International Affective Picture System

LC – Locus coeruleus

LTEQ – Godin Leisure Time Exercise Questionnaire

MCI – Mild cognitive impairment

NE – Norepinephrine

R-K – Remember-Know paradigm

RPE – Ratings of Perceived Exertion

sAA – Salivary Alpha-Amylase

SAM-A; SAM-V – Self-Assessment Manikin, Arousal/Valence dimensions

SD – Standard deviation

SEM – Standard error of the mean

STAI – State-Trait Anxiety Inventory

VO_2 max – Maximal volume of oxygen consumption

CHAPTER I: REVIEW OF LITERATURE

Overview

There is a large body of existing literature demonstrating the effects of exercise and physical activity on many aspects of cognition. It is well documented that long-term aerobic exercise training positively influences cognition in both healthy and patient populations across the lifespan (Kramer & Erickson, 2007; Smith, Alfini, Smith, & Weiss, 2015). Exercise training contributes to the maintenance of memory, cognitive abilities and brain volume in aging populations (Colcombe & Kramer, 2003; Smith et al., 2014), and has even been shown to increase the size of the human hippocampus (Erickson et al., 2011). While a substantial amount of evidence supports these benefits of exercise training, our understanding of acute exercise is more limited. Specifically, the effects of acute exercise on memory processes are not well understood.

A single session of exercise can have moderate-to-large effects on long-term memory, demonstrating the potential for appropriately prescribed exercise to improve memory for specific "chunks" of information (Roig, Nordbrandt, Geertsen, & Nielsen, 2013). Additionally, psychological and neurobiological investigations have elucidated an important role of arousal in the processes supporting memory for emotional events (Cahill & McGaugh, 1998). Methods of manipulating endogenous arousal, such as exercise, can be utilized in a laboratory setting to progress our knowledge of this relationship. In the case of acute exercise, its benefit as a model is compounded by its value for many aspects of physical and mental health extending beyond memory. The current project aimed to integrate research from psychology, neuroscience, and

kinesiology to further our understanding of the relationship between acute exercise, arousal, and long-term memory.

Memory for Emotional Material

Episodic Long-Term Memory

Episodic memory, the ability to remember specific events and the details surrounding them, is a fundamental and central process that supports even the most mundane human behaviors, such as finding one's car in a busy parking lot or remembering where a meeting is being held (Ghetti, Lyons, & DeMaster, 2012). The phases encompassing our capacity to remember include encoding, consolidation, and retrieval. *Encoding* refers to the initial experience of the event to be remembered, which is subsequently *consolidated* into a lasting (in some cases) representation of the experience (Kensinger, 2009). We remember an experience only when these two phases, and the final *retrieval* phase, occur successfully. This 3-stage model is a simple and accepted means of decomposing memory into smaller working parts. In the case of long-term memory, which is the focus of this review, the consolidation phase can take place for a handful of minutes, hours, or even years from the time of encoding (McGaugh, 2000). It has been suggested that an emotional event or experience sets into motion a series of events which influences a memory during all three of these phases (Kensinger, 2009). Based on this reasoning, the emotional qualities of a given stimulus or event can be considered intrinsic modulators of the memory for its experience.

Valence and Arousal

The emotional perception of stimuli includes a process of affective evaluation. Research on affect and perception has shown that this evaluation relies on the extent to

which stimuli are judged on pleasantness (pleasant or unpleasant) and activation (activating or inactivating) values (Lang, Greenwald, Bradley, & Hamm, 1993). A well-accepted model of affect, the value assigned to a given feeling state, suggests that these components of affective experience vary on two orthogonal dimensions: valence and arousal (Lang et al., 1993). *Valence* refers to the pleasant or unpleasant value assigned to a stimulus. The *arousal* dimension represents the intensity, or activation, assigned to a given stimulus. These dimensions are separate, but related: judgments that have greater affective impact on the valence spectrum tend to elicit a higher arousal than less valenced experiences. In other terms, a highly pleasant or highly unpleasant stimulus is also likely to be highly arousing in quality. Stimuli that fit these criteria can be labeled as *emotional* stimuli. Stimuli that evoke a lower level of activation or arousal are conversely referred to as *neutral.* For the remainder of this review, memory for emotional stimuli will be referred to as "emotional memory."

Recognition and Recollection of Emotional Memory

It is well established that emotional experiences are better remembered than neutral events. Flashbulb memories, the subjectively vivid and long-lasting memories of surprising and consequential events, are an extreme example of this memory enhancement for emotionally charged experiences (Brown & Kulik, 1977). Investigations of episodic memory, the ability to form and retrieve information about past experiences, have found that memory for emotional words, pictures, and stories is better than memory for neutral stimuli (Bradley, Greenwald, Petry, & Lang, 1992; Cahill & McGaugh, 1995; Kensinger & Corkin, 2003). Although both valence and arousal are contributors to the emotional appraisal of a stimulus or experience, it has been repeatedly

demonstrated that it is arousal, rather than valence, which provides the memory benefit to emotional material (Buchanan & Adolphs, 2002). This benefit is evident in examinations of both free-recall and recognition memory, and has been confirmed through the direct manipulation of arousal, as will be discussed in the next section of this review.

The role of valence and arousal in emotional memory can be further decomposed by differentiating between recollection and familiarity, the memory retrieval processes contributing to recognition (Yonelinas, Aly, Wang, & Koen, 2010). This differentiation can be approached using Tulving's Remember-Know (R-K) paradigm (1985). The R-K paradigm expands on tests of recognition memory by eliciting subjective judgments of the memory experience. Upon recognition of a stimulus, participants indicate that they "Remember" or "Know" an item. *Remembering* signifies the specific recollection of the encoding or experience with a stimulus, while feeling a sense of familiarity in the absence of recollection corresponds to *knowing*. Investigations of recollection in conjunction with objective measures have verified that subjective R-K responses align as expected with the number of correctly recollected contextual details (Dudukovic & Knowlton, 2006; Friedman & Trott, 2000).

Moving beyond recognition memory, additional studies have revealed a role for both valence and arousal in the recollection of emotional stimuli. The benefit of arousal for recollection has been supported by studies of source memory paradigms, indicating that both subjective vividness and accuracy for contextual details is enhanced for emotional stimuli (Kensinger & Corkin, 2003). Moreover, it has been demonstrated that people tend to make more *remember* responses for unpleasant than pleasant pictures, indicating a valence-related effect on the subjective vividness with which memories are

retrieved (Ochsner, 2000). Conversely, participants are more likely to *know* that they saw a pleasant picture, suggesting that valence might moderate the contributions of recollection and familiarity to memory (Kensinger, 2009; Mickley Steinmetz, Addis, & Kensinger, 2010).

Neurobiology of Emotional Memory

The enhancement for recognition and recollection of emotional stimuli has been attributed to the influence of psychological arousal on the neural processes involved in memory. Current opinion implicates hormones, released as a result of stress or emotional arousal, as important neuromodulators of long-term memory formation (McGaugh, 2004; Wolf, 2008). This review will focus on the role of the noradrenergic system as the link between arousal and emotional memory. Emphasis will be placed on studies investigating arousal during the encoding and consolidation phases of episodic memory, although evidence suggests that neural processes specific to emotion are also engaged during memory retrieval (Kensinger, 2009).

In response to an emotional experience, the sympathetic nervous system initiates a fast adaptive response, the results of which are well known as "fight or flight," or *arousal*. A hallmark of this response is the activation of the noradrenergic system. Norepinephrine (NE) is a hormone and neurotransmitter produced both peripherally, in the adrenal medulla, and centrally by neurons found primarily in the locus coeruleus (LC) of the pons (Sara, 2009; van Stegeren, 2008). The highly connected LC plays a critical role in the brain's response to emotionally salient information through noradrenergic projections to the forebrain, limbic system, and neocortex (McGaugh, 2004; van Stegeren, 2008). Although NE cannot pass the blood-brain-barrier, circulating peripheral

NE can indirectly stimulate noradrenergic activity in the LC via its action on adrenoceptors of the vagus nerve (Wolf, 2008). One of the LC's many noradrenergic projections is to the amygdala, which is believed to be an important locus of the neuromodulatory interactions influencing memory for emotional material (McGaugh, 2000, 2004; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006). It is in the basolateral amygdala (BLA) where the activation of β-adrenoceptors is critical to the memory-modulating effects of arousal (Tully & Bolshakov, 2010).

Endogenous Noradrenergic Activation and Emotional Memory

Much of the first evidence that noradrenergic activity in the brain can influence memory came from studies utilizing pharmacological intervention in rodents (e.g. Gold & van Buskirk, 1975). Since these initial works, several investigations of endogenous noradrenergic activity and its role in emotional memory have been conducted. An influential example is the work of McIntyre, Hatfield, and McGaugh (2002), which examined the relationship between noradrenaline release in the amygdala of rats and their subsequent retention of a stressful foot-shock avoidance learning task. Their findings revealed a direct association between the relative increase of NE and performance in the retention task. Rats with higher levels of NE in the amygdala after the stressful learning interval showed greater retention for the avoidance training than those with lower levels of NE activity. This study was among one of the first to provide evidence that long-term memory for emotionally arousing events is mediated by activation of the noradrenergic system.

Due to the methodological difficulties of assessing central noradrenergic activation in humans, it was not until recently that the role of endogenous NE in

emotional memory could be directly assessed. The development of an assay to measure salivary alpha-amylase has enabled the direct investigation of endogenous noradrenergic activation and emotional memory enhancement. Salivary alpha-amylase (sAA) has been validated as a useful biomarker for sympathetic nervous system activation (Nater & Rohleder, 2009). Studies of sAA in humans have shown a detectable elevation of the enzyme in response to psychological (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; van Stegeren, Rohleder, Everaerd, & Wolf, 2006) and physiological stress, including moderate-to-high intensity exercise (Allgrove, Gomes, Hough, & Gleeson, 2008). Importantly, these increases have been associated with parallel increases in plasma epinephrine and norepinephrine (Allgrove et al., 2008; Chatterton et al., 1996). Pharmacological intervention has strengthened these results by demonstrating that the increase in sAA observed after infusion with NE is prevented by the administration of the β-adrenoceptor blocker phentolamine (Kuebler et al., 2014; van Stegeren et al., 2006). These studies provide direct evidence for the utility of sAA as a noradrenergic activation-sensitive biomarker.

Early investigations of arousal-induced memory enhancement relied on indirect measures of sympathetic activity such as skin conductance at the time of encoding (e.g. Bradley et al., 1992). A study by Segal & Cahill (2009) was the first of its kind to examine the predicted relationship between endogenous noradrenergic activity, as indicated by sAA, and the enhancement of memory for emotional material. Participants were shown a selection of neutral and unpleasant images from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2008). Saliva was collected at baseline, immediately after, and at 3-, 8-, 18- and 28-minute intervals following picture viewing.

As expected, there was a significant memory enhancement for emotional images compared to neutral images. In participants that showed an increase in sAA as a result of picture viewing, there was a significant positive correlation between increase in sAA and the percentage of emotional images recalled. This association was not observed for neutral stimuli. These results support the hypothesis that the degree of endogenous noradrenergic activation is predictive of subsequent long-term memory for emotional stimuli, but not neutral stimuli.

Manipulation of Arousal and Emotional Memory

Despite the challenge of assessing noradrenergic activation in humans, researchers have made great strides in our understanding of arousal and emotional memory through the experimental manipulation of arousal. Studies in both rodents and humans have utilized pharmacological and psychological interventions to elucidate the time-sensitive relationship between arousal processes and memory. The following review is by no means comprehensive, but rather intends to outline the contributions of several of these studies. The first section will introduce studies that manipulated arousal before the encoding phase of memory. The subsequent section will summarize studies that manipulated arousal during the consolidation phase of memory immediately after encoding. Studies of memory consolidation have a rich history, and these investigations have drawn strong conclusions. Conversely, studies that have manipulated arousal by implementing an intervention before encoding have less consistent findings.

Manipulation of arousal before encoding

In a seminal study of emotional memory, Cahill and colleagues (Cahill, Prins, Weber, & McGaugh, 1994) investigated memory for the emotional content of a story in

subjects treated with propranolol, a β-adrenergic antagonist, compared to placebo Although subjective ratings of the emotional story phase did not differ across treatment groups, the results revealed a significant effect of β-blockade on memory for the emotional content. Recognition of the emotional narrative was higher than recognition for a neutral version of the story, and the emotional phase was remembered better than neutral parts of the same story. Both of these effects, which confirm the findings of a previous study using the same task (Cahill & McGaugh, 1995), were prevented by administration of propranolol. These results indicate that blockade of the β-adrenergic system prevents the memory enhancing effects of emotional arousal.

Building from this foundation, additional pharmacological studies have utilized a similar behavioral task to further investigate the influence of the noradrenergic system on emotional memory. Van Stegeren and colleagues (van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998) extended the design of Cahill et al. (1994) with the addition of a nadolol treatment group. Nadolol crosses the blood-brain barrier to a lesser extent than propranolol, therefore its effects on memory would suggest the notable contribution of peripheral β-adrenoceptor activity. The results duplicated the finding that propranolol prevented the enhancement of emotional recognition memory, but there was no effect of nadolol on any component of memory. These results demonstrate that it is the action of propranolol on central, not peripheral, β-adrenoceptors that blocks the benefits of arousal on emotional memory.

Another study aimed to explore the influence of exogenous noradrenergic activation on emotional memory for the same set of stimuli (Cahill et al., 1994; O'Carroll, Drysdale, Cahill, Shajahan, & Ebmeier, 1999). Over-activation of the noradrenergic

system would be expected to amplify the enhancement of emotional memory, but the noradrenergic activating agent yohimbine failed to have any specific effect on recognition memory for the emotional content. The memory benefit for emotional material was found even in the participants treated with a β-adrenergic antagonist. This result contradicts the findings of Cahill et al (1994), which demonstrated a prevention of emotional memory enhancement with administration of propranolol. The authors speculate that small treatment dosages may have contributed to the weak effects observed here, however; this study sets the stage for the mixed findings of studies manipulating arousal during encoding.

Manipulations of the endogenous arousal response have also been utilized to study the noradrenergic contribution to memory. Schwabe and colleagues (Schwabe, Bohringer, Chatterjee, & Schachinger, 2008) exposed participants to a cold pressor stress (CPS) test before encoding of emotional and neutral words. Although CPS has been shown to stimulate the sympathetic nervous system (Cahill, Gorski, & Le, 2003), the stress manipulation showed no lasting effect on free-recall or recognition for emotional words. These results also revealed that CPS stress before learning resulted in better free-recall memory for neutral words at both 1- and 24-hour assessments. This finding presents the possibility that external manipulations of arousal may substitute for the lack of intrinsic arousal in neutral stimuli, therefore increasing their memorability.

Another recent study demonstrated that the endogenous arousal stimulated by unpleasant picture viewing can facilitate detailed memory for neutral objects. Segal et al. (Segal, Stark, Kattan, Stark, & Yassa, 2012) manipulated arousal by exposing participants to a series of unpleasant and highly arousing pictures from the International

Affective Picture System (IAPS)(Lang et al., 2008). For the encoding task, participants were presented with a series of neutral images depicting everyday objects. Saliva samples were collected before and after both picture viewing and encoding for the measurement of sAA. In a delayed pattern separation task, which requires participants to recognize old and new objects amongst "lures" which closely resemble the old objects, performance was positively correlated with the increase in sAA measured between baseline and post-encoding. This correlation parallels the association found by Segal et al. (2009) between endogenous NE increase and memory for emotional pictures. These findings corroborate the evidence from Schwabe et al. (2008), and further the possibility that manipulating arousal before encoding may provide a benefit for otherwise non-arousing stimuli.

In contrast to the findings by Schwabe et al. (2008) and Segal et al. (2012), a study by Payne and colleagues (2006) found that pre-encoding stress is detrimental to memory for neutral words. This study utilized the Trier Social Stress Test (TSST), which requires subjects to deliver a speech that they believe is being recorded and evaluated by judges. An interesting difference arose between male and female participants, as the stress-induced enhancement of free-recall memory for pleasant and unpleasant words was observed only for females. Results of the recognition test revealed that pre-encoding stress caused an impairment of neutral memory with no effect on emotional memory. Because subjective reports of anxiety were the only measure used to assess the stress manipulation in this study, it is difficult to conclude why there is such a large discrepancy between these and other results. It is possible that the stress condition was insufficient to further enhance emotional memory. One could also speculate that changes in attention

caused the impairment of memory for neutral stimuli, however; this impairment was not observed in the other studies utilizing a similar task and design.

As evidenced by the studies included here, there is still much debate about the effects of pre-encoding arousal on memory for emotional and neutral material. There is a shortage of memory research utilizing the measurement of NE correlate (e.g. sAA), such that it is difficult to translate the meaning of pharmacological and behavioral interventions across studies. Lack of these measurements precludes the ability to draw direct conclusions about dose-response effects of central noradrenergic activation. Additionally, the varied modality and arousal characteristics of stimuli used across studies are problematic for comparison of results. In the case of repeated task design, such as the materials designed by (Cahill et al., 1994), the small number of stimuli allows only for crude indexes of memory to be calculated, also limiting the ability to interpret effects. The studies outlined here present the possibility that pre-encoding stress may enhance neutral memory, but these effects have not been consistently reported.

Manipulation of arousal after encoding

The role of the noradrenergic system in memory consolidation has been thoroughly investigated through the manipulation of post-learning arousal. Many of these investigations have utilized pharmacological interventions in rodents and humans to examine this relationship. In one of the earliest studies of hormonal modulation of memory consolidation, Gold & van Buskirk (1975) injected rats with epinephrine or a saline solution after training on a stressful inhibitory avoidance foot-shock task. Rats that received the epinephrine injection immediately after training cessation had better retention performance than those that received the injection later or did not receive

epinephrine. Follow-up studies confirmed and extended these findings, reporting that the pre-learning injection of a β-adrenergic antagonist prevented these effects, even in rats that received the epinephrine treatment during consolidation (Gold & van Buskirk, 1978; Gold, van Buskirk, & Haycock, 1977). This series of investigations was the first evidence suggesting that a stress hormone could be influencing the way memories are consolidated.

Further studies in rodents have led to the updated hypothesis that arousal-induced NE release is involved in memory formation during the initial exposure to a stimulus as well as during the subsequent phase of memory consolidation (Cahill & McGaugh, 1998). Blockade of the noradrenergic system by post-learning administration of a β-adrenergic antagonist reliably prevents the memory enhancing effects of stress or pharmacologically-induced arousal during learning in rodent models (Dornelles et al., 2007; Roozendaal et al., 2006). The capability to perform histological evaluations in rodent models has allowed these investigations to pinpoint the amygdala as the location of NE release most imperative for emotional memory enhancement (McGaugh, 2004; Roozendaal et al., 2006).

Interventions aimed at the modulation of memory during consolidation have also been conducted in humans. Cahill, Gorski, & Li (2003) studied the post-encoding effects of CPS on memory for emotional and neutral pictures. Those who underwent the CPS test recalled a higher number of emotional images and recalled more details about the emotional images than control participants. An interesting finding was that control participants remembered significantly fewer details about emotional stimuli than details about neutral stimuli, which conflicts with other research indicating that recollection is

stronger for emotional stimuli (e.g. Kensinger & Corkin, 2003). It is notable that control participants demonstrated a significant decrease in cortisol before and after arm immersion, which suggests the warm water may have produced a calming effect rather than serving as a true control.

The findings of Cahill and colleagues (2003) suggest that post-learning stress can enhance memory for emotional stimuli and confirms similar findings of pharmacological studies that have administered epinephrine or adrenergic activators (Cahill & Alkire, 2003; Southwick et al., 2002). The results of these studies combined have strengthened the suggestion that arousal during encoding is necessary for the successful modulation of memory during consolidation. In other words, increasing NE release during consolidation can only enhance memory for stimuli that elicit arousal during the encoding phase. Therefore, while the effects of pre-encoding arousal are possibly due, in part, to changes in arousal or attention processes during encoding, the effects of arousal during consolidation are dependent upon the inherent emotional characteristics of the to-be-remembered material.

Additional studies in humans have shown that memory for emotionally arousing stimuli can be enhanced by post-learning arousal, but this enhancement is not seen for neutral stimuli. Nielson and colleagues (Nielson, Yee, & Erickson, 2005) reported the enhanced recognition and free-recall for a list of moderately arousing words in participants that viewed an arousing video of oral surgery after encoding. A subsequent study revealed that either pleasant *or* aversive (unpleasant) arousal up to 30 minutes after learning can enhance memory for a similar word list (Nielson & Powless, 2007). The stimuli in these studies were of a low-to-moderate level of arousal based on normative

ratings, however; conclusions about the effects of neutral stimuli cannot be drawn from these findings because there was no delineation between different stimuli based on arousal ratings. An important contribution of the latter investigation is that memory for the words was enhanced regardless of the arousal manipulation's valence. Stress-inducing techniques, such as CPS or the TSST, have historically been used to manipulate arousal in these studies. It is an interesting consideration that arousal may serve its purpose independent of its pleasant or unpleasant value.

There is plentiful evidence to suggest that emotional memory can be enhanced through the manipulation of arousal at encoding. This effect has been demonstrated across studies utilizing pharmacological, psychological, or physiological stress interventions. The specific enhancement found for emotional, but not neutral, stimuli suggests that there is an interaction between arousal at encoding and during consolidation. Taken together with studies of pre-encoding arousal, we are presented with the importance of the time-dependent effects of arousal on specific memory processes. These findings have informed research that aims to utilize a single session (i.e. acute) exercise to manipulate arousal, as the following section will discuss.

Acute Exercise and Memory

The literature investigating the effects of acute cardiovascular exercise on memory has recently been reviewed and subjected to meta-analysis (Roig et al., 2013). General results of this meta-analysis indicate that acute exercise can have moderate-to-large effects on long-term memory. A very small proportion of these studies have specifically investigated memory for emotional content, however; the idea that exercise

can act to influence arousal is by no means new. In fact, the interaction between the sympathetic nervous system and exercise training forms the mechanistic basis for the cross-stressor adaptation hypothesis. This hypothesis suggests that exercise can act as a "stressor" to stimulate the adaptation of the stress-response system to challenges other than exercise (Sothmann et al., 1996). Here, it serves us to conceptualize acute exercise as a modulator of arousal, based on the observation that it can stimulate the sympathetic nervous system. It appears that high-intensity exercise, specifically, may be of value in manipulations of arousal. A single session of exercise $\geq$ 70% of VO_2 max has been shown to increase sAA, a correlate of central NE release (Allgrove et al., 2008; Koibuchi & Suzuki, 2014).

It has been demonstrated in studies of pharmacological and psychological arousal that the effects on memory depend heavily on the timing of the manipulation relative to encoding. The working principle of the current study is that these time-dependent effects should translate to the effects of acute exercise-induced arousal on long-term memory. An additional, equally important, consideration is the emotional quality of the to-be-remembered content. Keeping in line with memory consolidation research, it should be expected that a sufficient exercise bout after encoding would provide a selective enhancement of emotional memory. While the findings of studies with pre-encoding arousal have less consistent findings, acute exercise research has demonstrated the potential for a single session of exercise to influence memory. The studies outlined below have contributed theoretical and methodological reasoning to the current project.

Acute exercise before encoding

Although acute exercise has been studied in the context of several cognitive domains (e.g. working memory, executive function), very few studies have utilized exercise to specifically target encoding or consolidation processes. Some investigations have prescribed exercise before administration of a battery of examinations, often with the encoding and retrieval tasks nested amongst other examinations (e.g. Coles & Tomporowski, 2008). This design limits the ability to draw conclusions about the phase-specific effects of exercise. Several studies have shown no effects of pre-encoding exercise on long-term memory, however; these have included light (i.e. walking) exercise conditions (Hopkins, Davis, Vantieghem, Whalen, & Bucci, 2012; Schramke & Bauer, 1997). Studies that have shown memory improvements due to pre-learning exercise have utilized short to medium (20-40 min) duration and moderate or higher intensity (≥40% heart rate reserve) exercise protocols with close proximity to the learning event (Labban & Etnier, 2011; Roig et al., 2013; Winter et al., 2007). The meta-analysis conducted by Roig and colleagues (2013) also suggests that longer delays between encoding and retention have resulted in larger effects on memory. An important consideration is that many of these studies are investigating memory for neutral (i.e. non-arousing) stimuli, contributing to a body of research with varied results.

Winter and colleagues (2007) utilized a cross-over design to assess the effects of pre-encoding moderate- and high-intensity running on immediate and delayed retention for an associative vocabulary learning task. The moderate-intensity condition consisted of 40 minutes of running at an individually prescribed heart rate, based on a field fitness test. The high-intensity exercise condition consisted of two 3-minute sprints separated by

a 2-minute break. Blood plasma catecholamines (i.e. epinephrine and norepinephrine) were measured at baseline, post-exercise, and post-encoding as measures of physiological arousal. Recognition and free-recall of the novel words was assessed after a 1-week delay and again 8+ months after learning. The only reported difference was better recognition after 1 week post-learning for the high-intensity condition compared to moderate-intensity running. No beneficial memory effects were found for moderate-intensity exercise compared to rest. Plasma epinephrine increase during the high-intensity exercise session was significantly and positively related to accurate retention at 1 week and 8+ months after learning, although this peripheral measure is not an ideal indicator of central stress hormone activity. A strength of this study is the substantial delay before the retrieval session(s), which allows the reasonable assumption that the exercise manipulation did not influence arousal during retrieval. The results suggest that high-intensity exercise before encoding can enhance delayed recognition of an associative vocabulary task. The cross-over design, which utilized the moderate- and high-intensity exercise conditions as a within-subject variable, limits the conclusions that can be made from this study, although the intention to compare exercise intensities should be pursued further in future studies.

Acute exercise after encoding

Investigations of post-encoding exercise, including resistance training and hand-grip exercises, have reported enhanced long-term memory (Nielson, Wulff, & Arentsen, 2014; Segal et al., 2012; Weinberg, Hasni, Shinohara, & Duarte, 2014), however; these results have only been reported for emotionally arousing stimuli (i.e. pleasant or unpleasant), supporting earlier findings that some degree of arousal at encoding is

necessary for post-learning enhancement of memory (Cahill & Alkire, 2003). An exception is a study by Nielson and colleagues (2014), which reported memory enhancement for logical and visual memory tests as a result of post-encoding ergonomic hand-grip exercises.

The effects of exercise-induced noradrenergic activation on emotional memory have also been observed in older individuals. A study by Segal et al. (Segal, Cotman, & Cahill, 2012) investigated the effects of acute exercise on endogenous noradrenergic activation in both healthy older adults and individuals with mild cognitive impairment (MCI). Participants were exposed to a series of 20 pleasant and moderately arousing IAPS pictures. After picture viewing, participants in the exercise group cycled on a stationary bike for 6 minutes at 70% of estimated VO_2 max while those in the control group rested quietly. Saliva samples for measurement of sAA were taken before picture viewing, after picture viewing, immediately after exercise or rest, and at 10-, 30-, and 50-minute intervals following treatment. A surprise free-recall test was administered one hour after the completion of rest or exercise.

As expected, healthy older adults had significantly higher free-recall performance than those with MCI. In both groups, sAA significantly increased as a result of exercise compared to the rest condition, suggesting that noradrenergic activation is preserved even in individuals with cognitive decline. Memory enhancement was observed as a result of post-learning exercise in both healthy and MCI participants, with those in the exercise condition demonstrating a 1.3-fold and 2-fold improvement in free-recall, respectively. These results indicate that a short bout of moderate-intensity exercise in healthy older

adults and elders with MCI increases sAA to a comparable extent and improves memory for pleasant images.

Time-dependent effects of acute exercise on memory

A study designed to investigate the time-dependent effects of acute exercise provided the foundation of this project's experimental design. Labban & Etnier (2011) aimed to determine the effects of a moderate-intensity bout of cycling on long-term recall of a paragraph learning task. Participants were randomly assigned to three groups: exercise-prior, exercise-after, and a resting control group. Exercise consisted of a 30-minute session of moderate-intensity exercise on a cycle ergometer, and included 5-minute warm-up and cool-down periods. Exercise intensity was prescribed by instructing subjects to pedal at a rate that maintained a rating of 13-15 on Borg's ratings of perceived exertion (RPE) scale (Borg, 1982). Heart rate (HR) and RPE were assessed at five-minute intervals during both rest and exercise intervals for all subjects, and mean HR during the moderate-intensity stage of exercise was 146.53 bpm.

After 30 minutes of rest or exercise, subjects were exposed to the standard New York University Paragraphs for immediate and delayed recall. After paragraph exposure, the exercise-after group completed the acute exercise session while subjects in the other group completed 30 minutes of quiet seated rest. Following this 35-minute delay, delayed recall for the paragraphs was assessed. The authors found significant between-group differences in mean delayed recall score. The exercise-prior group remembered significantly more items than the exercise-after group and the control group. Paragraph recall for the exercise-after group was not significantly different than the control group. Effect sizes for the exercise-prior group over the control group (d=1.04) and the exercise-

prior group over the exercise-after group (d=0.75) indicate a moderate-to-large positive effect of exercise on delayed recall memory. The delayed recall was administered after a 35-minute delay, however; the study design makes it impossible to separate the effects of exercise on consolidation and retrieval processes. Stress-induced hormone activity immediately before memory recall has been shown to hinder memory performance (Smeets, Otgaar, Candel, & Wolf, 2008). It is possible that exercise-induced arousal so close to retrieval may disrupt the memory enhancement that results from exercise during consolidation.

These findings suggest that an acute bout of moderate-intensity and duration cycling before encoding can improve performance on a delayed recall memory task. In the context of the current project, the New York University Paragraphs for immediate and delayed recall would be considered neutral material. This presents support for the hypothesis that pre-encoding exercise may benefit memory for stimuli without the necessity for emotional arousal at encoding. This study did not include emotionally arousing stimuli, so examination of the valence- and time-dependent memory effects is not possible. Additionally, the relatively short delay between encoding and recall precludes clear conclusions about what phase of memory the exercise intervention influenced. The current study aims to expand on this experimental design to determine the time-dependent effects of acute exercise on memory for emotional and neutral content.

CHAPTER II: EXPERIMENT

Introduction

The ability of exercise to influence sympathetic nervous system activity has important implications as a therapeutic tool to selectively enhance memory. Acute exercise-induced arousal may be effective in mimicking the effects of emotional arousal, even in the absence of inherently emotional content. While pharmacological administration of β-adrenergic agonists and exposure to stressful events are alternative means to induce arousal-based memory enhancement, exercise has greater potential as a model because it is non-invasive, inexpensive, and accessible.

Previous exercise studies have either utilized only neutral stimuli (e.g. Labban & Etnier, 2011; Winter et al., 2007), uniformly valenced stimuli (e.g. Segal, Cotman, et al., 2012), or only prescribed post-learning exercise (e.g. Weinberg, Hasni, Shinohara, & Duarte, 2014). Some investigations of this relationship have not found exercise-induced benefits for long-term memory, but the shortage of studies with direct measures of NE correlates leaves several questions to be asked. Therefore, more work is needed to further understand the temporal relationship between exercise-induced arousal and long-term memory for both emotional and neutral stimuli. The capacity for acute exercise to act as a stressor, or noradrenergic activator, in this context lends to its potential as an accessible memory-enhancing tool.

The purpose of this study was to determine the effects of acute-exercise timing on long-term memory for emotional and neutral stimuli. We also aimed to elucidate the relationship between acute exercise-induced noradrenergic activity, indicated by sAA, and memory enhancement for these stimuli. Based on previous research, we

hypothesized that high-intensity exercise after encoding would selectively enhance memory for emotional stimuli, while the same exercise condition before encoding would enhance memory for both emotional and neutral stimuli. A secondary hypothesis was that memory performance for the affected stimuli would be associated with increased noradrenergic activity. As an exploratory component of the study, estimates of subjective recollection were included in the study design in an attempt to delineate changes in objectively measured recognition from changes in subjective memory vividness. Because previous work has suggested an influence of valence on the subjective vividness of memory retrieval, pleasant and unpleasant arousing pictures were considered separately in our study design.

Specific Aims and Hypotheses

Specific Aim #1: To determine the influence of acute exercise timing on long-term memory for emotional (pleasant and unpleasant) and neutral pictures.

Hypothesis #1a: Acute exercise performed before encoding will improve recognition memory for pleasant, unpleasant, and neutral pictures.

Hypothesis #1b: Acute exercise performed after encoding will selectively improve recognition memory for pleasant and unpleasant pictures.

Specific Aim #2: To determine the relationship between acute exercise-induced changes in sAA and long-term memory for emotional (pleasant and unpleasant) and neutral pictures.

Hypothesis #2a: Exercise-induced sAA elevation prior to encoding will be correlated with enhancement of long-term memory for pleasant, unpleasant, and neutral pictures.

Hypothesis #2b: Exercise-induced sAA elevation after encoding will be correlated with the selective enhancement of long-term memory for pleasant and unpleasant pictures.

Method

Participants

Healthy young adults (n = 46) ages 18-30 were recruited through undergraduate courses and flyers at the University of Maryland, College Park. Extra credit for courses was offered at the discretion of the course instructor. Both males and females were recruited. A screening survey was administered to exclude individuals who reported: a history or current diagnosis of anxiety, depression or mood disorder, participation in moderate-high intensity physical activity < 2 days/week, contraindications to moderate-to-high intensity exercise, or participation in a previous study which used the same picture stimuli. Upon enrollment, participants were randomly assigned to an exercise-before (Ex-Before), exercise-after (Ex-After), or a control (Rest) group. Separate randomization sequences were generated for participants using Microsoft Excel (2013) to ensure an equal number of male and female participants in each group. All procedures were approved by the Institutional Review Board at the University of Maryland, College Park.

Participants signed an informed consent form prior to beginning the study procedures (Appendix B). Questionnaires were administered in uniform order to all participants: Health History & Demographic Questionnaire (Appendix C), State-Trait Anxiety Inventory Forms Y1-Y2 (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), Beck Depression Inventory-II (BDI-II; Beck, Steer, & Brown, 1996), Godin Leisure Time Exercise Questionnaire (LTEQ; Godin & Shephard, 1997), 7-Day Physical Activity Recall Interview Questionnaire (7-Day PAR; Blair, 1984). Any individual who scored higher than zero on Question 9 of the BDI-II, which screens for

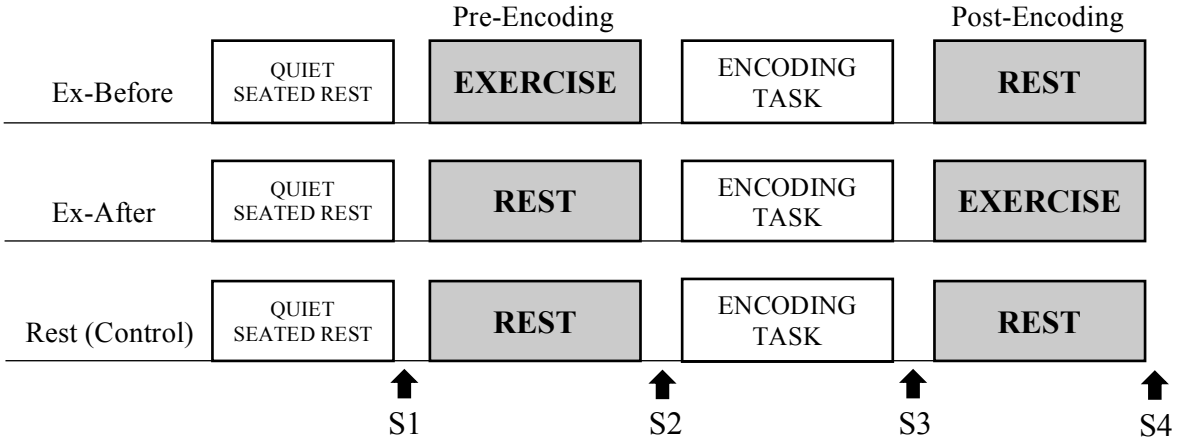
suicidal ideation, was excluded from participation and provided with contact information for the UMD Counseling Center and the Suicide Prevention Hotline.

Procedure

Study participation took place on two separate days separated by 48 hours. Participants were instructed to abstain from caffeine, nicotine, and strenuous exercise for the 2 hours before each session. On Day 1, participants signed the informed consent form. The purpose of the study was described in general terms to reduce the likelihood that participants would anticipate a memory test. Participants completed questionnaires in a pen and paper format. The 7-Day PAR was administered as an interview in accordance with standardized instructions (Blair, 1984). After the completion of questionnaires, all responses were reviewed to ensure that the participant met inclusion criteria.

The participants were given detailed instructions for the encoding task. A practice session with 6 stimuli was administered, and clarifying questions were answered. The stimuli used in the practice session were similar in arousal quality to those used in the memory task. All participants saw the same images for the practice session, which included 2 images each for pleasant, neutral, and unpleasant valence categories. Following completion of the practice session, participants were fitted with a Polar® heart rate monitor. Detailed instructions were provided regarding the use of the Self-Assessment Manikin valence and arousal dimension assessments (SAM-V, SAM-A) and Borg's Ratings of Perceived Exertion (RPE) scale (Appendices I-L; Borg, 1982; Bradley & Lang, 1994).

Figure 1. *Study design for Day 1*



Arrows indicate timing of saliva collection. Baseline sAA assessed at S1.

Study design is depicted in Figure 1. The procedures for all participants consisted of quiet seated rest, a pre-encoding interval, encoding task, and post-encoding interval. Heart rate (HR) was recorded during all procedural intervals on Day 1. For all rest intervals, participants were seated in a comfortable chair without access to phones, reading, or other materials. Participants in all groups completed 10 minutes of quiet seated rest before providing the baseline saliva sample (S1). The pre- and post-encoding intervals were completed in accordance with group assignment. Participants in the Rest group completed 20 minutes of quiet seated rest for both intervals. Those assigned to the exercise-before and exercise-after groups completed cycling exercise during the appropriate interval and rested for the other interval. The encoding task was administered in an identical fashion for all participants. Saliva samples were collected at baseline (S1), before and after the encoding task (S2, S3), and after the post-encoding interval (S4).

On Day 2, participants completed STAI Form Y-2 and were then fitted with the heart rate monitor. HR was recorded for the remainder of Day 2. Participants first

completed 10 minutes of quiet seated rest. Scripted instructions were provided for the retrieval task, with emphasis on the remember-familiar distinction (Appendix I). A 6-trial practice session was completed, including 3 pictures that were seen during practice on Day 1. The experimenter ensured the participant's understanding of the task before continuing. The retrieval task was administered in an identical fashion for all participants. Completion of the task marked the completion of study participation, at which point a post-experimental questionnaire (Appendix J) was administered. Participants were then debriefed and fully informed of the complete purpose and hypotheses of the study (Appendix K).

Exercise Protocol

The exercise interval consisted of a single session of acute high-intensity aerobic exercise on a Monark Ergomedic 828E Cycle Ergometer. Cycling was chosen based on the findings of meta-analysis, which have found a stronger effect for cycling than treadmill exercise (Lambourne & Tomporowski, 2010; Roig et al., 2013). Participants in the Ex-Before and Ex-After groups cycled for 20 minutes at a self-selected intensity corresponding to 15 ("hard") on Borg's RPE scale (Borg, 1982). A 2-minute warm-up and 2-minute cool-down were provided. Borg's RPE scale has been validated as a practical and affordable way to monitor exercise intensity across exercise modalities (Chen, Fan, & Moe, 2002; Scherr et al., 2013). Participants controlled pedaling speed and resistance on the cycle ergometer during the entirety of the exercise session, and received prompting for the appropriate intensity level from the experimenter. Resistance, power output, and HR were recorded at 0-, 5-, 10-, 15-, and 20-minute time points during the exercise. At the same time points, participants were asked to provide RPE, SAM-V,

and SAM-A responses. A saliva sample was collected at the beginning of the 2-minute cool-down. Immediately after the cool-down, participants were escorted to the exercise physiology laboratory and blood lactate concentration was measured using a Lactate Plus Lactate Analyzer.

Memory Task

A recognition task incorporating the R-K paradigm (Tulving, 1985) was administered to assess memory for emotional (i.e. pleasant and unpleasant) and neutral picture stimuli. We chose to use a Remember-Familiar (R-F) distinction, a variant of the R-K paradigm (Tulving, 1985; Yonelinas, Kroll, Dobbins, Lazzara, & Knight, 1998). This choice was made to reduce the likelihood that participants would confuse the concept of "knowing" with response confidence. A total of 180 pictures from the International Affective Picture System (IAPS) were selected as stimuli based on normative affective ratings from the IAPS instruction manual (Lang et al., 2008). For picture selection, emotional pictures were defined as those with an arousal rating ≥ 6.0 (i.e. highly arousing). Pleasant and unpleasant pictures were further defined as having a valence rating ≥ 6.0 or ≤ 4.0, respectively. Neutral pictures were defined as having an arousal rating ≤ 4.0. The selected images were used to create two lists including 90 pictures each: 30 pleasant, 30 neutral, and 30 unpleasant (Appendix A). Lists were matched on semantic content (e.g. both lists had a comparable selection of scenes, people, and animals) as well as overall valence and arousal characteristics (Table 1). A separate selection of 9 pictures was used for the encoding and retrieval practice tasks.

For all components of the memory task, participants were seated at a desk with a button response box positioned at a comfortable distance from their right hand. Stimuli

and task prompts were presented electronically on a 15" monitor using E-Prime 2.0 Software (Psychology Software Tools, Pittsburgh, PA). To enhance attention to the task and improve visibility, the lights were turned off during the encoding and retrieval tasks. Participants were instructed to rate each picture as "Arousing," "Somewhat Arousing," or "Not Arousing" using the button response box. Arousal was described according to the standard instructions for the administration of the SAM, specifically as "a feeling of activation or stimulation you get when looking at a picture." Participants were instructed to press the button at any point between the onset of the current and subsequent stimulus. Visual prompts for the arousal ratings remained on the screen for the duration of the task.

Table 1. *Normative affective ratings of selected IAPS stimuli*

Valence Ratings			
	Pleasant	Neutral	Unpleasant
List A	7.34 (0.54)	5.17 (0.46)	2.55 (0.67)
List B	7.21 (0.52)	5.19 (0.50)	2.63 (0.60)
Total	7.28 (0.53)*ǂ	5.18 (0.48)	2.59 (0.63)*ǂ
Arousal Ratings			
	Pleasant	Neutral	Unpleasant
List A	5.01 (0.63)	3.24 (0.57)	6.13 (0.69)
List B	4.57 (0.66)	3.24 (0.55)	6.00 (0.82)
Total	5.02 (0.64)*	3.24 (0.56)	6.06 (0.75)*

Normative affective ratings for each list and valence category calculated from values reported by Lang et al. (2008). Values are reported as Mean (SD).
** $p < .05$ (one-tailed), compared to neutral pictures*
ǂ $p < .001$ (one-tailed) comparison between pleasant and unpleasant pictures

The encoding phase took place on Day 1. The procedure for the encoding task was identical to the practice procedure. Participants were reminded of the task

instructions by prompts provided on the computer screen. During the task, participants were exposed to one list (90 pictures) in a standard order. The order of stimulus presentation for each list was pseudo randomly predetermined to ensure that no more than 3 pictures of a given valence were presented sequentially and that temporally related pictures did not contain semantically related content. The use of each list as the encoded set of pictures was counterbalanced. Pictures were presented one at a time for a duration of 3000 ms. A fixation cross was displayed during the inter-stimulus interval, which lasted for a randomly selected duration of 3000, 3500, or 4000 ms. The encoding task lasted approximately 10 minutes.

The retrieval phase took place on Day 2. Scripted instructions (Appendix M) were provided to participants before completion of the practice task. For each picture, participants were first asked to report if the picture was "Old" or "New," using the button response box. If "Old" was selected, the task continued to a screen that prompted to participant to report if the picture was "Remember[ed]" or "Familiar." The distinction between responses was described using instructions adapted from Dudukovic & Knowlton (2006):

> "When we *remember* something, we consciously recollect and become aware of aspects of its occurrence. For example, you might remember a recent movie and be able to recall *specific details* about it, like where and with whom you saw it. At other times, we simply know that something has occurred before, but without being able to consciously recollect what was experienced at the time of its occurrence. For example, you might recognize a person and be certain that you know him or her, but be unable to recall any specific details about the person, such as the person's name or how you met."
>
> "For this test, remembering a picture would signify that the picture evokes specific memories of what was experienced during its presentation, such as how it looked on the screen, the way in which it was presented, or even what you were thinking or doing at the time it was shown. Feeling a picture is familiar would

mean that you believe the picture appeared, or have a sense of having seen it before, but cannot recollect any aspects or details of its presentation."

The experimenter asked the participant to describe the meaning of each response until it was clear that the difference was understood. Participants then completed a practice test, which included 6 pictures: 3 from the encoding practice task, and 3 novel pictures.

The retrieval task included the total selection of 180 pictures from both lists presented to all participants in the same order. The order of stimulus presentation was pseudo randomly predetermined to ensure that no more than 3 pictures from a given list were presented sequentially. In this form of recognition task, a participant can correctly classify a previously encoded item as Old (i.e. hit) or may incorrectly classify an old item as New (i.e. miss). A novel item can be correctly identified as New (i.e. correct rejection) or incorrectly identified as Old (i.e. false alarm). The appropriate Old/New response for each picture was dependent on which list was viewed during the encoding phase. All responses were self-paced. The retrieval task lasted approximately 15 minutes, and was programmed to include a break after the first 90 pictures. Participants were allowed to report button-pressing errors (e.g. "I accidentally pressed 1 when I meant to press 2,") to the experimenter only before making the next response. These errors were manually corrected in the experimental output during data entry.

Saliva Collection and Analysis

The non-invasive measurement of sAA served as a marker for sympathetic nervous system activity. Saliva samples were collected at 4 time points across Day 1 using the passive drool method (S1-S4, Figure 1). To avoid sample dilution, water was restricted for the 10 minutes before sample collection. The passive drool method requires

participants to allow saliva to pool in the floor of the mouth and gently direct saliva into a tube through a saliva collection aid. This method is recommended over the alternative oral swab method as a means of collecting unstimulated whole saliva from adults in a supervised laboratory setting (Rohleder & Nater, 2009).

Analysis of sAA was conducted using the Salimetrics® (State College, PA) kinetic enzyme assay kit. Samples were collected using SalivaBio Saliva Collection Aids from Salimetrics®. After collection, samples were immediately stored on ice and subsequently frozen at -40˚ C. On the day of the assay, samples were thawed and diluted with the provided alpha-amylase diluent (1:200). Absorbance was measured with a 405 nm filter on a BioTek ELx808™ Absorbance Microplate Reader set to incubate at 37˚C. Strips were tested two at a time. For quality control purposes, the first and second wells of each strip contained the High and Low controls supplied with the Salimetrics® kit. Each saliva sample was analyzed in duplicate across the pair of strips during the same absorbance reading cycle (Table 2).

Table 2. *Sample 2-strip layout for sAA absorbance measurement*

	1	**2**
A	Ctrl L	Ctrl L
B	Ctrl H	Ctrl H
C	T1 - R1	T1 - R1
D	T1 - ExB1	T1 - ExB1
E	T1 - ExA1	T1 - ExA1
F	T1 - R2	T1 - R2
G	T1 - ExB2	T1 - ExB2
H	T1 - ExA2	T1 - ExA2

Ctrl L = Low control; Ctrl H = High control; T = time point; R, ExB, ExA = Rest, Ex-Before, Ex-After.

Statistical Analysis

Statistical analyses were performed using Microsoft Excel (2013) and SPSS (IBM Corp., Version 23.0). An alpha value of 0.05 was set as the threshold for significance in all statistical tests. For analysis of variance (ANOVA) procedures with repeated measures, Huynh-Feldt adjustment was used when Mauchly's test of sphericity met significance. In these cases, corrected *p*-values are reported with the original degrees of freedom. If multiple tests were performed for post hoc analyses, the false discovery rate (FDR) was calculated to control the family-wise error rate.

Memory Data

Recognition memory indices were calculated within each valence category using the P_r discrimination index, which relies on hits and false alarms (Snodgrass & Corwin, 1988). Recognition scores were calculated as the difference between hit rate and false alarm rate:

$$Pr(Recognition) = \left(\frac{hits}{\#\ old\ items}\right) - \left(\frac{false\ alarms}{\#\ new\ items}\right)$$

Recollection and familiarity were indexed as the percentage of hits with "Remember" and "Familiar" responses, respectively. Recognition and recollection data were submitted to a 3 (Group: Rest, Ex-Before, Ex-After) × 3 (Valence: pleasant, neutral, unpleasant) × 2 (List: List A, List B) mixed ANOVA. Follow-up contrasts were assessed with independent or paired *t*-tests.

Recognition and recollection memory were also assessed using participants' subjective arousal ratings from the encoding task. Retrieval stimuli were categorized as Very Arousing, Somewhat Arousing, or Not Arousing. Previously encoded ("Old")

pictures for each participant were assigned to arousal categories based on that participant's rating, provided using the button response box during encoding. Individual stimuli were excluded from scoring if a participant failed to provide a response. Subjective arousal ratings were not available for "New" pictures presented for the first time during the retrieval task. However, because the stimuli were presented in two sets that were counterbalanced within each group for encoding and retrieval, the categorization based on arousal for "New" pictures was determined by the rating most commonly assigned to each picture by participants within the same experimental group who were exposed to the alternate list during encoding. Recognition and recollection indices were calculated as described above. Recognition and recollection data were submitted to a 3 (Group: Rest, Ex-Before, Ex-After) × 3 (Arousal Rating: Very Arousing, Somewhat Arousing, Not Arousing) × 2 (List: List A, List B) mixed ANOVA. Follow-up contrasts were assessed with independent or paired *t*-tests.

Salivary Alpha-Amylase Data

Saliva samples were assessed for sAA only from participants included in the memory performance analyses. Samples were excluded from the analysis if there was an insufficient volume of saliva. The coefficient of variation (CV) was calculated between duplicates of each sample. An *a priori* value of 15% was chosen as a threshold value for acceptable between-sample variation. Data points were calculated from the average of the duplicate samples that fell below the CV threshold. Only subjects with intact data points for all four saliva samples were included in statistical analyses. SAA values were submitted to a 3 (Group: Rest, Ex-Before, Ex-After) × 4 (Time) mixed ANOVA. Follow-up contrasts were assessed using paired samples *t*-tests.

Correlation Analysis

To explore the association between changes in sAA and memory, correlations were calculated between memory indices and sAA change, both for the entire sample and within Rest, Ex-Before, and Ex-After groups. The change in sAA across the pre-encoding (S2-S1) and post-encoding (S4-S3) intervals was calculated as an indicator of exercise-induced NE release. Correlations were computed between recognition scores (pleasant, neutral, unpleasant) and sAA change at both intervals. Because a main effect of Valence on recollection was revealed in the previous analyses, correlation coefficients were also calculated for recollection indices (pleasant, neutral, unpleasant) and sAA change at both intervals. Extreme outliers were defined as any data point falling more than three times the interquartile range above the 3rd quartile or below the 1st quartile. Two data points were identified as outliers and excluded based on this criterion. For the whole-sample analysis, the Shapiro-Wilk test of normality indicated that sAA change indices were not normally distributed; therefore the correlations were calculated using a Spearman's correlation. The Shapiro-Wilk test did not meet significance for when the dependent variables were evaluated within experimental groups. Pearson's product-moment correlation coefficients were calculated for within-group correlation analyses.

Results

Participant Characteristics

Table 3 reports characteristics and questionnaire results for included participants within all experimental groups. Of the 46 participants (30 female) enrolled, 2 participants were excluded from participation because of scores that exceeded exclusion criteria on the BDI-II. Three additional participants' data were excluded from all analyses due to computer errors (n = 2) or failure to complete both visits (n = 1). Differences between groups were assessed using independent samples *t*-tests (two-tailed) where appropriate. No significant differences in age, self-reported anxiety or depression symptoms, or self-reported physical activity levels were found between groups.

Table 3. *Participant demographics and questionnaire results*

	Rest (Control)	**Exercise Before**	**Exercise After**
n	11	15	14
Age (years)	20.3 (1.7)	22.1 (3.2)	22.8 (4.7)
Gender (% female)	72.7	60	71.4
Trait Anxiety	36.5 (10.9)	32.5 (7.4)	34.4 (5.8)
BDI-II	5.1 (6.1)	4.7 (4.0)	5.3 (4.9)
LTEQ Activity (hrs/wk)	70.8 (22.3)	54.9 (22.8)	56.1 (27.6)
7-Day PAR (kJ/kg/day)	134.9 (12.7)	127.6 (57.0)	114.9 (41.0)
State Anxiety, Day 1	33.3 (10.5)	31.1 (9.7)	28.1 (6.8)
State Anxiety, Day 2	27.9 (7.4)	29.9 (7.6)	26.1 (6.5)

Values are reported as Mean (SD); Trait Anxiety = State-Trait Anxiety Inventory Form Y-2; BDI-II = Beck Depression Inventory-II, LTEQ = Leisure Time Exercise Questionnaire; 7 Day PAR = 7 Day Physical Activity Recall Interview Questionnaire; State Anxiety = State-Trait Anxiety Inventory Form Y-1.

Exercise Manipulation Check

Data from the exercise and rest conditions for each group are reported in Table 3. Participant values for exercise and rest sessions were calculated using data recorded at 10-, 15-, and 20-minute time points to capture the interval in which exercising subjects had reached the prescribed exertion rating and cardiovascular adaptations to the onset of exercise had reached relative stability. Within-group comparisons between rest and exercise sessions were calculated using paired samples *t*-tests (two-tailed). Between-group comparisons of rest sessions and exercise sessions were calculated using independent samples *t*-tests (two-tailed).

Table 4. *Exercise and rest session data*

		Group		
		Rest (Control)	Exercise-Before	Exercise-After
Rest Session(s)	HR	63.9 (1.5)	88.8 (6.4) §ǂ	54.6 (1.39) §ǂ
	RPE	7.0 (0.4)	6.1 (0.1)	6.6 (0.6)
	SAM-V	6.3 (0.3)	5.8 (0.8)	5.0 (0.5) §
	SAM-A	1.3 (0.2)	3.4 (0.9)	1.8 (0.4)
Exercise Session	Watts	-	135.8 (11.3)	127.0 (7.3)
	HR	-	159.3 (5.0) **	159.2 (4.6) **
	RPE	-	14.8 (0.1) **	15.0 (0.1) **
	SAM-V	-	5.6 (0.4)	6.3 (0.4)
	SAM-A	-	5.6 (0.4) *	5.4 (0.6) *
	Lactate (mmol/L)	-	6.17 (2.7)	5.35 (2.9)

Values calculated from recorded data at 10, 15, and 20-minute time points. Values are reported as Mean (SEM). HR = heart rate; RPE = ratings of perceived exertion, SAM-V = Self-Assessment Manikin-Valence; SAM-A = Self-Assessment Manikin Arousal.
§ $p < .05$, compared to control group
ǂ $p < .001$, comparison between exercise groups
** $p < .05$, compared to rest session*
*** $p < .001$, compared to rest session.*

Within both exercise groups, HR and RPE ($p < .001$) as well as SAM-A ratings ($p < .05$) were higher during exercise compared to rest. Between-group differences were observed for HR and SAM-V ratings during the rest session. Both exercise groups had a higher HR during the rest session than the control group ($p < .05$). The Ex-Before group had a higher HR during the rest session (post-encoding) than the Ex-After group (pre-encoding; $p < .001$). The Exercise-After group reported lower ratings on the SAM-V during rest than the control group ($p < .05$). No differences in blood lactate concentration, SAM-V ratings, or power output were found between exercise groups.

Memory Performance

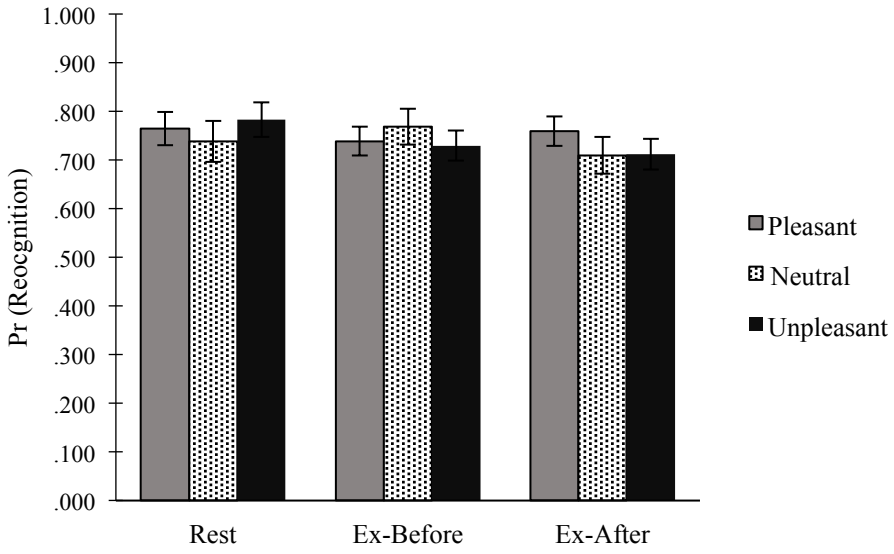
Recognition Memory

None of the participants reported anticipation of a memory test. Data from an additional subject was also excluded because of memory recognition performance that was > 3 SDs below the group mean. There was no main effect of Group or Valence on recognition (Figure 2). Between-group comparisons confirmed no effect of Group on recognition memory for pleasant, neutral, or unpleasant pictures (all $p > 0.1$). There was a significant interaction of List and Valence on recognition memory, $F(2,33) = 9.170$, $p < .001$, $\eta^2 = 0.357$ (Figure 3). Post hoc comparisons indicate that interaction is driven by the recognition of pleasant pictures. Recognition memory for pleasant pictures was significantly higher for participants who were exposed to List B on Day 1 than those exposed to List A ($p = .004$).

Recollection

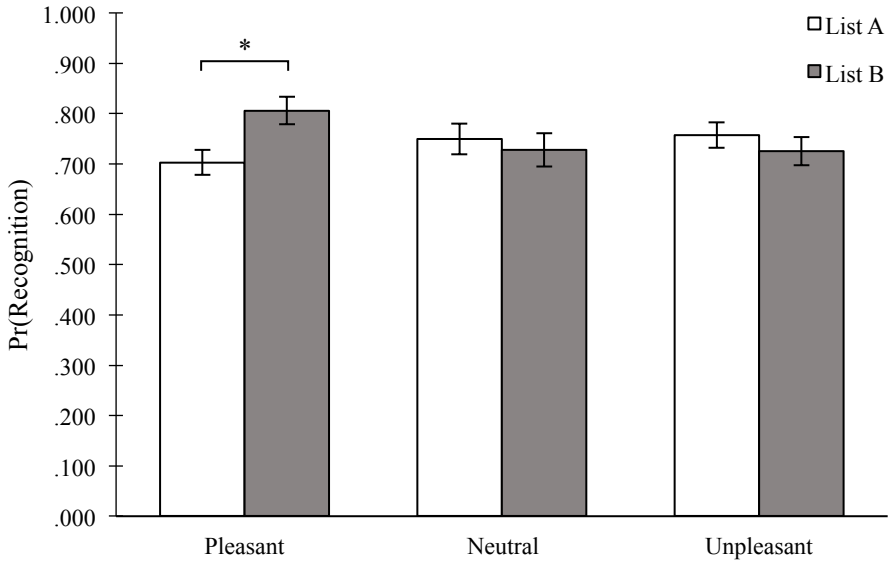
Analysis of recollection indices revealed a main effect of Valence on recollection, $F(2,74) = 16.580$, $p < .001$, $\eta^2 = .309$ (Figure 4). Post hoc comparisons within groups

Figure 2. *Recognition memory scores for all groups and valence categories*



Recognition scores calculated using Pr discrimination index (hit rate – false alarm rate). Error bars denote SEM.

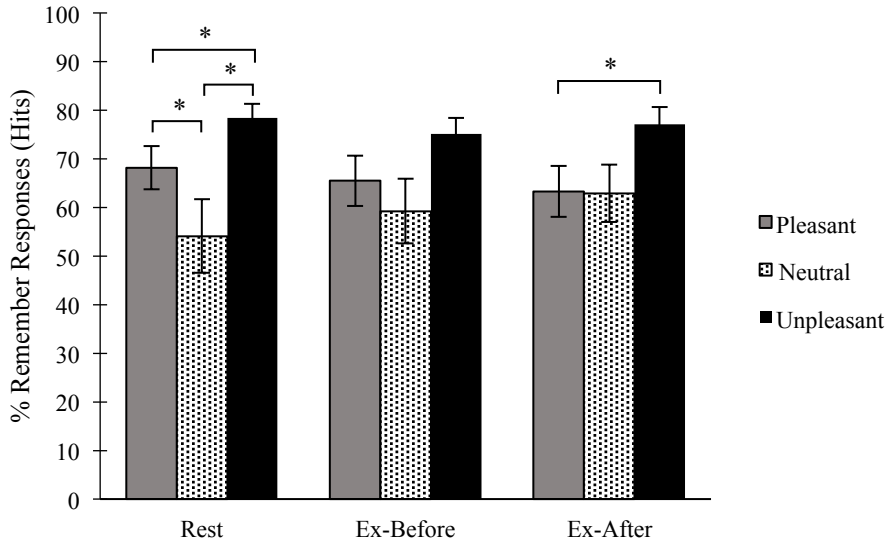
Figure 3. *Recognition memory scores for all valence categories and lists*



Recognition scores calculated using Pr discrimination index (hit rate - false alarm rate). Error bars denote SEM.
** p < .05, comparison between lists.*

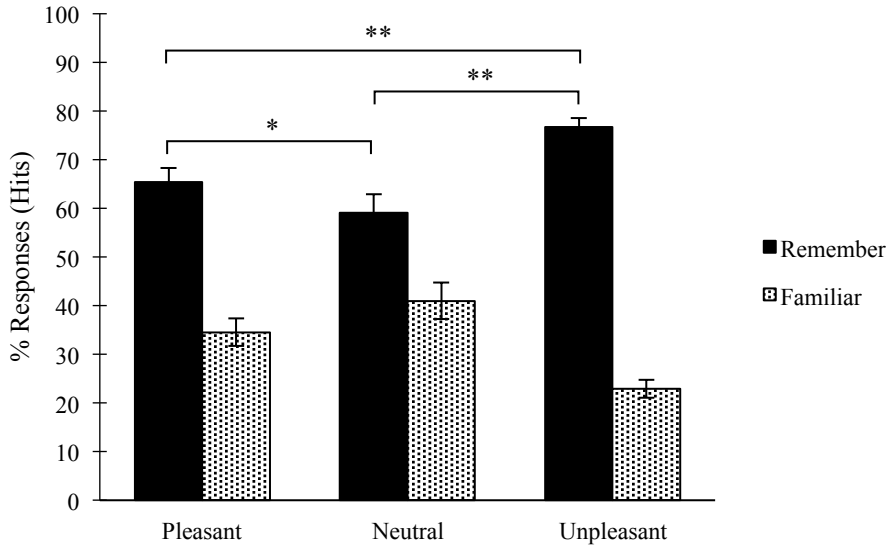
revealed that, within the Rest group, the percentage of *remember* responses to unpleasant hits was greater than that for pleasant (p = .048) and neutral (p = .005) hits. Additionally, recollection was higher for pleasant pictures compared to neutral pictures (p = .013). These comparisons survived FDR correction. In the Ex-Before group, there were more *remember* responses for unpleasant hits than neutral hits (p = .020). In the Ex-After group, there were more *remember* responses for unpleasant hits than both pleasant (p = .007) and neutral (p = .037) hits. Only the difference between pleasant and unpleasant recollection in the Ex-After group survived FDR correction. Collapsing across groups, pairwise comparisons showed that the number of *remember* responses to unpleasant hits was higher compared to pleasant and neutral hits (p < .001, Figure 5). There was no main effect of Group or interaction of Group and Valence on recollection.

Figure 4. *Recollection memory scores for all groups and valence categories*



Recollection scores calculated as % of Hits that received a remember *response. Error bars denote SEM.*
**p < .05, comparison between valence categories.*

Figure 5. *Recollection and familiarity memory scores for all valence categories*



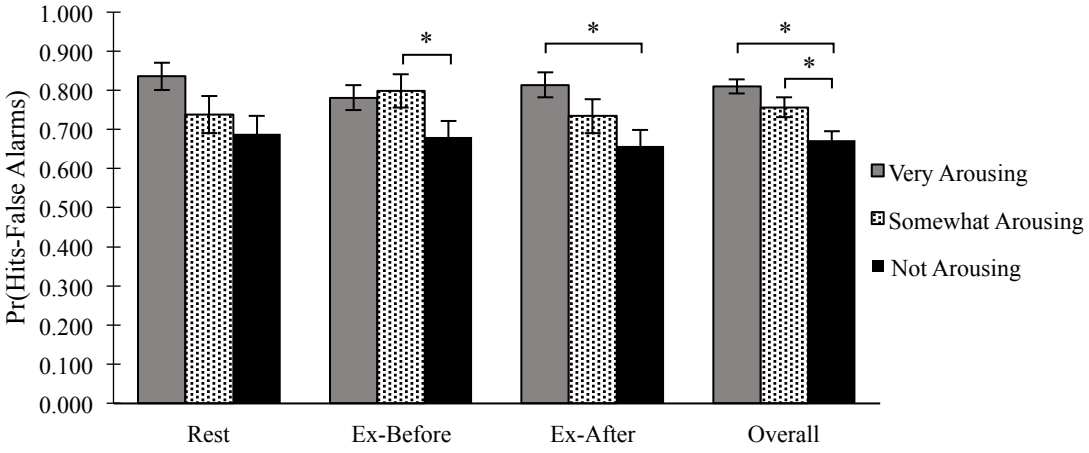
Recollection scores calculated as % of Hits that received remember *response. Familiarity scores calculated as % of Hits that received* familiar *response. Error bars denote SEM.*
** $p < .05$, comparison between valence categories*
*** $p < .001$, comparison between valence categories*

Subjective Arousal Ratings

When retrieval data were grouped and scored using participants' subjective arousal ratings, a main effect of Arousal Rating was revealed for recognition memory, $F(2,72) = 10.562$, $p < .001$, $\eta^2 = .227$ (Figure 6). Post hoc paired *t*-tests conducted within groups revealed that very arousing pictures were better recognized than not arousing pictures in the Rest group ($p = .049$), but this effect did not survive FDR correction. In the Ex-Before group, very arousing ($p = .043$) and somewhat arousing ($p = .009$) pictures were both better recognized than not arousing pictures, but only the comparison between somewhat arousing and not arousing pictures survived the FDR threshold. In the Ex-After group, significantly better recognition for very arousing pictures compared to not

arousing pictures (p = .006) survived FDR correction. Comparing recognition memory for the different Arousal Rating categories collapsed across groups revealed a significant improvement of recognition for very arousing and somewhat arousing pictures compared to not arousing pictures that met significance at the FDR threshold (Figure 6, Overall). There was no main effect of Group or List or interaction of Group and Arousal Rating on recognition memory.

Figure 6. *Recognition memory scores for all groups and arousal rating categories*

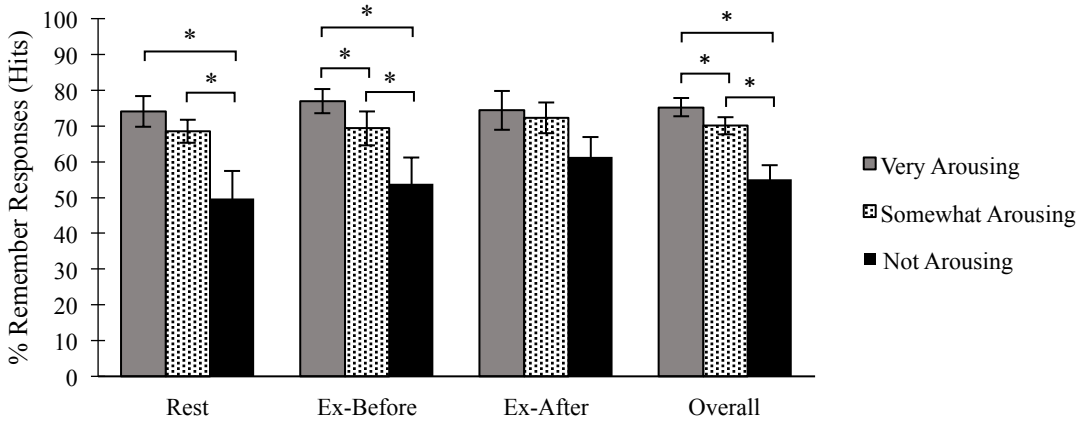


Recognition scores calculated using Pr discrimination index (hit rate – false alarm rate). Error bars denote SEM.
** p < .05, comparison between arousal rating categories*

Mauchly's test of sphericity was significant (p < .001) for the mixed ANOVA on recollection. Huynh-Feldt's corrected p-values are reported with original degrees of freedom. A main effect of Arousal Rating on recollection memory was also revealed, $F(2,72)$, p < .001, η^2 = .353 (Figure 7). Post hoc paired t-tests conducted within groups revealed more *remember* responses for very arousing (p = .001; p = .008) and somewhat arousing (p = .01; p = .027) hits than not arousing hits in Rest and Ex-Before groups.

Additionally, there were more *remember* responses to hits rated as very arousing than those rated as somewhat arousing in the Ex-Before group (p = .048). All of these differences met significance at the FDR threshold. While there were more *remember* responses to somewhat arousing hits than not arousing hits in the Ex-After group (p = .019), this difference did not survive FDR correction. Comparing recollection memory for the different Arousal Rating categories collapsed across groups revealed a significantly higher number of *remember* responses to somewhat arousing hits over not arousing hits, and to very arousing hits over both somewhat and not arousing hits, which survived the FDR threshold.

Figure 7. *Recollection memory scores for all groups and arousal rating categories*



Recollection scores calculated as % of Hits that received remember *response. Error bars denote SEM.*
** $p < .05$, comparison between arousal rating categories*

Salivary Alpha-Amylase

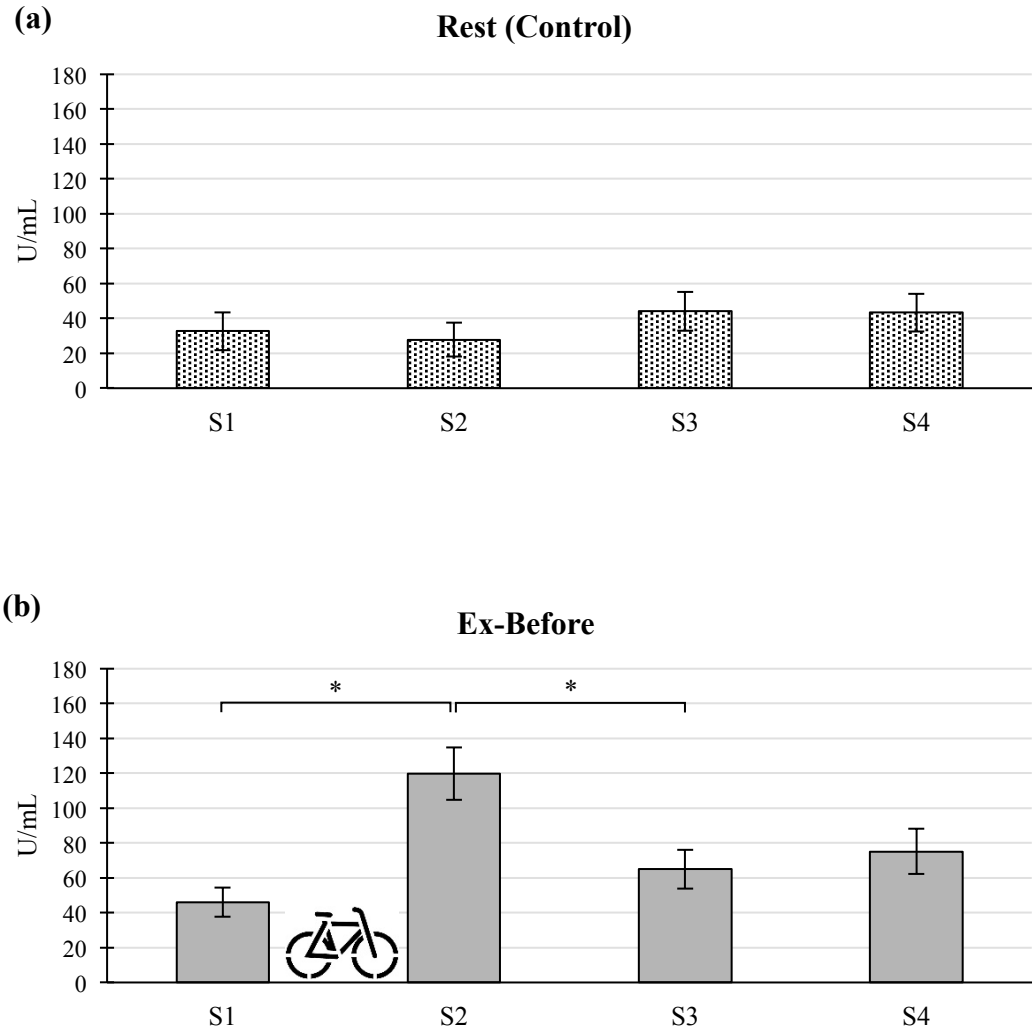
Out of 41 participants included in the memory analysis, saliva samples from an additional 7 participants were excluded from statistical analysis due to an insufficient

volume of saliva (n = 4) or a between-duplicate CV > 15% (n =2). Data from an additional subject was excluded because of a data point that was > 3 SDs from the group mean and exceeded the highest absolute value reported by the Salimetrics® kinetic enzyme assay protocol. Statistical analyses were performed on data from a total of 33 participants (11 Rest, 10 Ex-Before, 12 Ex-After). Mauchly's test of sphericity was significant ($p < .001$) for the mixed ANOVA. Huynh-Feldt's corrected p-values are reported with original degrees of freedom.

There was no difference in baseline sAA between groups (all $p > 0.1$). As expected, there was a significant interaction of Group and Time, $F(6,90) = 6.995$, $p = .001$, $\eta^2 = 0.318$. The main effect of Time was further decomposed using paired t-tests. Significant comparisons were submitted to FDR correction. In the Rest group, there was no significant effect of time on sAA. While sAA at S3 (post-encoding) was higher than both baseline ($p = .043$) and S2 ($p = .019$), these differences did not survive FDR correction (Figure 8a). There was a significant effect of Time on sAA in the Ex-Before group, $F(3,27) = 11.312$, $p < .001$, $\eta^2 = 0.557$ (Figure 8b). Concentration of sAA increased during exercise ($p = .001$) and subsequently decreased during the picture-viewing interval ($p = .003$). Both of these differences survived FDR correction. Uncorrected p-values suggest that sAA after the rest interval (S4) was higher than baseline ($p = .043$) and lower than the post-exercise time point (S2; $p = .031$), but these values did not survive FDR correction. There was also a significant effect of time on sAA in the Ex-After group, $F(3,33) = 7.304$, $p = .019$, $\eta^2 = 0.399$ (Figure 8c). Concentration of sAA increased during exercise ($p = .023$). Post-exercise sAA was

significantly higher than baseline (p = .018) and the pre-encoding time point (p = .020). All of these differences survived FDR correction.

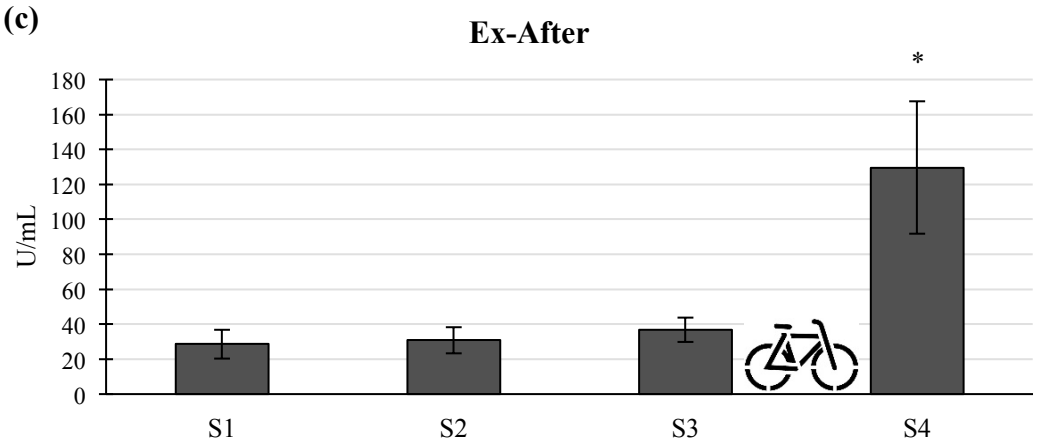
Figure 8. *Average salivary alpha-amylase for Rest, Ex-Before, and Ex-After groups*



Data labels correspond to saliva collection time points: S1 = baseline, S2 = after pre-encoding interval, S3 = post-encoding, S4 = after post-encoding interval. Error bars denote SEM.

** p < .05, comparison between time points*

Figure 8. *Average salivary alpha-amylase for Rest, Ex-Before, and Ex-After groups*



Data labels correspond to saliva collection time points: S1 = baseline, S2 = after pre-encoding interval, S3 = post-encoding, S4 = after post-encoding interval. Error bars denote SEM.
** $p < .05$, comparison between time points*

To explore the association between changes in sAA and memory, the correlation between memory indices and change in sAA was calculated. The Shapiro-Wilk statistic indicated that sAA change indices were not normally distributed in the whole-sample analysis; therefore these correlations were calculated using a Spearman's correlation. There were no significant correlations between recognition scores and sAA change at either interval. There was a moderate and significant positive correlation between post-encoding change in sAA and recollection for neutral pictures ($\rho = 0.394$, $n = 32$, $p = .012$, Suppl. Figure 1) that did not survive FDR correction.

Pearson's product-moment correlation coefficients were calculated for within-group correlation analyses because the Shapiro-Wilk test did not meet significance when the dependent variables were evaluated within experimental groups. There were no significant correlations between recognition scores and sAA change within any of the

groups at either interval. There was a strong and significant positive correlation between post-encoding change in sAA and recollection for unpleasant pictures in the Ex-Before group (r = 0.816, n = 10, p = .007, Suppl. Figure 2). This correlation did not meet significance at the FDR threshold.

Discussion

This study aimed to expand on the existing literature by determining the time-dependent effects of acute exercise on memory for pleasant, neutral, and unpleasant pictures. To our knowledge, this is the first study to utilize acute exercise to manipulate arousal both before and after encoding of emotional and neutral material. Evaluation of recognition indices revealed no effect of exercise or picture type on recognition after a 48-hour delay. Similarly, there was no effect of acute exercise on recollection, the subjective vividness of memory for the pictures. Despite the lack of exercise effects on recognition and recollection, this study did reveal an effect of both valence and subjective arousal on subsequent recognition and recollection. Unpleasantly valenced pictures that were correctly identified as "Old" received more *remember* responses than pleasant pictures, which were recollected better than neutral pictures. These results suggest a higher degree of subjective memory vividness for unpleasant over pleasant, and pleasant over neutral pictures.

The subjective arousal rating results indicated a clear pattern of influence on recognition and recollection, with higher subjective arousal ratings at encoding being associated with better recognition and recollection at retrieval. Exercise before encoding of pictures resulted in better subsequent recognition of pictures that were rated as "Somewhat Arousing" than those that were rated as "Not Arousing". Similarly, exercise after picture encoding resulted in better memory for "Very Arousing" than "Not Arousing" pictures. These differences in recognition based on the subjective arousal of the stimuli were not observed in the Rest group, suggesting the exercise may have augmented the influence of arousal on recognition performance. While there was no

statistically significant main effect of exercise revealed in our analyses, these findings may suggest differential contributions of exercise to emotional arousal and memory processes.

One important consideration is the possibility that exercise influences how the arousal quality of pictures is perceived. Studies of acute exercise have demonstrated that attentional bias is shifted away from unpleasant faces and towards pleasant faces during moderate-intensity exercise (Tian & Smith, 2011). Additionally, moderate-intensity exercise before emotional picture viewing prevents the increase in state anxiety observed when pictures are viewed after a rest condition (Smith, 2013). These studies indicate that exercise before picture viewing may have attentional, perceptual, or anxiolytic influences that influence encoding and subsequent consolidation of emotional material. In line with this reasoning is the observation that emotional arousal in response to unpleasant pictures was significantly decreased after moderate-intensity exercise relative to rest (Crabbe, Smith, & Dishman, 2007). Further analyses of the present results should compare the normative arousal and valence characteristics of pictures in the arousal rating categories for each experimental group. Interestingly, exercise after encoding resulted in improved recognition memory only for the pictures rated as "Very Arousing," which could reflect a threshold of arousal during encoding for arousal-induced memory enhancement (similar to the suggestion of Cahill & Alkire, 2003). An investigation of exercise-induced changes in affective evaluation separate from the effects of exercise on memory processes, as well as their interaction, is warranted.

The current project intended to extend on the work of Labban & Etnier (2011) and others by including emotionally arousing stimuli. Labban & Etnier (2011) executed a

similar design to assess the effects of acute exercise on long-term memory. Memory was operationalized as performance on the New York University Paragraphs for immediate and delayed recall. Their findings suggest that memory for non-arousing content can be enhanced by a moderate-duration and intensity session of cycling before, but not after, encoding. The failure of post-encoding exercise to enhance memory of the paragraphs provides further support for the hypothesis that arousal during encoding is necessary for the success of post-learning memory modulation (Cahill & Alkire, 2003). While some studies of emotional memory have included pleasant and unpleasant emotional stimuli (Liu, Graham, & Zorawski, 2008; Schwabe et al., 2008), acute exercise studies have typically included only neutral stimuli (Labban & Etnier, 2011; Nielson et al., 2014; Winter et al., 2007) or limited the emotional content to include either pleasant or unpleasant categories (Segal, Cotman, et al., 2012). An exception to this is a study by Weinberg and colleagues (2014) that investigated the effects of post-learning resistance exercise on memory for pleasant, neutral, and unpleasant stimuli. This investigation also employed the R-F paradigm and sAA measures as correlates of noradrenergic activation. These strengths were translated to the current study, however; the absence of an Ex-Before group and the use of resistance rather than aerobic exercise should be considered upon comparing the results.

Our hypotheses, reflecting the state of research on arousal and memory, predicted an interaction of Group and Valence on recognition for pleasant, neutral, and unpleasant stimuli. We predicted that exercising immediately after encoding would selectively enhance recognition of emotional pictures. Conversely, exercise before encoding was expected to improve recognition of both emotionally arousing and neutral pictures. The

latter hypothesis was derived from the results of Labban & Etnier (2011), as we predicted that exercise before encoding would provide an enhancement for neutral memory in addition to memory for emotional pictures. The recollection data, while exploratory, exposed the need for further investigation into the interaction between acute exercise, subjective arousal during encoding, and subsequent memory.

Recognition indices in the current study revealed no main effect of Group or Valence on long-term memory for the IAPS pictures, failing to support either of our hypotheses. The only main effect or interaction on recognition was an interaction of List and Valence. Both lists were systematically matched on semantic content and average normative affective ratings (Lang et al., 2008). Therefore, it is unknown why the P_r recognition index for pleasant pictures was higher for List B than and List A. This interaction did not extend to the recollection results, suggesting a difference between lists that contributes to memory for gist rather than detail. For example, other studies have accounted for the visual complexity of pictures (Ochsner, 2000) or ratings of dominance (dominant or submissive) assigned to visual stimuli (Bradley et al., 1992). Analysis of recollection indices did reveal a main effect of Valence, supporting previous findings that suggest enriched memory vividness for emotional stimuli (Kensinger & Corkin, 2003; Ochsner, 2000). There was no main effect of Group, demonstrating that exercise before or after encoding did not successfully enhance memory vividness compared to rest.

Similar predictions were made by Weinberg et al. (2014) based on a similar study design. Their study reported significant main effects of Group and Valence on recognition indices. Emotional pictures were remembered better than neutral pictures, and participants in the exercise group displayed higher accuracy than the control group.

The memory task used for the current study was highly similar to that used by Weinberg et al. (2014) with comparable normative affective ratings for each valence category. A notable difference in the protocol was the duration of the inter-stimulus interval. In the current study, pictures presented during encoding were separated by a fixation cross for a duration of 3000 – 4000 ms to allow adequate time for rating responses, while the previous investigation used a drastically shorter inter-stimulus interval of 500 ms. It is reasonable to conclude that this difference may have influenced the difficulty of the memory task, but further investigation would be necessary to determine the effects of inter-stimulus interval on long-term memory. The deviation of our findings from those of Labban & Etnier (Labban & Etnier, 2011) may be due to differences between memory for pictures and paragraphs; while pictures may include incidentally related content, paragraphs are bound by semantic relatedness and may contain more continuous themes or features across a given narrative.

Recognition performance was exceedingly high for all participants across all valence categories. The possibility exists that a "ceiling effect" is being observed. The average value of all P_r recognition indices calculated by Group and Valence falls between 0.710 and 0.783. A recognition index of 0.767 results from a combined total of 7 misses and false alarms, demonstrating the scarcity of incorrect answers in our participants' overall performance. These data can be compared to the control group of Weinberg and colleagues (2014). The average P_r recognition values for neutral and emotional stimuli fell within a range of 0.4-0.5 and 0.5-0.6, respectively. These differences were enough to reveal main effects of both Group and Valence. The lack of variability between recognition indices in the current study limits the ability to detect effects of within- or

between-subject factors. Recommendations for continued use of this memory task would necessitate an increase in difficulty, which could be achieved by a) increasing the number of encoded and novel stimuli, b) decreasing the inter-stimulus interval, or c) increasing the delay beyond 48-hours, among other changes.

Despite the limitations of the memory task, this study demonstrated that a 20-minute bout of self-paced high-intensity cycling increases sAA in male and female participants. Previous studies of acute exercise have reported increases in sAA following long-lasting (1-2 hours) or high-intensity exercise prescribed relative to VO_2 max or to the point of exhaustion (Allgrove et al., 2008). This is the first study to our knowledge that has demonstrated an increase in sAA resulting from exercise prescribed using Borg's RPE scale (1982). There were no differences in average HR during exercise or post-exercise blood lactate concentration between exercise groups. While further analysis of the relationship between physiological measures and sAA during exercise should be pursued, these data support the validity of RPE as a means of prescribing acute exercise intensity.

While several studies have assessed the ability of different exercise manipulations to increase sAA, there is a lack of systematic investigation regarding sex, duration, fitness and intensity-dependent characteristics of the sAA response to acute exercise. Allgrove and colleagues (2008) assessed the sAA response to cycling at a set duration to exhaustion, at 50% of VO_2 max, and at 75% of VO_2 max. Results indicated that cycling at 75% of VO_2 max or to exhaustion for an average of 22.3 min (SEM = 0.8). This study and others have been limited to men to reduce variability due to sex differences in adrenergic response (Allgrove et al., 2008; Chatterton et al., 1996; Ditzen, Ehlert, &

Nater, 2014). As the utility of sAA to characterize arousal responses develops, it is imperative to expand this line of study to both male and female participants.

Limitations & Future Directions

Some limitations of the current study should be considered. First, recruitment was limited to young adults with no history of anxiety or depression. The findings regarding emotional memory and exercise-induced sAA cannot be generalized to older adults or patient populations. Future investigations should consider the recruitment of participants with high anxiety to explore differences in exercise-induced arousal or emotional memory. Additionally, this sample was composed almost entirely of undergraduate students during the academic semester. There were no measurements in this study to account for stress or physical activity levels in the 48 hours between testing sessions. We also did not ask participants to report the quantity or quality of their sleep between sessions, a factor that could be considered as sleep has been shown to play a part in memory consolidation.

The memory task, while derived from previously utilized tasks, was not difficult enough to draw conclusions about the memory-enhancing effects of exercise. Importantly, the task utilized in this study differed from the task in Weinberg et al. (2014) in two ways: 1) the interstimulus interval was between 6-8 times longer than the 500 ms interval utilized in their task, and 2) participants in this study were asked to provide subjective arousal ratings, while Weinberg and colleagues asked participants to passively view the pictures at they appeared on the screen. The discrepancy between recognition memory performance in these two studies suggests that passive vs. active engagement with emotional stimuli may influence subsequent memory. Another consideration is the

use of valence as a between-subjects variable rather than within subjects. It may be that the interaction of valence categories during the encoding task plays a role in long-term memory, through attentional differences, for example.

Further investigations should explore the potential modulators of exercise-induced sAA activity. In the current study, there were not enough participants to perform statistical comparisons between males and females. Exploration of individual differences in the increase and profile of sAA increase should also be examined. Another limitation of this study is that we do not have precise indicators of cardiorespiratory fitness. We were able to exclude participants based on self-reported physical activity, but the lack of exercise testing precludes us from making conclusions about fitness-related differences in memory or sAA changes. Prescribing exercise using RPE is valuable in its accessibility, however; to further understand the relationship between acute exercise and sAA response, exercise prescribed based on exercise testing outcomes should be utilized.

Conclusions

Current opinion in neurobiology implicates noradrenergic activation as the link between arousal and memory enhancement. The current study aimed to contribute to the existing literature on emotional memory by investigating the time-dependent effects of acute exercise-induced arousal on memory for pleasant, neutral, and unpleasant pictures. There was no effect of exercise on measures of recognition or recollection, possibly due to exceedingly high performance on these assessments. While the results of the memory task were limited, salivary analysis methods demonstrated a modest but reliable increase of sAA in response to a 20-minute session of perceived high-intensity exercise. Therefore, a single session of exercise is sufficient to induce central noradrenergic

activity. This presents evidence for the utility of acute exercise as a model to study arousal-modulated memory processes. This study also revealed the importance of considering subjective measures of arousal in addition to normative groupings. Further exploration of the factors moderating the effects of exercise on sAA is imperative, and will lend to the use of exercise as a model and its potential as a therapeutic enhancer of long-term memory.

APPENDICES

Appendix A. IAPS Stimuli

List A

Pleasant
1460, 1600, 1659, 1710, 1721, 1920, 2071, 2151, 2154, 2156, 2209, 2250, 2310, 2345, 2347, 4600, 4606, 4611, 4624, 4677, 4680, 5199, 5210, 5260, 5623, 5660, 5825, 7492, 7502, 7580

Neutral
2026, 2210, 2214, 2372, 2377, 2390, 2411, 2488, 2579, 2580, 2594, 5250, 5740, 7003, 7030, 7031, 7035, 7056, 7057, 7080, 7095, 7100, 7130, 7186, 7205, 7217, 7224, 7235, 7595, 7820

Unpleasant
1120, 1200, 1525, 1932, 2053, 2457, 2683, 2703, 2717, 3059, 3060, 3130, 3180, 3195, 3250, 3530, 6230, 6312, 6370, 6560, 6821, 6834, 7359, 9181, 9291, 9301, 9405, 9414, 9426, 9810

List B

Pleasant
1440, 1463, 1590, 1660, 1720, 1722, 2070, 2150, 2158, 2165, 2208, 2260, 2311, 2340, 2341, 4601, 4612, 4625, 4670, 4676, 4694, 5215, 5270, 5600, 5626, 5829, 5836, 5849, 7489, 7530

Neutral
2036, 2200, 2215, 2235, 2374, 2383, 2394, 2489, 2593, 2840, 5130, 5720, 7004, 7009, 7014, 7017, 7025, 7032, 7036, 7058, 7096, 7140, 7161, 7187, 7207, 7234, 7242, 7550, 7705, 7830

Unpleasant
1050, 1205, 1300, 1930, 2301, 2691, 2694, 2799, 3019, 3030, 3071, 3120, 3150, 3181, 3185, 3350, 3500, 6231, 6315, 6510, 6550, 6571, 7380, 9102, 9140, 9290, 9320, 9413, 9425, 9800

Appendix B. Consent Form for Participation

University of Maryland College Park

*Initials*_____ *Date*_____

Project Title	Acute Exercise and Emotional Processes
Purpose of the Study	This research is being conducted by Dr. J. Carson Smith at the University of Maryland, College Park. We are inviting you to participate in this research project because you are a healthy adult between 18 and 35. The purpose of this research project is to examine how acute exercise affects emotional responsiveness and the influence of emotional processes on subjective response to aerobic exercise. **Your participation in this study is completely voluntary and you may choose to discontinue your participation any time you wish**.
Procedures	This research study will take place on two separate days, and will involve about 3 hours of your time over the two days. On the first day, you will be asked to complete some questionnaires about your health and physical activity habits. Some questions will ask about current or recent alcohol or drug use. If any question makes you uncomfortable, you are free to not answer it. Please ask the experimenter if you have any questions about any of the survey items. **Picture viewing** On each day you will be asked to view a series of pictures, lasting about 20-30 minutes. These pictures have been judged previously as subjectively pleasant, neutral, or unpleasant. You may be asked to provide information about your judgments of the pictures. Some of the pictures contain content that may be considered objectionable, such as disfigured bodies and threatening people. You will be shown examples of the types of pictures you may see. If for any reason you feel uncomfortable viewing any of the pictures, you may discontinue your participation without penalty. **Exercise or Rest** You will be randomly assigned to a group which will determine whether you participate in rest or exercise during each visit. There is the possibility that you may exercise during one or both visits. Each exercise or rest condition will last 15-20 minutes. You will wear a heart rate monitor (a strap around your chest) and the experimenter will ask you to rate how you feel during the exercise and rest conditions. Exercise sessions will consist of a 20-minute bout of self-paced moderate-to-high intensity cycling on a stationary cycle ergometer. You will be in control of your work rate and cycling speed for the duration of the exercise session. The exercise sessions will start with a warm-up and end with a cool-down. After exercise, your blood lactate levels will be measured via a small finger prick to collect blood. **Saliva Collection** A small amount, about one ml, of saliva will be collected. You will be provided with a vial for use during the saliva collection. The experimenter will wear protective gloves when handling saliva collection vials. A total of 4 vials will be collected over the course of participation.

	No monetary or other compensation will be provided.
Potential Risks and Discomforts	There are inherent risks involved with exercise, such as the risk of muscle or joint injury, and in rare circumstances cardiovascular events may occur that lead to serious injury or death. You will be asked about your personal and family history of heart disease and other factors that may make it unsafe for you to participate in this study. Please inform the experimenter if you have ever had difficulty breathing during exercise, or have ever had chest pain while exercising or walking. You will be provided with a warm-up and cool-down, and this will help minimize your risk of injury during and after exercise. There is a risk of minor bruising of the fingertip from finger pricks for the blood lactate measurement. To minimize the risks associated with the finger prick, only trained personnel will perform this procedure using sterile equipment that will be disposed after use. Your skin will be sterilized with an alcohol swab prior to the finger prick. The risk associated with viewing these pictures of either neutral or emotional material is no greater than a routine medical or psychological exam. You may experience feelings of discomfort upon viewing some of the pictures. If you are experiencing strong physical or mental discomfort or if you feel too uncomfortable to continue at any time, please inform the epxerimenter and she will discontinue the picture viewing task. There are minimal risks associated with completing the surveys. It is possible that some questions may make you uncomfortable. You may skip any questions you do not want to answer. The researchers will be available to clarify the instructions for you if you have any questions about the surveys. There are no right or wrong answers to the questions. None of the information from these surveys will be shared with persons outside of the research team, including information about recent drug and/or alcohol use. You should provide your honest response to each question. In the event you are injured while participating, you will be provided with immediate medical care either by calling 911 or referring you to your physician or the University Health Center. You will be responsible for any costs related to injury you may experience as a result of your participation. Throughout the study, the researchers will notify you of new information that may become available and might affect your decision to remain in the study. If you wish to discuss the information above or any discomforts you may experience, you may ask questions now or call the Principal Investigator listed on the front page of this form.
Potential Benefits	There are no direct benefits of participation in this study. After the final laboratory session you will have the opportunity to ask questions about the hypotheses for this experiment. Thus, you may benefit by learning something about psychological research. Also, your participation will benefit the researchers conducting this study by providing data that could be used to publish scientific papers or make presentations at scientific conferences. This research will contribute to our

	general knowledge of acute exercise and how it interacts with emotional processes.
Confidentiality	All information collected about you during the course of this study will be kept confidential to the extent permitted by law. Only the PI, his co-investigators, or laboratory assistants will have access to the information. However, the Institutional Review Board at UM-College Park or appropriate federal agencies like the Office for Human Research Protections may review your records. Your information will be coded, and only the PI, co-investigators, and research assistants will have the key to this code. Your information will be stored in a locked cabinet and a password protected computer. Your saliva samples will be coded and stored in a laboratory where access will be limited to research team members. After all evaluation of these samples is completed, they will be discarded using appropriate biohazard waste disposal procedures. After all of the data is collected, we may decide to present the results of the study to others, or publish our results in scientific journals or conferences. If they are published, we will always report an average of all people tested, not the results from just one person. Your name will never be associated with any of the data collected, and your identity will always remain strictly confidential. If we write a report or article about this research project, your identity will be protected to the maximum extent possible. Your information may be shared with representatives of the University of Maryland, College Park or governmental authorities if you or someone else is in danger or if we are required to do so by law.
Medical Treatment	The University of Maryland does not provide any medical, hospitalization or other insurance for participants in this research study, nor will the University of Maryland provide any medical treatment or compensation for any injury sustained as a result of participation in this research study, except as required by law.
Right to Withdraw and Questions	Your participation in this research is completely voluntary. You may choose not to take part at all. If you decide to participate in this research, you may stop participating at any time. If you decide not to participate in this study or if you stop participating at any time, you will not be penalized or lose any benefits to which you otherwise qualify. Grades, employability, or standing within the University will not be positively or negatively affected by your decision to participate in the study. If you decide to stop taking part in the study, if you have questions, concerns, or complaints, or if you need to report an injury related to the research, please contact the investigator: **Lauren Weiss, phone: 301-405-0448; email: lafisher@umd.edu** **J. Carson Smith, PhD, phone: 301-405-0344; email: carson@umd.edu**

Participant Rights	If you have questions about your rights as a research participant or wish to report a research-related injury, please contact: **University of Maryland College Park** **Institutional Review Board Office** **1204 Marie Mount Hall** **College Park, Maryland, 20742** **E-mail: irb@umd.edu** **Telephone: 301-405-0678** This research has been reviewed according to the University of Maryland, College Park IRB procedures for research involving human subjects.	
Statement of Consent	Your signature indicates that you are at least 18 years of age; you have read this consent form or have had it read to you; your questions have been answered to your satisfaction and you voluntarily agree to participate in this research study. You will receive a copy of this signed consent form. If you agree to participate, please sign your name below.	
Signature and Date	**NAME OF PARTICIPANT [Please Print]**	
	SIGNATURE OF PARTICIPANT	
	DATE	

Appendix C. Health History and Demographic Questionnaire

Study ID # AXM_____	**HEALTH HISTORY and DEMOGRAPHIC QUESTIONNAIRE**	Date _____

ID#: _____

PHONE: _____ DATE OF BIRTH: _____ AGE: ____

SEX: ___ M ___ F OCCUPATION: _____ FULL TIME: ____ Y ___ N

MARITAL STATUS: (circle one) SINGLE MARRIED DIVORCED WIDOWED

EDUCATION: (check highest level completed)

ELEM. _____ HIGH SCHOOL _____ COLLEGE _____ GRADUATE _____

HISPANIC: ____ YES ____ NO

RACE: (check all that apply)

____WHITE
____AMERICAN INDIAN
____ASIAN
____BLACK/AFRICAN AMERICAN
____NATIVE HAWAIIAN/PACIFIC ISLANDER
____DO NOT WISH TO DISCLOSE

PERSONAL PHYSICIAN: _____ LOCATION: _____

Are you taking any prescription or over-the counter medication? ____ YES ____ NO

Name of Medication Reason for Taking For How Long?

_____

_____

_____

_____

For female participants:

Are you currently pregnant or is there a possibility you could be pregnant? _____YES _____NO

PAST HISTORY

Have you ever had?

_____ High blood pressure
_____ Stroke
_____ Any heart problems
_____ Blood Clots
_____ Arthritis
_____ Cancer
_____ Recurring leg pain (not related to arthritis)
_____ Liver or Kidney Disease
_____ Psychiatric
_____ Any breathing or lung problems
Disorder
_____ Ankle swelling (not related to twisting)
_____ Low back or joint problems

FAMILY HISTORY

Have any immediate family (brothers/sisters, children, parents) had?

_____ Heart attacks
_____ Stroke
_____ High blood pressure
_____ Early death
_____ High cholesterol
_____ Diabetes
_____ Congenital heart defect
_____ Psychiatric
_____ Heart operations
Disorder
_____ Other family illnesses _____
_____

PRESENT SYMPTOMS

Have you ever had? (please check)

_____ Chest pain / discomfort
_____ Cough on exertion
_____ Shortness of breath
_____ Coughing of blood
_____ Heart palpitations
_____ Dizzy spells
_____ Skipped heart beats
_____ Frequent headaches
_____ Back pain
_____ Orthopedic / joint problems
_____ Problems sleeping or sleeping too much
_____ Depressed mood for more than 2 weeks

Have you been hospitalized in the last year? _____ Yes _____ No

If yes, for how many days were you in hospital? _____ days

Have you ever had your cholesterol measured? YES _____ NO _____; If yes, (value) _____

Do you currently smoke? YES ___ NO ___ If so, what? Cigarettes _____ Cigars _____ Pipe _____

How much per day: < 0.5 pack _____ 0.5 to 1 pack _____ 1.5 to 2 packs _____ >2 packs _____

Have you ever quit smoking? YES ____ NO ____ When? ____ How many years did you smoke? _____

Do you drink alcoholic beverages? YES _____ NO _____ If yes, how much in 1 week? _____

Do you drink caffeinated beverages? YES _____ NO _____ If yes, how much in 1 week? _____

When did you consume your last alcoholic beverage? _____Caffeinated beverage?_____

Have you used a recreational drug in the past 48 hours? YES ____ NO _____

Experimenter Notes:

Appendix D. Data Collection Sheet

AXM Study Date:_____

Subject ID: _____ Experimenter: _____ Others Present: _____

Group: Ex-Before Ex-After Rest Task: List A List B

Saliva Collection:

- ❑ S_1 collection time: _____
- ❑ S_2 collection time: _____
- ❑ S_3 collection time: _____
- ❑ S_4 collection time: _____

Exercise Start: _____ Exercise End: _____

Time	Kp/Watts	HR	RPE	SAM-V	SAM-A
Pre					
Warm-up					
5 min					
10 min					
15 min					
20 min					
Cool-down					

[Lactate] measurement:

_____mmol/L Time: _____

Notes:

Appendix E. Borg's Ratings of Perceived Exertion Scale

Borg Perceived Exertion Scale

6

7 Very, very light

8

9 Very light

10

11 Fairly light

12

13 Somewhat hard

14

15 Hard

16

17 Very hard

18

19 Very, very hard

20

Appendix F. Borg's RPE Scale Instructions

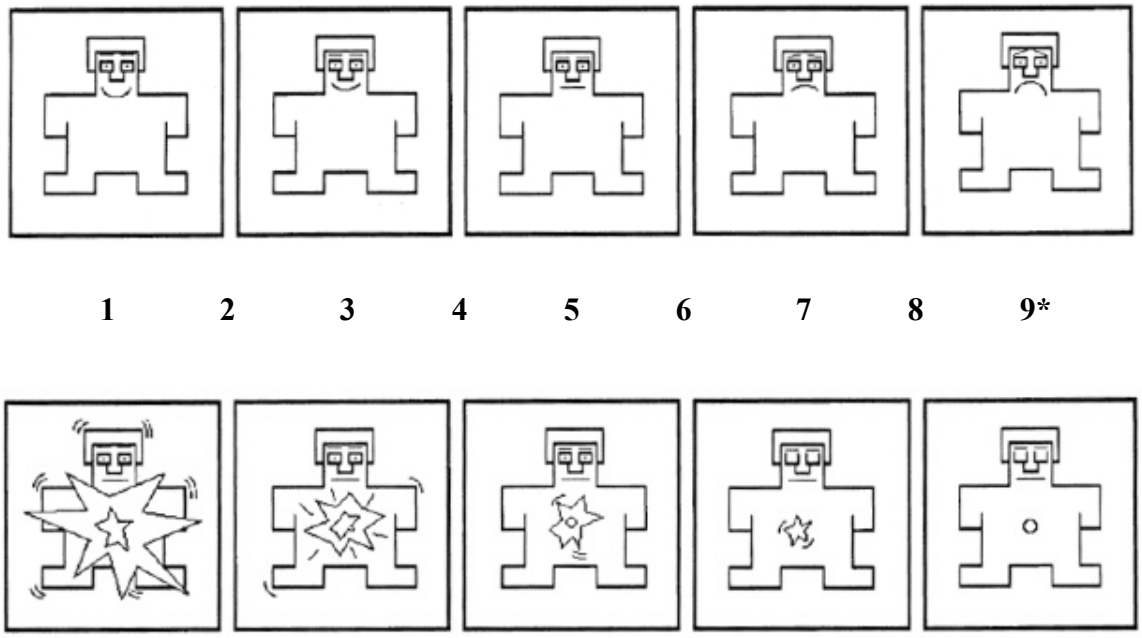
This is Borg's RPE scale. You'll be using this scale to adjust your exercise intensity while you're pedaling. The scale goes from 6-20, with 6 corresponding to feelings of no exertion—what you feel when you are just at rest. A 20 on the scale represents the maximum exertion you can do or imagine yourself doing. For example, if you were running as fast as you could up a mountain and at the brink of exhaustion.

In between these two ends of the scale, you can assess your exercise intensity using these verbal anchors. We want you to be exercising at a 15, which corresponds to working "hard." Working hard might be indicated by heavier breathing or having difficulty maintaining a conversation, as opposed to somewhat hard which is a more comfortable pace where you could probably maintain a conversation fairly well.

If you find yourself moving beyond this 15 or "hard," level of exertion, you may need to reduce your resistance to stay at the desired level of exertion. We'd like you to keep your pedaling speed at about 70 rpms, which is shown on the screen—so try your best to stay between about 65-75 rpms as you pedal at this level of intensity.

Do you have any questions?

Appendix G. Self-Assessment Manikin Valence & Arousal Scales



1 2 3 4 5 6 7 8 9*

*Numbers denote scoring system. Not displayed on scales used by participants.

Appendix H. Self-Assessment Manikin Instructions

SAM Instructions:

*** Modified to include only Valence and Arousal scales**

On the page in front of you, you will see **2** sets of 5 figures, each arranged along a continuum. We call this set of figures SAM, and you will be using these figures to rate how you feel. SAM shows **two** different kinds of feelings: Happy vs. Unhappy and Excited vs. Calm.

You can see that each SAM figure varies along each scale. The first SAM scale is the happy-unhappy scale, which ranges from a smile to a frown. At one extreme of the happy vs. unhappy scale, you feel happy, pleased, satisfied, contented, hopeful. If you feel completely *happy,* you can indicate this by **pointing at** the figure at the left, like this (demonstrate). The other end of the scale is used when you feel completely unhappy, annoyed, unsatisfied, melancholic, despaired, bored. You can indicate feeling completely *unhappy* by **pointing at** the figure at the right, like this (demonstrate). The figures also allow you to describe intermediate feelings of pleasure, by **pointing at** any of the other pictures. If you feel completely neutral, neither happy nor sad, **point at** the figure in the middle. If, in your judgment, your feeling of pleasure or displeasure falls *between* two of the pictures, then **point** between the figures, like this (demonstrate). This permits you to make more finely graded ratings of how you feel.

The excited vs. calm dimension is the second type of feeling displayed here. At one extreme of the scale you feel stimulated, excited, frenzied, jittery, wide-awake, aroused. If you feel completely *aroused*, **point at** the figure at the left of the row, like this (demonstrate). On the other hand, at the other end of the scale, you feel completely relaxed, calm, sluggish, dull, sleepy, unaroused.

Your rating should reflect your immediate personal experience, and no more.
Do you have any questions?

Please rate how you feel **right now, at this moment**.

Appendix I. Remember/Familiar Task Instructions

For this task, you will see pictures like the ones you saw the other day. Some of the pictures will be ones you saw before, and some will be new pictures that we did not show you. You will be asked if each picture is "Old" or "New." If it is a picture that you saw the other day, you should respond "Old." If you did not see the picture the other day, you should respond "New." If you're unsure, I encourage you to take your best guess. If you select "Old," you will also be asked to provide a more specific answer about the vividness of that memory. I'll tell you more about those answers next. Do you have any questions so far? *(Answer questions before continuing)*

When we *remember* something, we consciously recollect and become aware of aspects of its occurrence. For example, you might remember a recent movie and be able to recall *specific details* about it, like where and with whom you saw it. At other times, we simply know that something has occurred before, but without being able to consciously recollect what was experienced at the time of its occurrence. For example, you might recognize a person and be certain that you know him or her, but be unable to recall any specific details about the person, such as the person's name or how you met.

For this test, remembering a picture would signify that the picture evokes specific memories of what was experienced during its presentation, such as how it looked on the screen, the way in which it was presented, or even what you were thinking or doing at the time it was shown. Feeling a picture is familiar would signify that you believe the picture appeared, or have a sense of having seen it before, but cannot recollect any aspects of its presentation.

(To participant):
"What does it mean to *remember* a picture?"

- ❑ Participant correctly answered first time
- ❑ Participant needed at least one reminder, but then correctly answered question
- ❑ Participant does not understand

"What does it mean to *know* a picture?"

- ❑ Participant correctly answered first time
- ❑ Participant needed at least one reminder, but then correctly answered question
- ❑ Participant does not understand

Are you clear on the distinction between remembering and knowing a picture? Do you have any questions about this task before we begin the practice run?

Appendix J. Post-Experimental Questionnaire

AXM Study Date:_____

Subject ID: _____

AXM Questionnaire

Age _____ Sex: M F

Year in school _____

Major_____

Left or Right Handed? LEFT RIGHT

RATE DIFFICULTY OF MEMORY TEST

0	1	2	3	4	5
Very Easy					Very Difficult

RATE CONFIDENCE IN HOW WELL YOU PERFORMED THE MEMORY TEST

0	1	2	3	4	5
Very Poorly					Very Well

What did it mean if an image was "Remembered?"

What did it mean if an image was "Familiar?"

What did you think about when you saw the images during the first part of the experiment?

What did you think about when you saw the images today?

Had you ever seen any of these images before participating in this study?

Did you anticipate a memory test? YES NO
If YES, Explain:

How did you hear about our study?

Appendix K. Debriefing Script & Mental Health Resources

AXM Debriefing Script

You were not informed that you would be taking a memory test today or that memory performance was a component of this study. We also told you to expect an exercise session on one or both days of the study. In reality, it was known that you would only be asked to exercise on the first day. This misinformation was provided in order to reduce the chance that you would expect a memory test today. You also may have noticed that we were not very specific about the purpose of the study. This was the only part of the study that you were not completely informed about as part of the consent process. We didn't tell you about the memory test because we wanted to minimize the effects of strategy or rehearsal on your memory performance. We kept this information from you because we felt it was the best way to perform the study while answering our research question.

The complete purpose of this study is to investigate the interaction between acute exercise, arousal, and memory. We think that physical arousal elicited by exercising may interact with memory processes that occur when you see or experience emotional things. It is important that future study participants do not expect a memory test. Should you talk to any other students or friends that are interested in participating, **please avoid discussing the memory component of the study** with them.

If you are feeling upset or become upset in the future thinking about the pictures you saw today, we encourage you to reach out to resources on the University of Maryland Campus.

Mental Health Services at the University Health Center

Phone: 301-314-8106
Website: www.health.umd.edu/mentalhealth

University of Maryland Counseling Center

Phone: 301-314-7651
Website: www.counseling.umd.edu

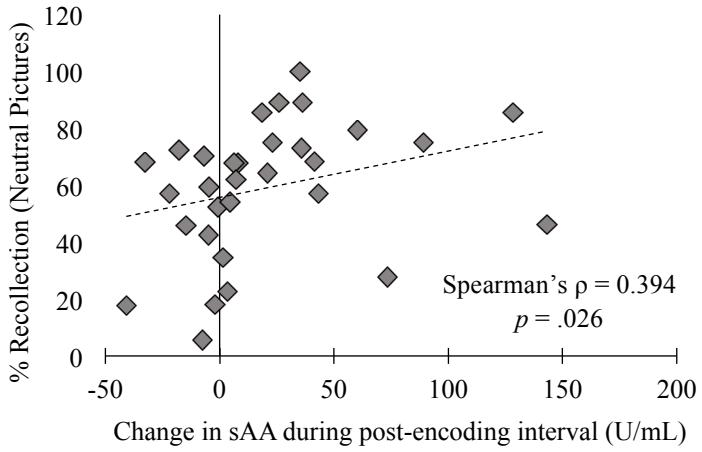
Please contact the study investigator if you have any questions, concerns, or complaints:

Lauren Weiss, phone: 301-405-0448; email: lafisher@umd.edu

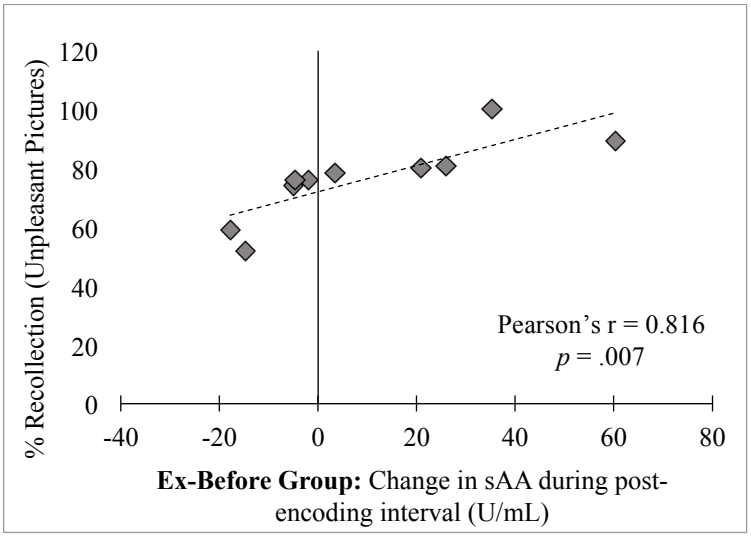
J. Carson Smith, PhD, phone: 301-405-0344; email: carson@umd.edu

Appendix L. Supplementary Figures

Suppl. Figure 1. *Relationship between post-encoding change in sAA and recollection for neutral pictures*



Suppl. Figure 2. *Relationship between post-encoding sAA and recollection for unpleasant pictures in Ex-Before Group*



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