Summer 2019

Yoga to Decrease the Stress Response: Gentle Yoga Encourages Faster Decline in Salivary Cortisol Concentrations Following Participation in TSST

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YOGA TO DECREASE THE STRESS RESPONSE: GENTLE YOGA ENCOURAGES FASTER DECLINE IN SALIVARY CORTISOL CONCENTRATIONS FOLLOWING PARTICIPATION IN TSST

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Psychology

By

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Psychology

Missouri State University, August 2019

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ABSTRACT

Short-term, activation of the human stress response system is beneficial as it prepares the body to deal with stressors at hand (McEwen & Stellar, 1993). If this system is overactive or chronically active however, it can negatively impact health and longevity (Cohen, Janicki-Deverts, & Miller, 2007). According to a review conducted by Ross & Thomas (2010) implementation of a yoga practice has been shown to down-regulate the stress response system. The present study aimed to expand on current research involving yoga for stress reduction by exploring whether participation in gentle yoga could decrease the stress response more quickly than naturally occurs after psychological stress exposure. Participants were exposed to a psychological stress test, then randomly assigned to take part in a gentle yoga sequence or watch a neutral video. Analyses through independent sample *t*-tests indicated faster decline in salivary cortisol concentrations from initial stress response for participants that took part in gentle yoga. Results suggest participation in a gentle yoga practice helps decrease the stress response more quickly than occurs naturally following psychological stress exposure.

KEYWORDS: yoga, stress, TSST, cortisol, stress response, stress reduction, gentle yoga

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ACKNOWLEDGEMENTS

I would like to thank my friends and family for their continued support throughout the course of my graduate studies. I could have never survived the late nights and stress without the love and laughter you've given me. I would also like to thank those working in Dr. Amber Abernathy's lab this past semester, for without you, data collection to this extent would have never been possible. Thank you for your dedication, attention to detail, and patience. Last, but not least I would like to thank my committee members for their support and advisement over the course of this project, and not dismissing my out-of-the-ordinary research venture.

I dedicate this thesis to Kelly Hopke,

the woman who fostered my desire to learn since the very beginning.

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INTRODUCTION

Short-term, activation of the human stress response system is beneficial as it prepares the body to deal with stressors at hand (McEwen & Stellar, 1993). If this system is over-active or chronical active however, it can negatively impact health and longevity (Cohen, Janicki-Deverts, & Miller, 2007). Therefore, finding methods to decrease the stress response when no longer necessary could be beneficial to overall well-being. One simple, non-invasive treatment that has been suggested to help calm the stress response system is participation in yoga (Ross & Thomas, 2010).

Human Stress Response

The human stress response system is a complex and intertwined network which responds to anything the body perceives as causing harm or having the potential to cause harm, referred to here as ‘stressors’ (Selye, 1950). This means that the stress response system is activated with exposure to both physical stressors (i.e., blood loss, pain, etc.) and psychological stressors (i.e., public speaking, test taking, etc.) (Ulrich-Lai & Herman, 2009). The process by which the body deals with stressors, through activation of the stress response system, is often referred to as ‘allostasis’ (Sterling & Eyer, 1988). In this process, when either physical or psychological stressors are perceived, the brain stem (physical stressors) and/or limbic system (psychological stressors) will signal the hypothalamus to initiate a stress response (Chrousos, 2009). The two main pathways that are activated in this response are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (McEwen, 1998).

The Autonomic Nervous System (ANS) is the first to respond when a stress response is initiated; this response occurs almost immediately and is acute in nature (Ulrich-Lai & Herman, 2009). The ANS consists of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS). The SNS is the branch of the ANS activated when a stimulus is perceived as a stressor. This system puts the body in a state of 'fight or flight' to prepare for strenuous activity through increase in heart rate and vasoconstriction in smooth muscle to direct resources to skeletal muscle (McCorry, 2007). The PSNS works to put the body in a state of 'rest and digest' through decreased heart rate and direction of resources to systems for bodily maintenance (McCorry, 2007). The PSNS also works to deactivate the SNS when stress response is no longer necessary (Ulrich-Lai & Herman, 2009). These systems communicate at many points to regulate this stress response (Ondicova & Mravec, 2010; Ulrich-Lai & Herman, 2009).

The hypothalamic-pituitary-adrenal (HPA) axis produces a slower response, but one that lasts longer and is more controlled than that of the ANS (Ulrich-Lai & Herman, 2009). When the stress response is initiated, the paraventricular nucleus (PVN) of the hypothalamus synthesizes and releases corticotrophin releasing hormone (CRH) to act on the anterior pituitary. The anterior pituitary then releases adrenocorticotrophic hormone (ACTH) which acts on the adrenal cortex to cause the release of cortisol into the bloodstream. The release of cortisol leads to changes which allow access to oxygen and energy for those systems deemed necessary to deal with stressors (Ulrich-Lai & Herman, 2009). The HPA axis is regulated through a negative feedback mechanism in which the increase in free cortisol signals the initial HPA components to cease release of hormones that would later lead to cortisol being released into the blood stream (Chouros, 2009).

Together the SNS and HPA axis work to create a very efficient stress response system, with the SNS generating the initial stress response and the HPA axis following shortly after the stress response may be maintained if necessary (Ulrich-Lai & Herman, 2009). Another feature of these two components is that their effects can be influenced by the type of stressors encountered. Where the SNS responds more effectively to physical stressors, the HPA axis responds more effectively to psychological stressors (Ulrich-Lai & Herman, 2009). According to Ulrich-Lai & Herman (2009) this is because physical stressors are immediately interpreted as harmful, whereas psychological stressors require processing and interpretation of potential harm.

When functioning correctly, this process of allostasis is beneficial to regulating body systems during stress exposure. Activation of the stress response system allows us to effectively deal with any stressors that may be encountered (McEwen & Stellar, 1993). However, when this system is activated frequently or for long periods of time, as occurs with chronic stress exposure, it can lead to negative effects on both health and longevity (Cohen, Janicki-Deverts, & Miller, 2007; McEwen 1998; McEwen, 2008). This dysregulation of the stress response system has been termed ‘allostatic load’ (Sterling & Eyer, 1988; McEwen, 1998), or ‘allostatic overload’ when resulting in disease (McEwen, 2008). According to McEwen (2008) allostatic overload can lead to disease in the same systems regulated during allostasis including neural, cardiovascular, autonomic, immune, and metabolic systems. In these systems, dysregulation of allostasis contributes to increased risk of depression (Cohen, Janicki-Deverts, & Miller, 2007), cardiovascular disease (Cohen, Janicki-Deverts, & Miller, 2007), neural degeneration (McEwen, 1998), autoimmune disease (Black, 1994), and Type II diabetes (Cohen, Janicki-Deverts, & Miller, 2007; McEwen, 2008). With such vast consequences for over-all health due to over activation of this system, it is important to examine the stress response and interventions that could assist in regulation of this response.

Trier Social Stress Test (TSST)

One way to evaluate the stress response and interventions is to examine how the response decreases following activation. As psychological stress has the greatest contribution to chronic dysregulation of this system (Cohen, Janicki-Deverts, & Miller, 2007), psychological stressors are often used to initiate a stress response. However, some psychological stressors are more effective at activating a measurable stress response than others (Dickerson & Kemeny, 2004). In a meta-analysis conducted by Dickerson & Kemeny (2004) characteristics of psychological stressors (i.e., uncontrollability, social-evaluative, cognitive task, etc.) and their influences on cortisol concentrations were compared. Through this meta-analysis, it was determined that situations in which “an important aspect of the self-identity is or could be negatively judged by others" resulted in the largest and most reliable stress response (as measured through cortisol) (Dickerson & Kemeny, 2004). This type of stress is termed ‘social-evaluative threat,’ and includes tasks completed in front of an evaluative audience or those in which task performance is comparison based (Dickerson & Kemeny, 2004). One well-established stressor that includes social-evaluative threat is the Trier Social Stress Test (TSST).

The Trier Social Stress Test (TSST) was designed by Kirschbaum, Pirke, & Hellhammer, 1993) specifically to elicit a stress response through exposure to a social-evaluative threat. In the original design, participants were not told about the task until arrival. After arrival, they experienced a rest period in which baseline measures of cortisol were obtained. They were then introduced to the task. After a 10-minute preparation period, participants were directed to a room with judges (‘managers’) and a recording system. Here, participants were asked to give a five-minute speech for a mock job interview. Participants were directed to introduce themselves and convince the ‘managers’ that they were the perfect candidate for the position. If participants finished before the five-minute mark, the were instructed to continue. After the speech, participants were asked to serially subtract the number 13 from 1,022 as quickly and accurately as possible until five minutes had passed. Each time the participant made an error, they were instructed to start over from 1,022 (Kirschbaum, Pirke, & Hellhammer, 1993).

Results revealed that the TSST causes a stress response as demonstrated through increases in stress markers including salivary cortisol Kirschbaum, Pirke, & Hellhammer, 1993). Since its first utilization, this test has been shown to reliably induce a psychological stress response as noted by both perceived stress test questionnaires and biological stress markers (Dickerson & Kemeny, 2004; Kudielka & Wüst, 2010). This information makes the TSST a valuable tool in stress research.

Since chronic activation of this system can lead to disease, it is beneficial for the stress response to decrease quickly when stressors are no longer present. As the TSST can reliably induce a measurable stress response, it can also be used to measure the decline in stress response after exposure to psychological stressors. Extending this idea, the TSST can also be used to compare stress response decline under normal conditions versus interventions. One simple intervention that could prove useful in decreasing the stress response is participation in a yoga practice (Ross & Thomas, 2010; Broad, 2012).

Yoga and Stress

Though yoga practices vary greatly, the most popular type of yoga in the United States is Hatha Yoga (Broad, 2012). Hatha Yoga encompasses any yoga practice which involves asana (postures/movement) and pranayama (breath control) and can range from strenuous to low impact in nature (Broad, 2012). Due to its dynamic nature and common claims of stress reduction through utilization of the practice, yoga has continued to grow in the number of studios, teachers, and students in recent years.

In a review conducted by Ross & Thomas (2010) several studies were evaluated to examine the effectiveness of yoga and exercise on health outcomes including heart rate variability, blood glucose, blood lipids, salivary cortisol, and oxidative stress (Ross & Thomas, 2010). Findings from this review suggested that health outcomes of yoga treatment groups were better than or equal to those experienced in exercise treatments in each of these areas, but not in areas of physical fitness. Although these results are promising, it must be noted that the studies reviewed varied greatly in design. Types of yoga utilized were not consistent in utilization of both asana and pranayama, intensity of practice, or length of practice implemented (ranging from one single session up to six months of consistent practice).

Of the studies included, only two were single session studies, and only one single session study measured cortisol concentrations in response to Hatha Yoga. This single session study evaluated changes in perceived stress and salivary cortisol from baseline after a single session of Hatha Yoga, African dance, or a college lecture (West et al., 2004). Results suggest a reduction in perceived stress from participation in Hatha Yoga and African dance, while decreases in salivary cortisol were only observed from participation in Hatha Yoga (West et al., 2004).

This difference in response is of importance when designing a study to measure stress response through cortisol. Although exercise has been shown to have long term health benefits (Hackney, 2006), the immediate changes that occur in the body during physically challenging exercise can result in short term increases in cortisol (Hackney, 2006). A study conducted by Hill and colleagues (2008) however, suggests that this increase depends on the intensity of exercise. Results of this study showed that cortisol levels dropped during low intensity sessions (exercise at $\leq$ 40% VO_{2MAX}), while levels increased for moderate and high intensity exercise (exercise at $\geq$ 60% VO_{2MAX}) (Hill et al., 2008). This indicates taking part in low intensity exercise does not cause the same short-term cortisol increase seen during moderate to high intensity exercise (Hill et al., 2008).

Another way to avoid cortisol increase during exercise, that is commonly utilized during a yoga practice, is diaphragmatic breathing (Gerritsen & Band, 2018; Hazlett-Stevens & Craske, 2003). This type of respiration involves deep, purposeful breath using the diaphragm (Hazlett-Stevens & Craske, 2003), and has been shown to activate the PSNS through vagus nerve stimulation (Hazlett-Stevens & Craske, 2003). This PSNS activation works to decrease the stress response, as demonstrated through lowered cortisol concentrations (Ulrich-Lai & Herman, 2009). Gerritsen & Band (2018) suggest that this focus on controlled breathing can also act to signal the HPA axis that a stress response is no longer necessary via the limbic system. In this way, diaphragmatic breathing can have both indirect (i.e., bottom-up) and direct (i.e., top-down) effects on the stress response system that encourage decreased activation (Gerritsen & Band, 2018).

The Present Study

Chronic activation of the stress response system has been linked to many negative health aspects (Cohen, Janicki-Deverts, & Miller, 2007). One simple, non-invasive treatment that has been suggested to help calm the stress response system is participation in yoga (Ross & Thomas, 2010). Although it has been shown that a single session of Hatha Yoga could decrease cortisol, this decrease was observed from baseline measures (West et al., 2004). The present study aimed to expand on current research by exploring whether participation in a single gentle yoga sequence following stress exposure could decrease the stress response more quickly than occurs naturally. This gentle yoga practice was defined as low intensity Hatha Yoga, with beginner level asana to avoid cortisol increases observed in physically challenging exercises (Hill et al., 2008). The practice also utilized diaphragmatic breathing which has been shown to down-regulate the stress response system (Gerritsen & Band, 2018).

MATERIALS AND METHODS

Participants

This study utilized a sample of college students from a midwestern university (N = 63). Participant ages ranged from 18 to 34 years (M =19.89, SD = 2.83), with 22 participants identifying as male and 40 participants identifying as female. One participant chose to not specify gender. Participants were primarily of Caucasian descent (73% Caucasian and < 1% American Indian, African-American/Black, Biracial, Hispanic/Latino, Asian/Asian-American, and Other). Participants were randomly assigned to conditions resulting in 32 participants in the yoga condition and 31 participants in the control condition.

Procedure

This study was approved by Missouri State University's Institutional Review Board on January 17, 2019 (study number IRB-FY2019-380, see Appendix A). Two lab members were present to run each participant; one of which took the role of the judge and one which took the role of the researcher. The researcher was there to guide the participant through the study with instructions and collect saliva samples. The judge was there to facilitate social-evaluative stress during the TSST and set timers throughout the course of the study. To begin the study, the participant was taken into a room with a two-way mirror through which they could be observed when the researcher was not in the room. This two-way mirror was used to monitor participants for assurance they were not compromising the study (i.e., using electronics, not participating, etc.). Participants were instructed to leave all belongings included electronics at a table by the door and were unable to access them during the study. They were then asked to sign an informed consent which described the study (Appendix B) and give a saliva sample to measure baseline cortisol concentrations (Time 1~ zero minutes). Next, they took part in a revised version of the TSST to induce a stress response measurable through salivary cortisol.

For the TSST, participants were given a pen and piece of paper, then told that they would have 10 minutes to prepare before giving a five-minute speech about why they were qualified for their dream job in front of judges. The researcher then left the participant alone in the room to prepare (anticipatory stress period). After the 10-minute preparation, the researcher and the judge entered the room. The judge then asked the participant to begin their speech and told them that a timer would go off when their time was up. If the participant stopped speaking before the time was up, the judge would encourage them to keep talking by saying, "You still have some time left. Please continue." Each subsequent time they stopped before the time was up, the judge would wait ~20 seconds then say, "Please continue." When the five-minute speech was up, the participant would undergo five minutes of mental arithmetic. For this portion, the judge would ask the participant to begin at the number 1,051 and count backwards, subtracting 13 each time. Any time the participant gave an incorrect answer, the judge would say, "That is incorrect. Please start again at 1,051." Both the judge and researcher were instructed to have neutral expressions throughout this portion of the study to facilitate social evaluative stress for the participant. Upon completion of the TSST, both the judge and researcher left the room. Participants were told to wait quietly until the researcher returned.

The researcher then returned 10 minutes later and asked the participant to give a second saliva sample measuring cortisol levels from stress response (Time 2 ~ 30 minutes) as this has been shown to be the time frame in which salivary cortisol peaks after the TSST (Gordis, Granger, Susman, & Trickett, 2006, Kirschbaum, Pirke, & Hellhammer, 1993). After this sample collection, the conditioned segment of the study commenced. Participants were randomly assigned to the yoga or control condition. Participants assigned to the yoga condition were asked to take part in a 30-minute gentle yoga sequence. Before beginning the video for the participant, the researcher laid out two yoga mats lengthwise touching and placed a woven blanket on top which covered the mats. Participants were then instructed they could remove their shoes and have a seat on the mat where a laptop was placed in front of them with the yoga video pulled up via a web link. The video included visual demonstration as well as audio instruction for participants to follow and was specifically designed for this study by a certified yoga therapist (Sweere, 2019). Participants were asked to play the video and follow along when the researcher left the room. They were also instructed to simply skip anything in the sequence they found uncomfortable and to simply focus on breathing during that time. Participants assigned to the control condition watched a 30-minute segment of the movie *March of the Penguins* (Jacquet, 2005). The segment shown was considered a non-emotion inducing and essentially showed penguins walking/swimming with narration of day to day life. It was decided that a neutral movie would be the best option for the control group in this study as both visual and audio were used in the experimental condition. Participants were asked to play the video and watch quietly when the researcher left the room.

When the conditioned segment was complete, the researcher returned to the room and asked the participant to give a third saliva sample. This sample was taken to measure the initial decreases in stress response after stress exposure (Time 3 ~ 60 minutes) as this has been shown to be the time at which cortisol levels begin to decline following the TSST (Gordis, Granger, Susman, & Trickett, 2006, Kirschbaum, Pirke, & Hellhammer, 1993). The researcher then accessed a Qualtrics link with all questionnaires to be completed including a demographic questionnaire; an acceptance and action questionnaire; a depression, anxiety, and stress scale; and a personality inventory (Appendices C-F). They asked the participant to take their time completing the questionnaires and let them know that they would return in 30 minutes to collect one last saliva sample, then left the room again. Although participants were asked to complete these questionnaires, analysis regarding the questionnaire results outside of demographic information was not a focus of this thesis. Exploratory analysis will be performed at a later date to determine whether relevant differences exist between any factors evaluated and stress response.

Thirty-minutes after the third sample, the researcher returned and asked the participant to give a fourth sample (Time 4 ~ 90 minutes). This sample was used to measure a secondary decrease in stress response as this is the time at which salivary cortisol has been shown to decline to approximately baseline following the TSST (Gordis, Granger, Susman, & Trickett, 2006, Kirschbaum, Pirke, & Hellhammer, 1993). After the sample was collected, the researcher debriefed the participant, telling them that the speech and mental arithmetic portions of the study were intended to elicit a stress response, while the yoga condition was intended to reduce stress response. The researcher then ensured all samples were labeled with the correct participant number and collection time. Samples were frozen for later immunoassay analysis. A summary of all time-markers for saliva samples may be found in Table 1 below.

Salivary Cortisol

According to Salimetrics, LLC (2019), many factors can influence salivary cortisol including: consumption of foods with high sugar, acidity, or caffeine close to the time before sample collection; consumption of alcohol, caffeine, nicotine, or medications within 12 hours before collection; brushing teeth or eating meals within 60 minutes before collection; and drinking water within 10 minutes before collection. Due to these many possible confounds, participants were instructed to not have food, caffeine, alcohol, or tobacco within two hours before the start of their session.

Table 1. Time-markers for saliva samples.

Component	Time-Marker
Time 1 (Baseline)	0 minutes
Anticipatory Period	0 – 10 minutes
TSST	10 – 20 minutes
Time 2 (Peak)	30 minutes
Time 3 (Initial Decline)	60 minutes
Time 4 (Return to Baseline)	90 minutes

Time of day can also confound results when measuring salivary cortisol (Kelly, et al., 2008). This is relevant as salivary cortisol concentrations follow a circadian rhythm, peaking approximately 30 minutes after awakening and falling off after about two hours (Kirschbaum, Pirke, & Hellhammer, 1993; Kelly et al., 2008). For this reason, the present study was only conducted between the hours of 12:00 PM and 6:00 PM, and conditions were randomized between time slots.

Saliva collection was accomplished using a passive drool method. To collect passive drool sample, the participant was given a small plastic Sallivite tube with volume markings (in mL) and a straw. They were then instructed to not 'work-up' saliva in their mouth, but instead to wait for saliva to collect in their mouth, then add it to the tube using the straw provided.

After the participant completed the study, their saliva samples were grouped and put in the freezer at -20˚C to be preserved for later analysis (Salimetrics LLC, 2019). Samples were then picked up by a local company for immunoassay analysis. This analysis is used to measure unbound, active, cortisol which Gozansky, Lynn, Laudenslager, & Kohrt (2005) argue better represents the dynamic changes that occur with HPA axis activation.

Questionnaires

Although participants were asked to complete the following questionnaires, analysis regarding the questionnaire results outside of demographic information was not a focus of this thesis. Exploratory analysis will be performed at a later date to determine whether relevant differences exist between any factors evaluated and stress response.

Demographic Questionnaire. This questionnaire asked participants to disclosure basic information about themselves, such as age, gender, grade, ethnicity, etc. Other questions included questions about when the participant had eaten, consumed caffeine or nicotine, whether they practiced meditation, etc. For a complete list of questions please see Appendix C.

Acceptance and Action Questionnaire II (AAQ-II). This questionnaire includes seven items in which participants were asked to rate how truthful each statement was for them on a scale of 1 (*never true*) to 7 (*always true*). This questionnaire assesses psychological inflexibility with a mean alpha coefficient of .84, and 3- and 12-month test-retest reliability of .81 and .79 (Bond et al., 2011). To score, ratings are added together to find a total, with higher total scores indicating more psychological inflexibility (Bond et al., 2011).

Depression, Anxiety, and Stress Scale (DASS 21). This questionnaire includes a list of 21 items which are classified as symptoms of depression, anxiety, or stress (i.e. seven items that relate to each disorder). When completing this questionnaire, participants were asked to rate how often each of these symptoms applied to them over the past week on a scale of 0 (*never*) to 3 (*almost always*). To score, ratings for each disorder are summed based on the seven items considered symptoms of that disorder for a result of three separate severity scores (i.e. one severity score for depression, one for anxiety, and one for stress). Higher scores indicate higher severity in symptoms for the associate factor. These totals are each multiplied by two to find scores in the same scale as that of the original 42 item DASS created by Lovibond & Lovibond (1995). The DASS-21 has been shown to have an excellent three factor structure, and actually seems to have lower intercorrelations between factors as well as higher mean loadings and fewer cross-loading items than the 42 item DASS (Antony, Bieling, Enns, & Swinson, 1998). Cronbach's alphas for the DASS 21 subscales have been noted as .94, .87, and .91 for depression, anxiety, and stress respectively (Antony, Bieling, Enns, & Swinson, 1998).

International Personality Item Pool (IPIP). This questionnaire consists of 100 items; 20 items for each of the "Big Five" personality traits Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness. To complete this scale participants were asked to rate how accurate each item was to them on a five-point scale ranging from *very inaccurate* to *very accurate*. Coefficient alphas for Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness for the 100 item IPIP were found to be .91, .88, .88, .91, and .90 respectively, with low intercorrelations (mean intercorrelation = .31) (Goldberg, 1992). Reverse scoring is conducted on denoted items and scores calculated for each "Big Five" trait, with higher scores indicating higher associations with traits.

RESULTS

In total, 63 students participated in this study (*N* control = 31, *N* yoga = 32). Salivary cortisol concentrations for each participant, at each time point, were obtained though immunoassay analysis performed by a local laboratory. Data cleaning was performed using these cortisol concentrations prior to statistical analysis. One participant was excluded from the study due to missing data, and five participants were excluded due to outlier status.

The present study defined outliers as participants with cortisol concentrations more than two standard deviations away from the mean at any time point. This cutoff of two standard deviations has been utilized previously to lessen the impact of the many factors which can affect cortisol concentration measurements (Herbert et al., 2012). Exclusion of participants due to missing data and outlier status resulted in resulted in *N* = 57 to be included in subsequent analyses (*N* control = 29, *N* yoga = 28). Cortisol concentrations were found to be positively skewed. To correct for this, a natural log transformation was performed for concentrations at each time point (Gordis, Granger, Susman, & Trickett, 2006). To help account for these confounds and natural variation in cortisol concentrations between individuals, change scores between collection times were utilized (Kelly et al., 2008). These change scores were calculated by difference in natural log concentrations between time points.

Comparisons of focus were Time 2 – Time 1, Time 3 – Time 2, Time 4 – Time 3, and Time 4 – Time 2. The change score for Time 2 – Time 1 measured ‘activation’ of stress response (i.e., peak stress response observed due to the TSST). The change score for Time 3 – Time 2 measured ‘initial decrease’ in the stress response (i.e., decrease in stress response 30 minutes after peak). The change score for Time 4 – Time 3 measured ‘secondary decrease’ in stress response (i.e. decrease in stress response from 30 minutes after peak to 60 minutes after peak). The change score for Time 4 – Time 2 measured 'total decrease' in stress response (i.e., decrease from peak stress response to 60 minutes after peak). A summary of average change scores can be found below in Table 2, and a graph displaying these change scores can be found in Figure 1.

Table 2. Change scores utilized and descriptives for condition change scores.

Change Score	Calculation	Condition	*N*	*M*	*SD*	*SE*
Activation	ln(Time 2) – ln(Time 1)	Control	29	0.19	0.59	0.11
		Yoga	28	0.39	0.57	0.11
Initial Decrease	ln(Time 3) – ln(Time 2)	Control	29	-0.08	0.34	0.06
		Yoga	28	-0.27	0.39	0.07
Secondary Decrease	ln(Time 4) – ln(Time 3)	Control	29	-0.04	0.20	0.04
		Yoga	28	-0.21	0.29	0.05
Total Decrease	ln(Time 4) – ln(Time 2)	Control	29	-0.12	0.35	0.06
		Yoga	28	-0.45	0.48	0.09

In order to assess whether the TSST did induce a stress response in participants, a paired sample t-test was utilized. Results of the test indicate a significant increase in cortisol concentrations from Time 1 (M = -1.723, SD = 0.485) to Time 2 (M = -1.438, SD = 0.0.572), $t(56) = 3.70$, $p < .001$, $d = 0.49$. This indicates a stress response was initiated by the HPA axis as indicated by an increase in cortisol concentrations. In order to assess whether this stress response differed between conditions, an independent t-test was utilized. Results of the test indicate no significant difference in change scores for Time 2 – Time 1 between conditions, $t(55) = 1.31$, $p = .196$, $d = 0.347$. This indicates the control (M = 0.187, SD = 0.589) and yoga (M = 0.387, SD = 0.567) conditions exhibited an equal increase in cortisol after exposure to the TSST.

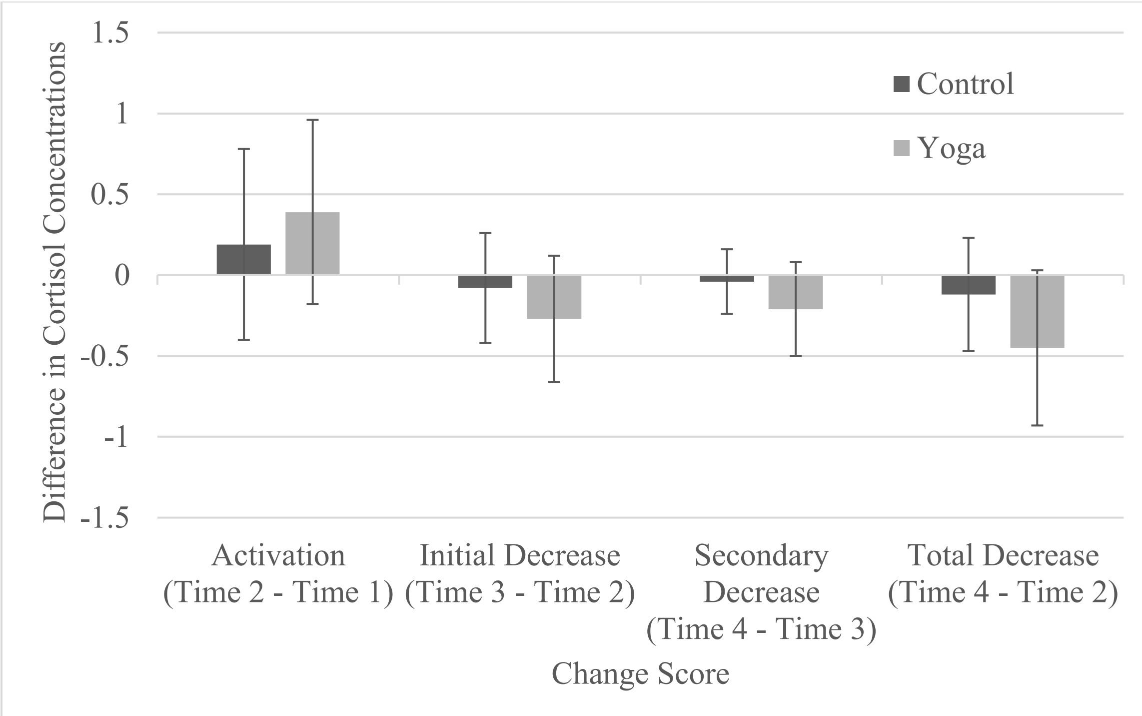

Figure 1. Average cortisol change scores by condition. Negative change scores denote decreases in cortisol concentration.

A series of independent sample *t*-tests were conducted to compare the change scores measuring the decline of cortisol after stress exposure between the yoga and control conditions. The 'initial decrease' was found to be greater in the yoga condition ($M = -0.27$, $SD = 0.39$) than the control ($M = -0.08$, $SD = 0.34$), $t(55) = 2.01$, $p = .050$, $d = 0.53$. This indicates that cortisol concentrations decreased more in the yoga condition than the control during the 'initial decrease' between Time 2 and Time 3. The 'secondary decrease' was also greater in the yoga condition ($M = -0.21$, $SD = 0.29$) than the control ($M = -0.04$, $SD = 0.20$). For this comparison, a Welch's corrected *t*-statistic was used to account for unequal variances (Levene's $p = .028$), $t(48.10) = 2.49$, $p = .016$, $d = 0.66$. This indicates that cortisol concentrations decreased more in the yoga condition than the control during the 'secondary decrease' between Time 3 and Time 4. In addition, the 'total decrease' was greater in the yoga condition ($M = -0.48$, $SD = 0.48$) than the control (*M* = -0.12, *SD* = 0.35). A Welch's corrected *t*-statistic was used again to account for unequal variances (Levene's $p = .044$), $t(48.79) = 3.21$, $p = .002$, $d = 0.85$. This indicates that cortisol concentrations decreased more in the yoga condition than the control during the 'total decrease' between Time 2 and Time 4. A summary of independent *t*-test results for all comparisons made may be found below in Table 3.

Table 3. Independent sample *t*-tests comparing change scores.

Change Score	*t*	*df*	*p*	*M* diff	*SE* diff	95% CI for *M* diff	Cohen's *d*
Activation (Time 2 – Time 1)	-1.31	55	.196	-0.20	0.15	[-0.51, 0.11]	-0.35
Initial Decrease (Time 3 – Time 2)	2.01	55	.050	0.20	0.10	[4.32 e-4, 0.39]	0.53
Secondary Decrease (Time 4 – Time 3)	2.49*	48.10	.016	0.16	0.07	[0.03, 0.29]	0.66
Total Decrease (Time 4 – Time 2)	3.21*	48.79	.002	0.36	0.11	[0.13, 0.58]	0.85

*Welch's corrected *t*-statistic

Analysis regarding the questionnaire results outside of demographic information was not a focus of this thesis. Exploratory analysis will be performed at a later date to determine whether relevant differences exist between any factors evaluated and stress response.

DISCUSSION

Conclusions

The human stress response system serves an important purpose in preparing the body to deal with stress in the short term (McEwen & Stellar, 1993). However, when this system is activated too frequently or for extended periods of time, disease can result (Cohen, Janicki-Deverts, & Miller, 2007). For this reason, it is important that this stress response be regulated efficiently. Recently, yoga has gained popularity among a wide variety of people, largely due to claims of stress reduction and improved health (Broad, 2012). Although it has been shown that a single session of Hatha Yoga could decrease cortisol (West et al., 2004), this decrease was observed from baseline measures, not after stress exposure. The present study aimed to expand on current research by exploring whether participation in a single gentle yoga sequence following stress exposure could decrease the stress response more quickly than occurs naturally.

Findings support the research hypothesis that taking part in a gentle yoga sequence following exposure to a psychological stressor could help decrease the stress response more quickly. This was shown by larger decreases in cortisol concentrations for participants in the yoga condition during the ‘initial decrease,’ ‘secondary decrease,’ and ‘total decrease,’ as indicated by independent *t*-test results. These results suggest that taking part in one 30-minute gentle yoga practice can down-regulate the stress response system more effectively than occurs naturally. Larger decreases observed also indicate more efficient cessation of the stress response after activation.

Results from this study add to current information by suggesting a gentle yoga practice can not only reduce stress from baseline but can also help reduce the stress response more efficiently after activation. Although more research is needed to determine the exact mechanisms that lead to this efficient decrease in stress response, it is hypothesized that both the focus on linking breath to movement and utilization of diaphragmatic breathing played a role. Gerritsen & Band (2018) previously suggested top-down effects on the HPA axis through focus on controlled breathing in addition to the bottom-up effects that occur through vagal stimulation (Ulrich-Lai & Herman, 2009). The heightened focus it takes to link controlled breath with movement that is utilized in gentle yoga could lead to increased top-down regulation of the HPA axis, and more efficient regulation of the stress response.

This increased regulation of the stress response system through gentle yoga could be beneficial in preventing disease that occurs due to 'allostatic overload' (McEwen, 2008; Cohen, Janicki-Deverts, & Miller, 2007). In the present day, many individuals experience frequent, high levels of psychological stress causing chronic stress system activation. Utilization of a short gentle yoga sequence following stress exposure is an efficient, accessible method to counteract chronic stress system activation and lead to improved health and well-being.

Limitations

Using a broader population (i.e., larger age range, more even male/female ratio, etc.) would be beneficial in generalizing these results to a larger population. More confounds that affect samples could also have been accounted for to ensure samples were not affected such as asking about when participants had brushed their teeth, last consumed water, consumed alcohol, taken medications, and had foods with high sugar or acidity (Salimetrics LLC, 2019). This study was conducted by primarily female researchers and judges, which could influence social evaluative stress experienced by participants as well. Although conditions were randomized between times lab members could be there, this was unavoidable as the lab group was almost entirely female. Another potential influence is that there was no initial rest period to ensure that initial cortisol levels were actually at baseline and no awakening time was recorded to determine how this could have influence initial cortisol concentrations.

Future Directions

Future directions for this area of research include performing exploratory analysis using questionnaire data gathered, as well as doing a comparison study for a biofeedback stress reduction study which utilized this same design. It would also be beneficial to perform this study with more participants and evaluate whether participants respond better to different treatments at different times in the day.

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APPENDICES

Appendix A. IRB Approval

Date: 2-27-2019

IRB #: IRB-FY2019-380
Title: Learning to Relax: Stress Reduction Strategies
Creation Date: 11-30-2018
End Date: 1-18-2020
Status: Approved
Principal Investigator: Amber Abernathy
Review Board: MSU
Sponsor:

Study History

Submission Type Initial	Review Type Expedited	Decision Approved

Key Study Contacts

Member Amber Abernathy	Role Principal Investigator	Contact amberabernathy@missouristate.edu
Member Dallas Robinson	Role Primary Contact	Contact Robinson772@live.missouristate.edu
Member Dallas Robinson	Role Co-Principal Investigator	Contact Robinson772@live.missouristate.edu
Member Tabetha Hopke	Role Co-Principal Investigator	Contact tabetha6789@live.missouristate.edu

Initial Submission

1. General Information

1A. **What is the full title of the research protocol?**

Learning to relax: Examining the impact of stress reduction techniques on salivary cortisol

Abstract/Summary

1B. **Please provide a brief description of the project (no more than a few sentences).**

College students have a high prevalence of mental health problems and that number seems to be increasing (Lipson et al., 2015; Reetz et al., 2017). According to the Healthy Minds Study, 34.4% of college students surveyed (n = 42,210) had at least one mental health problem like anxiety, depression, or serious thoughts of suicide (Lipson et al., 2015).

There is evidence that relaxation techniques can be useful in overall stress management (Van Rhenen et al., 2005), and short-term interventions like computer-based biofeedback or yoga could be ways to give students a time friendly and independent way to manage the physiological symptoms of stress and anxiety. The purpose of this study is to compare the impact of a biofeedback intervention, a yoga intervention, and a control on the stress response as measured by salivary cortisol levels and self-report variables like anxiety and distress, as well as examining factors that may influence participant response to the intervention.

Who is the Principal Investigator?

1C. *This MUST be a faculty or staff member.*

Name: Amber Abernathy
Organization: Psychology
Address: 901 S National Ave , Springfield, MO 65897-0027
Phone:
Email: amberabernathy@missouristate.edu

Who is the primary study contact?

1D. *This person may be the Principal Investigator or someone else (faculty, staff, or student). This person, in addition to the PI, will be included on all correspondence related to this project.*

Name: Dallas Robinson
Organization: Psychology
Address: 901, S. National Avenue , Springfield, MO 65897-0027
Phone:
Email: Robinson772@live.missouristate.edu

Select the Co-Principal Investigator(s).

1E. *This MUST be a faculty or staff member.* ***Persons listed as Co-PIs will be required to certify the protocol*** *(in addition to the PI). This person will also be included on all correspondence related to this project.*

Name: Dallas Robinson
Organization: Psychology
Address: 901, S. National Avenue , Springfield, MO 65897-0027
Phone:
Email: Robinson772@live.missouristate.edu

Name: Tabetha Hopke
Organization: Psychology
Address: 901, S. National Avenue , Springfield, MO 65897-0027
Phone:
Email: tabetha6789@live.missouristate.edu

Select the Investigator(s).

1F. ---

An investigator may be faculty, staff, student, or unaffiliated individuals.

Describe the proposed project in a manner that allows the IRB to gain a sense of the project including:

- **the research questions and objectives,**
- **key background literature (supportive and contradictory) with references, and**
- **the manner in which the proposed project will improve the understanding of the chosen topic.**

Cortisol and the Stress Response

Self-regulation is the integrative functioning of emotional and physiological regulation to maintain or reestablish homeostasis, where the physiological systems in the body are all at their optimal levels (Sapolsky, 2004). This can be measured through the functioning of the autonomic nervous system (ANS) (Crockett et al., 2011). The ANS is responsible for regulating bodily systems like respiration, heart rate, etc. Maintaining a physiological balance is essential to maintaining good health, and homeostasis can be disrupted by prolonged activation of the stress response, which can be defined as a network of physiological responses in reaction to a threat or stressor, whether real or perceived (Sapolsky, 2004; Crockett et al., 2011). This prolonged activation can lead to chronic stress and other associated problems.

Chronic stress has been linked to several health related problems in areas of fine motor performance, attention, and cognitive function (Carroll & Winslow, 2017). When energy is constantly mobilized in the stress response, fatigue occurs because the body is unable to store energy (Sapolsky, 2004). This can be even more detrimental when the stress-response is activated due to an imagined threat or a psychological stressor. There are several components to psychological stressors that contribute to anxiety, such as a lack of predictability, lack of control, and an inappropriate interpretation of the stressor (Sapolsky, 2004). Anxiety can be accompanied by physiological symptoms like increased heart rate, sweating, and rapid breathing, as well as distress and an inability to function. (Tabachnick, 2015).

One of the ways the stress response can be measured is through the analysis of cortisol levels from participant saliva samples. Psychological stressors cause the activation of the hypothalamus-pituitary-adrenal (HPA) axis, increasing levels of several hormones including cortisol. Cortisol is a glucocorticoid hormone released when stimuli interpreted as a potential physical or psychological threat stimulates the HPA axis (Byrd-Craven et al., 2012). Chronic dysregulation or excessive secretion of cortisol has been shown to have detrimental long-term effects on both physical and mental health (Raison & Miller, 2003).

The Trier Social Stress Test (TSST) has been shown to stimulate the stress response in a research setting. This test has been found to increase levels of salivary cortisol to 2 to 4 elevations above baseline levels (Kirschbaum et al., 1993). The TSST typically consists of a ten-minute anticipation period and a ten-minute test period where the participant delivers a free speech and performs mental arithmetic in front of an audience. When this test is administered, salivary cortisol has been shown to peak ten minutes after administration (Kirschbaum et al., 1993; Gordis et al., 2006).

Biofeedback

Biofeedback is a method of helping individuals regulate ANS functioning and learn how to become aware of physiological processes like muscle tension, breathing, heart-rate variability, etc., and then work to change them (Ratanasiripong, 2009; Ratanasiripong, 2015). Peripheral biofeedback provides feedback based on activity in the body, while neurofeedback provides feedback based on activity in the brain (Crockett et al., 2011). Peripheral biofeedback can be used to target the physiological symptoms associated with anxiety through a feedback loop in which information about the participant's experience can be used to inform the client's self-regulation (Crocket et al., 2011). This could eventually teach individuals to control and reduce those symptoms.

Biofeedback typically has three stages: 1) acquiring awareness of the maladaptive physiological responses 2) learning to control those responses using techniques like deep breathing and passive muscle relaxation 3) transferring that control to every day life (Carroll & Winslow, 2017). This technique has been shown to be effective in reducing stress associated with every day hassles
2A. and anxiety (Carroll & Winslow, 2017). In Carroll and Winslow's 2017 study, a condensed 90-minute biofeedback training method was used incorporating diaphragmatic breathing and stress inoculation training. The experimental group was compared to a control group using physiological measures of the ANS response and cortisol measures of the stress response, as well as perceived stress levels using the state portion of the State Trait Anxiety Inventory. Those in the biofeedback condition had a statistically significant reduction in cortisol, but not in ANS stress response or perceived stress from pre-training to post-training. These results support the efficacy of a condensed biofeedback method, but also show the importance of additional individual practice time (Carroll & Winslow, 2017).

In another study, participants in the biofeedback condition using a diaphragmatic breathing technique experienced a significant reduction in cortisol from pre-training to post-training, but the control group did not (Carroll & Winslow, 2017). In a different study, a four-week biofeedback intervention showed a reduction in both stress and depression, and a statistically significant reduction in anxiety when compared to the control group (Ratanasiripong, 2015). Those in the control condition actually had a slight increase in reported depression and stress.

Heart rate variability (HRV) is one method of biofeedback, looking at the beat-to-beat changes in heart rate. Low HRV has been linked to psychopathologies like anxiety disorders and substance use disorders during craving states (Henriques et al., 2011; Thurstone & Lajoie, 2013). HRV biofeedback has been shown to reduce stress and mean salivary cortisol levels, one of the more common ways of measuring the stress response (Thurstone & Lajoie, 2013). Research has suggested that computer-based biofeedback may be beneficial for stress management. The use of a computer based biofeedback program significantly decreased levels of both state and trait anxiety in college students (Henriques et al., 2011). This biofeedback program used a finger pad to track the user's heart rate and displayed a graph showing the user's real time heart rate, where a smooth line indicated relaxation. This intervention lasted four weeks and was individualized so the participants could practice biofeedback on their own. There is a need for more in depth research on computer-based biofeedback and its potential benefits, as well as how effective a short term intervention is.

One of the general guidelines for biofeedback is that to develop the skill it is necessary for participants to take an active role in practicing, but there is still debate on the amount of time, method of biofeedback, and sessions necessary for efficacy (Carrol & Winslow, 2017).

Yoga

In the broadest sense of the term, yoga could be considered a practice that involves postures (poses) of the physical body (asanas) and controlled breathing (pranayama). Gentle yoga, which will be the focus of this study, is a low intensity yoga practice. While this type of class still uses active poses, these poses are not meant to be physically demanding or increase heart rate. Gentle yoga classes are fairly slow-paced, maybe taking several breaths per pose instead of the "one breath one movement" style of a power vinyasa practice, and are meant to be accessible to students with a wide rage of physical activity levels.

Many times, there is a mindfulness meditation component to yoga practice. According to Kabat-Zinn (1982), meditation is the practice of directing the thoughts, while mindfulness mediation the practice of disconnecting from thoughts and simply noticing them without judgement. This mindfulness meditation component is what fosters the mind-body connection that is traditionally associated with yoga, and on its own has been shown to be beneficial in stress reduction (Carlson, Speca, Patel, & Goodeyhe, 2003). Although mindfulness meditation component of yoga classes can also vary greatly, classes frequently involve a focus on breath and connecting breath with postures in specific ways which can assist students in disconnecting from their thoughts.

Implementation of a yoga practice has previously been shown to decrease psychological stress measures as well as biological stress markers (Riley & Park, 2015; Ross & Thomas, 2010). It has also been shown that one session of Hatha yoga immediately decreased salivary cortisol, perceived stress, and negative affect (West et al., 2004). The duration and type of yoga practice used in studies involving yoga however varies greatly. This study aims to add to the existing base of research by examining whether one session of gentle yoga could assist in recovery of the stress response system as assessed through measurement of salivary cortisol.

2B. Check all research activities that apply:

✓ Audio, video, digital, or image recordings

Biohazards (e.g., rDNA, infectious agents, select agents, toxins)

✓ Biological sampling (other than blood)

Blood drawing

Class Protocol (or Program or Umbrella Protocol)

Data, not publicly available

Data, publicly available

Deception

✓ Devices

✓ Diet, exercise, or sleep modifications

Drugs or biologics

Focus groups

✓ Internet or email data collection

Materials that may be considered sensitive, offensive, threatening, or degrading

Non-invasive medical procedures

✓ Observation of participants

Oral history

Placebo

Record review

Specimen research

Surgical procedures

✓ Surveys, questionnaires, or interviews (one-on-one)

Surveys, questionnaires, or interviews (group)

Other

Describe the procedures and methods planned for carrying out the study. Make sure to include the following:

- site selection,
- the procedures used to gain permission to carry out research at the selected site(s),

- data collection procedures,
- and an overview of the manner in which data will be analyzed.

Provide all information necessary for the IRB to be clear about **all** of the contact human participants will have with the project.

2C. Participants will be recruited from the community through psychology courses in a large mid-western university. Students will receive course credit or extra credit for their participation. The study will be conducted in a research lab in the psychology building on campus. Participants will be randomly assigned to a control condition or a condition teaching ALIVE biofeedback software. The biofeedback software will measure heart rate and breathing smoothness through a finger pad.

Participants will be given an informed consent document explaining the study upon entry. Saliva will be collected immediately before beginning the Trier Social Stress Test (TSST) to establish a baseline stress response measured by cortisol levels. There will be two researchers in the room, one acting as the judge and the other collecting saliva and giving instructions. The TSST consists of a ten minute anticipatory period where participants will prepare a speech as if on a job interview followed by five minutes of the interview and five minutes of a mathematical activity. Saliva will be collected again ten minutes after the completion of the stress test. Participants will then be assigned to receive the ALIVE biofeedback intervention, the yoga intervention, or the control condition for thirty minutes. Saliva will be collected ten minute after completion of the intervention. Then, participants will be given several surveys through Qualtrics; the order in which they will be presented will be counterbalanced. Saliva will be collected for a final time 30 minutes after beginning the surveys.

Attach surveys, questionnaires, and other social-behavioral measurement tools, if applicable.

2D.

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C - DASS 21.pdf
IPIP formatted right.docx
Demographic Questionnaire - B.docx

3. Participants

3A. Specify the participant population(s). Check all that apply.

✓ Adults

Children (<18 years)

Adults with decisional impairment

Non-English speaking

✓ Student research pools (e.g. psychology)

Specify:

students enrolled in select courses in the psychology department

Pregnant women or fetuses

Prisoners

Unknown (e.g., secondary use of data/specimens, non-targeted surveys, program/class/umbrella protocols)

3B. Specify the age(s) of the individuals who may participate in the research.

Participants will be age 18 and older.

3C. Describe the characteristics of the proposed participants, and explain how the nature of the research requires/justifies their inclusion.

Participants will be college students from psychology classes. They will receive course credit for their participation, and potentially a useful intervention meant to decrease stress levels.

3D. Provide the total number of participants (or number of participant records, specimens, etc.) for whom you are seeking Missouri State IRB approval.

100 participants.

3F. Estimate the time required from each participant, including individual interactions, total time commitment, and long-term follow-up, if any.

Total: Approximately 1.5-2 hours of participation.

3G. Describe how potential participants will be identified (e.g., advertising, individuals known to investigator, record review, etc.). Explain how investigator(s) will gain access to this population, as applicable.

Participants will be identified based on their enrollment in psychology courses.

3H. Describe the recruitment process; including the setting in which recruitment will take place. Provide copies of proposed recruitment materials (e.g., ads, flyers, website postings, recruitment letters, and oral/written scripts).

Participants will be able to sign up for this study online and select from a variety of different research projects and timeslots available.

3H.1. Attach recruitment materials, if applicable.

3I. Will participants receive compensation or other incentives (e.g., free services, cash payments, gift certificates, parking, classroom credit, travel reimbursement, etc.) to participate in the research study?

✓ Yes

Describe the incentive, including the amount and timing of all payments.

Participants in an introductory psychology course will receive 4 research credits (1 per half hour of participation). Participants in other psychology classes will be offered extra credit.

No

4. Informed Consent

4A. From the list below, indicate how consent will be obtained for this study.

Check all that apply.

✓ Written/signed consent by the subject

Written/signed consent (permission) for a minor by a Parent or Legal Guardian

Written/signed consent by a Legally Authorized Representative (for adults incapable of consenting).

Request for Waiver of Documentation of Consent (e.g. Verbal Consent, Anonymous Surveys, etc.)

Waiver of parental permission

Consent will not be obtained from subjects (Waiver of Consent)

4B. Describe the consent process including where and by whom the subjects will be approached, the plans to ensure the privacy of the subjects and the measures to ensure that subjects understand the nature of the study, its procedures, risks and benefits and that they freely grant their consent.

Participants will be presented with a hard copy of the informed consent document and given time to read it. All participant information (survey responses, saliva samples) will be identified with a number to ensure privacy. The method of the study will be described, as well as any potential risks and benefits. Finally, they will sign the document if they wish to continue their participation, and if not, they will be excused from the study.

4B.1. Attach all copies of informed consent documents (written or verbal) that will be used for this study.

A -Consent .docxSample documents: Informed Consent Examples

4B.2. Attach all copies of assent documents that will be used for this study, if applicable.

5. Risks and Benefits

Describe all reasonably expected risks, harms, and/or discomforts that may apply to the research. Discuss severity and likelihood of occurrence.

5A. *Consider the range of risks - physical, psychological, social, legal, and economic.*

To elicit the stress response, participants will take the Trier Social Stress Test. This may cause mild discomfort or distress in some participants, but the components are not unlike what students are required to do in class: public speaking and performing mental arithmetic. Participants in the yoga group will be performing light physical activity but the activity is not meant to be challenging or increase heart rate.

Describe the steps that will be taken to minimize risks and the likelihood of harm.

5B.

Participants will be informed prior to arriving for the study that they may be asked to take part in a yoga practice or use biofeedback software. They will be given instructions during the test, and the researchers will explain the nature of the TSST to participants upon completion of the study.

List the potential benefits that participants may expect as a result of this research study. State if there are no direct benefits to individual participants.

5C.

The biofeedback and yoga interventions have been shown to reduce stress levels and feelings of anxiety.

Describe any potential indirect benefits to future subjects, science, and society.

5D.

This research will further the understanding of short-term biofeedback and yoga interventions and the impact they have on stress response recovery and self-report variables.

5E. Discuss how risks to participants are reasonable when compared to the anticipated benefits to participants (if any) and the importance of the knowledge that may reasonably be expected to result.

The TSST is a small stressor that most will have experienced before. If participants can better regulate their stress response when presented with this stressor, it is possible that ability to cope will translate into their every day lives making the use of the test reasonable.

6. Data Collection

Missouri State University is committed to keeping data and information secure. Please review the Missouri State Information Security policies. Discuss your project with the MSU Information Security Office or your College's IT support staff if you have questions about how to handle your data appropriately.

6A. <u>Statement of Principal Investigator Responsibility for Data</u>
The principal investigator of this study is responsible for the storage, oversight, and disposal of all data associated with this study. Data will not be disseminated without the explicit approval of the principal investigator, and identifying information associated with the data will not be shared.

✓ By checking this box, all personnel associated with this study understand and agree to the Statement of Principal Investigator Responsibility for Data.

6B. How will the data for this study be collect/stored?

Check all that apply.

✓ Electronic storage format

On paper

Describe where the data will be stored (e.g., paper forms, flash drives or removable media, desktop or laptop computer, server, research storage area network, external source) and describe the plan to ensure the security and confidentiality of the records (e.g., locked office, locked file cabinet, password-protected computer or files, encrypted data files, database limited to coded data, master list stored in separate location).

6C. *At minimum, physical data should always be secured by lock and key when stored. Electronic data should be stored on University secure servers whenever possible (Office 365 or other secure campus server). If data has to be stored off campus, the file should be encrypted and the device password protected. Additionally, any data to be shared outside the University network will require a SUDERS request be filed and approved. See https://mis.missouristate.edu/Central/suders/creat...*

Saliva samples will be stored in a refrigerator located in a private research lab on the fourth floor of the psychology building. Only the primary investigator and members of the research team will have access to this room. For questionnaires, the online survey system Qualtrics will be used. Participants will enter a participant number, the same number that will be linked to the saliva samples so that the participants name will only be found on the informed consent document. Informed consent forms will be stored in a locked cabinet in the research lab.

Describe how data will be disposed of and when disposal will occur.

6D. *At minimum, Federal regulations require research records to be retained for at least 3 years after the completion of the research (45 CFR 46). Research that involves identifiable health information is subject to HIPAA regulations, which require records to be retained for at least 6 years after a participant has signed an authorization. Finally, funded research projects may require longer retention periods, you may need to follow the sponsoring agency guidelines.*

Informed consent documents will be kept in a locked cabinet in the research lab for three years after the completion of the study and then will be shredded

7. Funding

7A. Is this study externally funded?

For example, this research is funded by a source outside Missouri State; a federal agency, non-profit organization, etc.

Yes

✓ No

Potentially (this study is being submitted for funding, but has not yet been awarded)

7B. Is this study internally funded?

For example, this research is funded by a source inside Missouri State; departmental funds, the Graduate College, etc.

✓ Yes

Please list the internal funding source.

Missouri State University Graduate College

No

Potentially (this study is being submitted for funding, but has not yet been awarded)

8. HIPAA

Does your study contain protected health information (PHI)?

8A.

PHI is any information in a medical record or designated record set that can be used to identify an individual and that was created, used, or disclosed in the course of providing a a health care service, such as a diagnosis or treatment.

Yes

✓ No

9. Supporting Documentation

Human Subjects Training Certificates

9A.

Attach human subjects training certificates for all listed personnel. To access your training documents, please go to CITI Training.

Dallas CITI.pdf
Dallas CITI 3.pdf
Dallas CITI 2.pdf
CITI training.pdf
1abernathyciti.pdf
2abernathyciti.pdf
3abernathyciti.pdf
4abernathyciti.pdf
5abernathyciti.pdf

HIPAA Training Certificates

9B.

Attach HIPAA training certificates for all listed personnel, if applicable. To get more information about HIPAA training and/or to access your training documents, please go to HIPAA Information for Researchers.

Informed Consent Documents

9C.

Attach all copies of informed consent documents (written or verbal) that will be used for this study.

A -Consent .docxSample documents: Informed Consent Examples

Assent Documents

9D.

Attach all copies of assent documents (written or verbal) that will be used for this study.

Sample documents: Assent Examples

Recruitment Tools

9E.

Attach copies of proposed recruitment tools.

Surveys/Questionnaires/Other Social-Behavioral Measurement Tools

9F. *Attach surveys, questionnaires, and other social-behavioral measurement tools.*

aaqii-7-item-scoring-original-working-3-reader.pdf

C - DASS 21.pdf

IPIP formatted right.docx

Demographic Questionnaire - B.docx

Other Documents

9G.

Attach any other documents that have not been specified in previous questions, but are needed for IRB review.

10. Additional Information

10A. Would you like to add additional information?

Yes

✓ No

Appendix B. Informed Consent

Informed Consent

TITLE: Learning to Relax: Changing the Stress Response
INVESTIGATORS: Dallas Robinson, B.S., Missouri State University
Tabetha Hopke, B.S., Missouri State University
Amber Abernathy, Ph.D., Missouri State University

PURPOSE: The purpose of the study is to better understand the stress response and how to reduce it through different relaxation techniques.

VOLUNTARY: This study is completely voluntary. You may refuse to answer any questions or choose to withdraw from participation at any time without any penalty or loss of benefits to which you are otherwise entitled.

WHAT DO YOU DO? Participants will be taking part in a mock job interview and a math-based activity. They will then be randomly selected to learn a computer based relaxation technique, a yoga based relaxation technique, or be allowed to relax on their own. Saliva will be collected four times to assess stress levels. Participants will then complete a series of questionnaires through Qualtrics, an online computer program.

RISKS: This project contains minimal risks. There may be some discomfort during the mock interview and math activity.

BENEFITS: Participants will learn and practice different ways to relax, which may lead to a reduction in overall stress levels and an increase in ability to handle stress. Participants will receive compensation in the form of research credit or extra credit.

CONFIDENTIALITY: Your answers are entirely confidential, and will not be revealed to anyone other than the researchers conducting the study. Only your arbitrary participant identification number will link you to data you provide. Your confidentiality will be maintained in that your name will not appear on the survey or in the published study itself. Any written results will discuss group findings and will not include information that will identify you. Research records will be stored securely and only researchers and individuals responsible for research oversight will have access to the records. Data will be stored on a secure computer with file encryption software for three years after the study completion.

CONTACTS: If you have any questions regarding the study, please contact Dallas Robinson at Robinson772@live.missouristate.edu

You have read and fully understand the consent form. You attest that you sign it freely and voluntarily.

____________________________________ ______________
Signature Date

Appendix C. Demographic Questionnaire

Please Circle The Answers Below That Best Represent You Currently

1.) I am 18 19 20 21 22 23 24 25 26+

2.) I am male female

3.) Relationship Status:

Married Cohabitating Divorced Widowed Committed

Relationship Dating Single

4.) Your ethnicity:

Caucasian American Indian African-American/Black Biracial

Hispanic/Latino Asian/Asian-American Other

5.) Your current grade level (select one):

___ Freshman ___ Sophomore ___ Junior ___ Senior
___ Other ___ Graduate student ___ Not applicable

6.) Please estimate your income:

___ $0 - $10,000 ___ $10,000 - $20,000 ___ $20,000 - $30,000

___ $30,000 - $40,000 ___ $40,000 - $50,000 ___ $50,000 - $60,000

___ $60,000 - $70,000 ___ $70,000 - $80,000 ___ $80,000 – 90,000

___ $90,000 - $100,000 ___ $100,000-$110,000 ___ $ Over $110,000

7.) Do you smoke or use nicotine? ____Yes ____No

8.) How long ago did smoke or use nicotine?

___ 0 – 30 min ___ 30 – 60 min ___ 1 – 2 hours ___ 2 – 3 hours

___3 – 4 hours ___ 4 – 5 hours ___ 5 – 6 hours ___ 6 – 7 hours ___ 7+ hours

9.) How long ago did you have caffeine?

___ 0 – 30 min ___ 30 – 60 min ___ 1 – 2 hours ___ 2 – 3 hours

___3 – 4 hours ___ 4 – 5 hours ___ 5 – 6 hours ___ 6 – 7 hours ___ 7+ hours

10.) How long ago did you eat?

___ 0 – 30 min ___ 30 – 60 min ___ 1 – 2 hours ___ 2 – 3 hours

___3 – 4 hours ___ 4 – 5 hours ___ 5 – 6 hours ___ 6 – 7 hours ___ 7+ hours

11.) How many days/ week do you typically exercise?

___ 1 ___ 2 ___3 ___ 4 ___ 5 ___ 6 ___ 7

12.) Do you practice any form of meditation or mindfulness? ___ Yes ___No

Appendix D. Acceptance and Action Questionnaire II (AAQII)

Below you will find a list of statements. Please rate how true each statement is for you by using the scale below to fill in your choice.

1	2	3	4	5	6	7
never true	very seldom true	seldom true	sometimes true	frequently true	almost always true	always true

1. My painful experiences and memories make it difficult for me to live a life that I would value.	
2. I'm afraid of my feelings.	
3. I worry about not being able to control my worries and feelings.	
4. My painful memories prevent me from having a fulfilling life.	
5. Emotions cause problems in my life.	
6. It seems like most people are handling their lives better than I am.	
7. Worries get in the way of my success.	
TOTAL	

Appendix E. Depression, Anxiety, and Stress Scale (DASS 21)

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend too much time on any statement.
The rating scale is as follows:

0 Did not apply to me at all - NEVER
1 Applied to me to some degree, or some of the time - SOMETIMES
2 Applied to me to a considerable degree, or a good part of time - OFTEN
3 Applied to me very much, or most of the time - ALMOST ALWAYS

FOR OFFICE USE

		N	S	O	AA	D	A	S
1	I found it hard to wind down	0	1	2	3			
2	I was aware of dryness of my mouth	0	1	2	3			
3	I couldn't seem to experience any positive feeling at all	0	1	2	3			
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3			
5	I found it difficult to work up the initiative to do things	0	1	2	3			
6	I tended to over-react to situations	0	1	2	3			
7	I experienced trembling (eg, in the hands)	0	1	2	3			
8	I felt that I was using a lot of nervous energy	0	1	2	3			
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3			
10	I felt that I had nothing to look forward to	0	1	2	3			
11	I found myself getting agitated	0	1	2	3			
12	I found it difficult to relax	0	1	2	3			
13	I felt down-hearted and blue	0	1	2	3			
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3			
15	I felt I was close to panic	0	1	2	3			
16	I was unable to become enthusiastic about anything	0	1	2	3			
17	I felt I wasn't worth much as a person	0	1	2	3			
18	I felt that I was rather touchy	0	1	2	3			
19	I was aware of the action of my heart in the absence of physicalexertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3			
20	I felt scared without any good reason	0	1	2	3			
21	I felt that life was meaningless	0	1	2	3			
					TOTALS			

Appendix F. International Personality Item Pool (IPIP)

	Very Inaccurate	Moderately Inaccurate	Neither Accurate nor Inaccurate	Moderately Accurate	Very Accurate
Often feel blue.					
Dislike myself.					
Am often down in the dumps.					
Have frequent mood swings.					
Panic easily.					
Am filled with doubts about things.					
Feel threatened easily.					
Get stressed out easily.					
Fear for the worst.					
Worry about things.					
Seldom feel blue.					
Feel comfortable with myself.					
Rarely get irritated.					
Am not easily bothered by things.					
Am very pleased with myself.					
Am relaxed most of the time.					
Seldom get mad.					
Am not easily frustrated.					
Remain calm under pressure.					
Rarely lose my composure.					

	Very Inaccurate	Moderately Inaccurate	Neither Accurate nor Inaccurate	Moderately Accurate	Very Accurate
Feel comfortable around people.					
Make friends easily.					
Am skilled in handling social situations.					
Am the life of the party.					
Know how to captivate people.					
Start conversations.					
Warm up quickly to others.					
Talk to a lot of different people at parties.					
Don't mind being the center of attention.					
Cheer people up.					
Have little to say.					
Keep in the background.					
Would describe my experiences as somewhat dull.					
Don't like to draw attention to myself.					
Don't talk a lot.					
Avoid contacts with others.					
Am hard to get to know.					
Retreat from others.					
Find it difficult to approach others.					
Keep others at a distance.					

	Very Inaccurate	Moderately Inaccurate	Neither Accurate nor Inaccurate	Moderately Accurate	Very Accurate
Believe in the importance of art.					
Have a vivid imagination.					
Tend to vote for liberal political candidates.					
Carry the conversation to a higher level.					
Enjoy hearing new ideas.					
Enjoy thinking about things.					
Can say things beautifully.					
Enjoy wild flights of fantasy.					
Get excited by new ideas.					
Have a rich vocabulary.					
Am not interested in abstract ideas.					
Do not like art.					
Avoid philosophical discussions.					
Do not enjoy going to art museums.					
Tend to vote for conservative political candidates.					
Do not like poetry.					
Rarely look for a deeper meaning in things.					
Believe that too much tax money goes to support artists.					
Am not interested in theoretical discussions.					
Have difficulty understanding abstract ideas.					

	Very Inaccurate	Moderately Inaccurate	Neither Accurate nor Inaccurate	Moderately Accurate	Very Accurate
Have a good word for everyone.					
Believe that others have good intentions.					
Respect others.					
Accept people as they are.					
Make people feel at ease.					
Am concerned about others.					
Trust what people say.					
Sympathize with others' feelings.					
Am easy to satisfy.					
Treat all people equally.					
Have a sharp tongue.					
Cut others to pieces.					
Suspect hidden motives in others.					
Get back at others.					
Insult people.					
Believe that I am better than others.					
Contradict others.					
Make demands on others.					
Hold a grudge.					
Am out for my own personal gain.					

	Very Inaccurate	Moderately Inaccurate	Neither Accurate nor Inaccurate	Moderately Accurate	Very Accurate
Am always prepared.					
Pay attention to details.					
Get chores done right away.					
Carry out my plans.					
Make plans and stick to them.					
Complete tasks successfully.					
Do things according to a plan.					
Am exacting in my work.					
Finish what I start.					
Follow through with my plans.					
Waste my time.					
Find it difficult to get down to work.					
Do just enough work to get by.					
Don't see things through.					
Shirk my duties.					
Mess things up.					
Leave things unfinished.					
Don't put my mind on the task at hand.					
Make a mess of things.					
Need a push to get started.					