The Efficacy of a Modified Trier Social Stress Test as Indicated by Heart Rate Variability

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The Efficacy of a Modified Trier Social Stress Test as Indicated by Heart Rate Variability

Matt Green
Honors Thesis

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Abstract

This study was conducted as part of a larger project assessing the impacts of meditation on state and trait physiological stress responses in a college population. College students face a tremendous amount of stress which could result in detrimental health implications and affect academic performance. The overall goal of the project is to assess the impacts of meditation on students' psychophysiological stress, academic performance, and overall well-being. In order to elicit a physiological stress response in the laboratory setting we used a modified version of the Trier Social Stress Test (TSST). Our version substituted the mock interview portion with verbal analogy questions from previous GRE exams. The math stressor section remained the same as in the original TSST. This paper specifically focuses on the efficacy of our modified TSST as indicated by normalized high frequency heart rate variability (HF HRV) using pulse photoplethysmography (PPG). This analysis method has been shown to be a measure of parasympathetic activity. Thus, we expected normalized HF HRV to decrease during verbal and math stressor intervals. Phase one of the study consisted of a one-day meditation workshop and testing days pre- and post-workshop. The normalized HF HRV data from post-workshop testing showed a significant decrease from baseline to the math stressor interval. However, no significant difference was seen between baseline and verbal stressor intervals (n=6). This indicates that our modified TSST was partially effective in eliciting a stress response. Phase two of the study involves a semester-long compassion seminar course entitled Religion 250: Seminar in Compassion. Testing was conducted during finals week preceding the course. Post-compassion seminar data will be collected in April during finals week, but pre-seminar data showed a significant decrease in normalized HF HRV from baseline to the verbal stressor interval. However, no significant difference was seen from baseline to the math stressor interval (n=17). This indicates that our modified TSST was partially effective in eliciting a stress response. When post-seminar data is collected we can analyze the effects of the compassion seminar course on state and trait physiological stress responses in a college population.
In an increasingly fast-paced and demanding world, humans are constantly exposed to various stressors. These stressors are defined by Chrousos and Gold (1992) as physical or psychological events that threaten an organism’s complex homeostasis. The inability to effectively deal with these forces can impose detrimental effects on physical and mental health. Problems arise when stressors constantly bombard an individual and affect his or her ability to function optimally. Cohen et al. (2007) defines this “threatening” type of stress as occurring when “an individual perceives that environmental demands...exceed his or her adaptive capacity.” This “adaptive capacity” differs for all individuals depending on unique biological, psychological, and social parameters. Adverse health effects occur when this capacity is reached and the individual can no longer utilize intrinsic adaptive mechanisms to cope in response. Psychological and physical resources are depleted thus increasing the probability of developing physical illness or psychological distress. Although many definitions exist for “stress,” in this paper the word will refer to the type of threatening psychosocial stress that causes detrimental effects and distress.

Chronic stress has been implicated as a risk factor for many health problems ranging from depression to immunodeficiency (Hammen, 2005; Stone et al., 1992). Antoni, et al. (2006) found that initiation, growth, and metastasis of certain tumors have also been attributed to chronic stress exposure. In addition, stress can cause decreased antiviral defense and DNA repair activity, and accelerated cellular aging, thus implicating it in cancer pathology. Hammen (2005) explains that relationship stressors are major risk factors in the development of depressive disorders. She also states that concurrent stressful events may reduce positive responses to treatment and may exacerbate negative symptoms. Stress has also been shown to increase the risk of progression from HIV to AIDS in men by as much as 50% (Leserman et al.,
Krantz and McCeney (2002) found that constant stress may cause myocardial ischemia and activate inflammatory mechanisms leading to cardiovascular disease. Krantz and McCeney's study focused on occupational stress, taking into account factors such as psychological job demands, employee autonomy, and job satisfaction.

Stress is extremely prevalent in an average college student population. These students face a wide array of specific challenges such as academic expectations, social integration, loss of home comforts, and career planning issues. In a study of 100 undergraduates, Ross et al. (1999) found via survey that some of the top stressors were change in sleeping habits, change in eating habits, new responsibilities, increased class workload, change in social activities, and financial difficulties. Misra and Mckean (2000) found that in the sample of 249 undergraduates, more students were stressed due to pressure and self-imposed stress than changes, conflict, and frustration. They also found that men experience less self-imposed stress, state and trait anxiety, and physiological responses to stress than women. State anxiety is defined as temporary and in response to a specific stressor whereas trait anxiety is naturally occurring and may not have specific causation. Interestingly, women scored higher than men in time-management and perceived control of their time. Regardless of what is causing them to be stressed, the fact remains that increasing numbers of students are reporting feeling stressed and overwhelmed. Sax (1997) used annual nationwide surveys to show that proportions of students reporting feeling overwhelmed increased from 1985 to 1995. Moreover, a study by The American College Health Association (2004) found stress to be the most common impeding factor on academic success. Over 32% of almost 50,000 students across 74 campuses cited stress as impairing academic performance.
In addition to depression and other aforementioned health problems, stress has been implicated in other maladies specifically within a college population. For example, in a study of 199 students, Laabè et al. (1997) reported greater instances of headaches in the population deemed stressed than in the non-stressed population. Lorrie et al. (1999) found that sleep disturbances were also correlated with high levels of stress, and Stone et al. (1992) showed that stress from life events may increase infection risk for the common cold.

The physiological explanations for some of these problems are due to the body’s natural stress response. The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) both distinctly respond to stressors. The HPA axis response is hormone-mediated and results in the production of the stress hormone cortisol. When the body detects a stressor, the paraventricular nucleus of the hypothalamus is activated and secretes corticotropin-releasing hormone (CRH) and adrenocorticotropin vasopressin (AVP) (Guilliams, 2010). These hormones signal genesis of adrenocorticotropin hormone (ACTH) in the anterior pituitary, which regulates the adrenal cortex production of cortisol, the archetypal “stress hormone” measured in many studies. Cortisol is a glucocorticoid that works to impede long-term metabolic processes used for “rest and digest,” while activating processes for immediate survival and maintenance of a stable internal environment. CRH also activates the locus coeruleus/norepinephrine (LC/NE) system, which regulates epinephrine/norepinephrine for the “fight or flight” response of the sympathetic nervous system. ACTH and CRH fluctuate normally based on circadian rhythms, and work via negative feedback with blood cortisol concentration. The negative feedback loop limits long-term exposure of tissues to cortisol because of its inflammatory and immunosuppressive effects. Dehydroxyepiandrosterone (DHEA) is also produced by the adrenal cortex and protects the hippocampus and other brain regions from
cortisol's damaging effects. Chronic stress can lead to dysfunction in the HPA axis and decreased DHEA production (Guilliams, 2010).

The autonomic nervous system (ANS) has a distinct mechanism for responding to stressors which is nerve-mediated. This system is part of the central nervous system (CNS) and regulates involuntary bodily functions. It innervates every tissue and organ in the body except for skeletal muscle fibers and it is composed of parasympathetic and sympathetic branches. Sympathetic nervous system (SNS) activation is commonly known as the "fight or flight" response. This occurs during stressful situations and its physiological manifestations are well known: eye dilation, heightened respiratory rate, stimulation of production and release of glucose, etc. The parasympathetic nervous system (PNS) is commonly known to be responsible for "rest and digest" actions. When the PNS is active salivation increases, peristalsis and release of digestive enzymes are stimulated, and respiration slows. The two divisions cause opposite physiological effects (Bear et al., 2007).

Both ANS branches innervate cardiac cells and influence heart rate via the sinoatrial (SA) node, which modulates heart rate. The parasympathetic system releases acetylcholine via the vagus nerve to slow the rate of SA depolarization, thus slowing pulse during the "rest and digest" response. The sympathetic system uses norepinephrine, which speeds pulse during the "fight or flight" response (Aubert et al., 2003). These natural responses to external stressors have developed for survival purposes. If a man had to run from a bear he would not need his digestive enzymes stimulated; he would need to see clearly, and have energy from glucose as in the sympathetic response.
Blood vessels also constrict during the sympathetic response and shunt blood to core vital organs (Bear et al., 2007). Blood pressure is a measure of the pressure exerted by blood on the walls of blood vessels and has been used to measure sympathetic activity. The systolic component indicates maximum pressure when the left ventricle is contracted and diastolic indicates minimum pressure when the heart is relaxed. A normal reading for a healthy adult is 120/80 mmHg and blood pressure has long been studied as an indicator for stress (Vrijkotte et al., 2000). Hypertensive individuals, as defined by having over 140 mmHg systolic pressure and 90 mmHg diastolic pressure, are known to be at a higher risk for cardiovascular disease. Hypertension is also implicated as a risk factor for stroke and chronic kidney disease. Hypertension can be caused by a variety of factors including genetics, age, lifestyle, diet, physical activity, and stress (Vrijkotte et al., 2000). Vrijkotte et al. (2000) found that in a population of 109 middle-aged white-collar men workers, those described as having high imbalance of extrinsic effort and reward at work had significantly higher systolic blood pressures at both home and work than those who did not report an imbalance. Systolic blood pressure was even shown to be increased at night, thus indicating that work stress may contribute to constant elevation of systolic blood pressure.

Galvanic Skin Response (GSR) has also been used to measure sympathetic nervous system stress response. GSR is sometimes called sympathetic skin response, and is defined by Vetrugno et al. (2003) as the skin’s momentary change in electric potential. This conductance change occurs depending on the presence of sweat. There are two types of sweating that occur: thermoregulatory and emotional. Both interact in some cases. Emotional sweating is mostly evident in the palms of the hands and fingers. It is thought to be controlled by the anterior cingulate cortex (ACC) of the central nervous system. The ACC is responsible for
visceral and somatic aspects of emotional response and arousal. The thalamus has also been shown to regulate significance of stimuli and degree of alertness. Increased GSR is thought to result from allotting attention to novel, significant stimuli, thus indicating a sympathetic response. The specific mechanisms for GSR response are still relatively understudied, but it is known that drowsiness and lowered sleep levels can decrease arousal (Vetrugno et al., 2003).

Heart rate variability (HRV) is a relatively new biomarker for measuring sympathetic and parasympathetic activity. Although accurate, the conventional model of pulse increasing during the sympathetic response and decreasing during the parasympathetic response is too simplistic. In normal conditions, pulse increases with inhalation and decreases with exhalation in a phenomenon called the respiratory sinus arrhythmia (Aubert et al., 2003). Variations in the intervals from beat to beat are expected because with each breath, sympathetic and parasympathetic influences fluctuate. These variations can be measured on an electrocardiogram (ECG) or pulse photoplethysmograph (PPG). Both of these graphs display heart activity at a given time in the form of peaks. ECGs are usually measured with electrodes on the chest, ankles, and wrists. They give a waveform called the QRS complex, which signifies rapid depolarization of both ventricles (Hurst, 1998). PPGs are measured with pulse oximeters usually worn on the index finger. These oximeters detect arterial blood flow rate by comparing absorbance at two different wavelengths being passed through the finger. This produces systolic peaks indicating ventricular contraction (Elgendi, 2012). Figure 1 (biochemtech.com, n.d.) shows an example of three R-R intervals from an ECG and three P-P intervals from a PPG, both signifying three cardiac cycles.
The P-P distance interval indicates the time between heartbeats. A greater variability of the time from peak to peak signifies greater parasympathetic modulation, and thus at rest variability is usually high (Berntson, 1997). The first step in measuring this variability, after filtering out equipment interference artifacts with an automatic software function, consists of converting raw PPG data into a tachogram. The tachogram displays the P-P interval duration in seconds on the y-axis versus the actual time of testing period on the x-axis as shown in figure 2. A small P-P interval duration indicates a high pulse rate, and vice-versa (Aubert et al., 2003).

The magnified version of the tachogram at the bottom of figure 2 shows respiratory sinus arrhythmia over the course of a minute: the function consistently alternates between larger and smaller P-P interval durations in a pseudo-sinusoidal form, thus indicating the fall and rise of pulse rate due to inhalation and exhalation.
Figure 2 Tachogram over total session of about thirty-five minutes (top) and over one minute (bottom). Both show the P-P interval duration (seconds) on the y-axis versus the actual testing time (seconds) on the x-axis.

The second step in determining heart rate variability is to conduct a Fourier transformation. This transformation decomposes the complex function of the tachogram into many simple oscillatory functions. All of these smaller functions have different frequencies and add together to make the complex function of the tachogram. The power of a specific frequency is defined as the proportion of simple waves with that frequency contributing to the overall complex function. These frequencies are divided into ranges of high frequency and low frequency as explained in table 1. High frequency (HF) HRV is from 0.15 to 0.4 Hz and signifies parasympathetic activity. Therefore, decreased levels of HF HRV mean less parasympathetic activity, which may be indicative of the stress response. Low frequency (LF) HRV is below 0.15 Hz and is less understood, but could signify a mix of sympathetic and parasympathetic activity.
(Berntson et al., 1997). The Fourier transformation results in a power spectrum displaying the power in each frequency range as shown in the example figure 3 (Vivonoetics, 2012).

Table 1: HRV frequency abbreviations, frequencies, and significance.

<table>
<thead>
<tr>
<th>Abbreviation:</th>
<th>HF</th>
<th>LF</th>
<th>LF/HF</th>
<th>Normalized HF</th>
<th>Normalized LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name:</td>
<td>High Frequency</td>
<td>Low Frequency</td>
<td>Ratio of Low/High Frequency</td>
<td>Normalized High Frequency</td>
<td>Normalized Low Frequency</td>
</tr>
<tr>
<td>Measurement</td>
<td>0.15 to 0.4 Hz</td>
<td>0.04 to 0.15 Hz</td>
<td>0&lt;Ratio&lt;1</td>
<td>0&lt;x&lt;1</td>
<td>0&lt;x&lt;1</td>
</tr>
<tr>
<td>Significance:</td>
<td>Parasympathetic Activity</td>
<td>Mix of Sympathetic/Parasympathetic Activity</td>
<td>Mix of Sympathetic/Parasympathetic Activity</td>
<td>Parasympathetic Activity</td>
<td>Mix of Sympathetic/Parasympathetic Activity</td>
</tr>
</tbody>
</table>
Figure 3: Example levels of frequency power resulting from the Fourier transformation (Vivonoetics, 2012).

There is no standardized way to analyze and display HRV data but most studies focus on normalized HF or LF/HF ratios. Low frequency and very low frequency (0.0033 to 0.04 Hz) HRV are present but are not commonly assessed because their relevance is yet to be confirmed. In some studies LF is attributed to sympathetic activation but substantial evidence to support this is still lacking (Berntson et al., 1997). High Frequency HRV has been widely substantiated as indicating parasympathetic activity. Chess et al. (1975) was the first to show this in a study of thirteen decerebrate cats. They used spectral analysis to determine the effects of different neural input to the heart. Two prominent peaks were seen in what we know of now as the high frequency range and a third in the “respiratory frequency”. The high frequency peak amplitudes were only increased when vagal input was present, and decreased with sympathetic input or no input. Thus, they concluded that parasympathetic activity influences those high frequency peaks whereas sympathetic activity has no effect. Furthermore, Fouad et al. (1984) found that parasympathetic control was correlated with high frequency HRV when beta-adrenergic supply
was pharmacologically blocked. Pomeranz et al. (1985) showed that the high frequency peak is affected by variations in respiration rate, thus linking it to the respiratory sinus arrhythmia.

Kollai and Mizsei (1990) argued that variations in the respiratory sinus arrhythmia do not necessarily correlate with parasympathetic cardiac activation. They used the drug atropine sulphate as a complete parasympathetic block and measured the relationship between change in "heart period" and RSA averaged over five minutes following a fifteen minute resting period. In the group of twenty-nine male subjects aged 18-26 who were breathing normally, correlation was significant but they described it as "not close" because r=.61.

HRV has been used in a variety of contexts. In their review article, Aubert et al. (2003) found that aerobically trained athletes had higher resting HF HRV, also called enhanced vagal tone, as compared to non-athletes. Wu and Lo (2008) found an increase of normalized HF and a decrease in LF/HF following twenty minutes of inward meditation, thus suggesting that parasympathetic influence/relaxation was increased as a result of the meditation. Similarly, Phongsupsup et al. (2008) showed distinct peaks in the HF region during the "Samadhi state" of concentration meditation. This was defined as the state of one-pointed concentration resulting from "successful" meditation. By the HF peaks they concluded that the HRV was synchronized to the respiration, which may improve efficiency of gas exchange in the lungs. They also concluded from the peaks that meditation may be able to increase parasympathetic activity. An interesting study by Libby et al. (2012) showed that acute increases in HF HRV from baseline to meditation were associated with smoking fewer cigarettes following a 4-week smoking cessation intervention. They concluded that the acute changes in HF HRV may be beneficial predictors of smoking cessation treatment responses. It is also important to note that
alterations to respiration may affect HRV. Bernardi et al (2000) concluded that both reading and free-talking decreased variability compared to spontaneous breathing.

In order to analyze the stress response there must be a way to elicit it in a laboratory setting. A great deal of current research supports a method called the Trier Social Stress Test (TSST) for this purpose. This test was developed in 1993 by Kirschbaum et al. at the University of Trier to promote a standard, reliable, quantifiable stress response in humans. The original test consisted of ten minutes of rest, ten minutes of preparation for a mock personal interview, five minutes of interview, and five minutes of continuously subtracting 13 from 1,022 as fast and accurately as possible. The preparation phase is considered the beginning of the stressor. Salivary cortisol levels were taken every ten minutes in six independent studies, and peak levels were seen in every study 10-20 minutes after the beginning of the stressor. This makes sense because cortisol has a latency period for dissolving in saliva. The TSST was deemed effective in eliciting stress response based on these results. This has been confirmed in numerous studies since, including one by Kudielka et al. (2008) which showed a twofold rise in salivary cortisol for 70% of subjects. Since its development, hundreds of studies have used the test and many modifications have resulted such as the TSST-C for children, a TSST for retired subjects, and a TSST for psychiatric patients (Foley, 2010).

With the rise in stress and its detrimental effects, people are searching for ways to alleviate it. Modern research has started to focus on an age-old practice that has been effective for thousands of years: meditation. The word is derived from Latin “meditari,” meaning to contemplate or reflect. Over 10 million people in the United States practice some form of meditation regularly, with that number reaching hundreds of millions throughout the world.
Traditionally, meditation has been cited as a spiritual, belief-based practice, but in recent years the benefits on mental and physical health have come to light and therapeutic applications have arisen. Cardoso et al. (2004) developed an operational definition encompassing both therapeutic and spiritual aspects. They claimed that a meditation practice usually has five specific characteristics: clearly defined technique, muscle relaxation, logic relaxation, self-induced state, and self-focus or anchor. Mancoha (2000) described meditation as a "thoughtless awareness."

There are many different kinds of meditation. In their meta-analysis Ospina et al. (2007) described that the differences arise in primary goal of practice, direction of attention, the anchor, and posture. They also found that out of 813 studies regarding meditation from 1956 to 2005, half were published after 1994 and the most common form was mantra based practice such as transcendental meditation (TM). This form involves using some sort of chanted sound, word, or phrase as the object of concentration anchor. The mantra may be recited aloud or silently, and in transcendental meditation it is a meaningless sound that comes from the ancient Vedic tradition of India. The meditative state is said to be achieved with the repetition of the mantra is no longer a conscious action, and the mind is "without thought" (Delmonte, 1980).

Mindfulness based meditation is also very common. It involves being fully aware of the present moment and usually puts emphasis on acceptance, nonjudgment, and openness. This practice aims to broaden perspective and self-understanding, encouraging reflection and non-impulsive response to situations (Bishop et al., 2004). This particular form of meditation has been widely studied and used due to the work of Kabat-Zinn (1982). He first developed a
program of mindfulness training for behavioral medicine application and in 51 subjects saw significant decrease in chronic pain and mood disturbances. In a larger clinical trial of 90 subjects, Kabat-Zinn et al. (1985) also found significant decreases in chronic pain, anxiety, depression, and mood disturbance following the program. Follow up surveys confirmed that the benefits were sustained up to fifteen months following the program. This training, now called mindfulness-based stress reduction (MBSR), involves an 8-10 week process with two-hour meetings centering on instruction and practice of mindfulness. The meetings also involve discussion of stress, coping, and similar topics. Participants are taught focused awareness, present attention, yoga, and nonjudgmental observation of thoughts. MBSR has now been effectively implemented in many occupational and clinical settings across the United States (Baer, 2003).

The efficacy of meditation-based courses on improving stress response in a college population is yet understudied. This group is of particular interest because as a whole they are very susceptible to high stress levels and also are generally more open to new experiences. Oman et al. (2008) found that perceived stress and rumination were significantly decreased in fifteen undergraduates following an eight-week MBSR program. Shapiro et al. (1998) also saw significantly decreased state and trait anxiety, depression, and increased empathy in a group of 35 premedical students and 38 medical students after an eight-week mindfulness-based program. Furthermore, Hall (1999) found improved academic performance in fifty-six undergraduates at Hampton University as a result of semester-long meditation instruction. The meditation group participated in weekly, hour-long study sessions which began and concluded with ten minutes of meditation practice. This group had significantly higher semester and cumulative grade point averages (GPA) than the control group. Nidich et al. (2009) also found
decreased levels of anxiety, depression, and anger in a group of 298 university students who participated in a Transcendental Meditation (TM) program.

Most of the current research on the effects of meditation in a college population is lacking because of flaws in study design and inconsistent data. Thus, the overall goal of our study is to determine if a semester-long compassion seminar will affect stress response, anxiety, academic performance, and overall well-being in a population of college students. We will use a modified TSST to evoke a stress response both pre- and post-compassion seminar. The magnitude of this response will be assessed by measuring the physiological parameters of cortisol, blood pressure, GSR, and HRV. Ultimately we hope to see a reduced stress response post-compassion seminar, but for now I will be analyzing the efficacy of our TSST stressors: verbal and math questioning. In particular, I hypothesize that the HRV data will show less HF power (less parasympathetic activation) during both stressor intervals as compared to baseline, thus confirming an elicited stress response.
Methods

Pilot workshop study

A three-day pilot study was conducted in order to test equipment and protocols with a small group of students. This included a one-day meditation workshop and two days (pre- and post-workshop) of physiological and psychological assessment.

Recruitment: University of Redlands students aged 18-22 were recruited with flyers (Appendix I) posted in visible areas throughout all of campus. The flyers instructed students to contact Dr. Celine Ko if they were interested in participating. The pre-screening process was initiated when they set up a meeting with her. In this meeting, Dr. Ko explained the informed consent (Appendix II) process and screened for any of the exclusion criteria: pregnancy, regular anxiolytic medications, or frequent cigarette smoking. Ten students were able to participate and were scheduled for assessments on the Friday and Sunday (pre- and post-workshop) of the study weekend. The timing was the same for both days in order to control for potential differences in circadian rhythm. Other confounding variables were controlled for as well: participants were asked to consume caffeine as they normally would, wake up at their normal time, not eat or drink anything but water an hour before testing, and not become intoxicated the night before testing.

Assessment: The protocol for both pre- and post-workshop testing was identical. Upon arrival, participants completed written psychological assessments (Hernandez, 2013) for thirty minutes and were then led into the physiological assessment room. After removing all metal
jewelry and nail polish that might interfere with the equipment, the following physiological assessment equipment was placed on the participants: a Nonin WristOx2™ Model 3150 Pulse Oximeter (Nonin Medical, Inc., Plymouth, MN), an Omron 10 Series™ Blood Pressure monitor (Omron Healthcare, Inc., Lake Forest, IL), GSR electrodes, and respiration monitor (iWorx Systems, Inc., Dover, NH).

The Nonin Pulse Oximeter was used to measure pulse and heart rate variability. It had to be synced to the Nonin OEM Software (Nonin Medical, Inc., Plymouth, MN) via Bluetooth prior to testing as per protocol in Appendix III. The right index finger of the participant was inserted into the sensor for the duration of testing and he or she was asked to keep hands as still as possible to avoid interference. At the end of testing, the file was saved into a private, password-protected folder as per protocol in Appendix III.

The Omron blood pressure monitor was used to store blood pressure readings four times throughout the testing. The cuff was wrapped around the participant’s left arm and “User A” was selected on the machine. Prior to testing, the monitor was checked for the correct time, date, and mode. Blood pressure data was uploaded to Microsoft HealthVault (Microsoft, Redmond, WA) at the end of the testing day. The data was then transferred into an Excel file and coded.

The GSR electrodes were placed on the participant’s right index and ring fingers and the respiration monitor was wrapped around the chest just below the sternum. Prior to the start of testing, the GSR electrodes and respiration monitor were synced with iWorx and Labscribe (iWorx Systems, Inc., Dover, NH) software with specialized settings to display GSR and
respiration channel. The software and equipment protocols were followed as per manufacturer instructions and our written protocol (Appendix III).

In order to assess physiological stress response, a modified version of the Trier Social Stress Test (Kirschbaum, 1993) was used. Our version replaced the speech portion with a five-minute verbal questioning section. During this time participants were asked analogies from previous GRE exams (Study Mode, Inc., 2012). They were orally presented with two associated words and asked to choose from a list of four pairs of words that demonstrated a similar association (i.e.: Mason is to stone as...A: soldier is to weapon, B: lawyer is to law, C: blacksmith is to forge, or D: carpenter is to wood). The arithmetic section was kept the same as the original TSST; participants were asked to serially subtract a two-digit number from a four digit number as fast and accurately as possible (i.e.: subtract 13 from 1,022).

In order to carry out the modified TSST and improve standardization between researchers we developed and followed a script (Appendix IV). Figure 4 shows the succession of events in the script. After the equipment was initially placed on the participant they underwent a five-minute period to establish baseline while listening to Seaside-Ocean Sounds from a Golden Sand Beach by Calmsound–Nature Sounds from iTunes. Following this period researchers left the room and asked the participant to sit quietly for ten minutes. This was done in order to account for the latency period of cortisol diffusion into saliva, which was sampled at the end of the ten minutes with a swab as per manufacturer protocol. A five minute period of neutral questions was then started (i.e.: What is your favorite color?). The five-minute verbal questioning section followed as indicated above, and the five minute arithmetic section followed that. The timing of all of the questions, sections, and blood pressure measurements
were marked with the iWorx software in order to synchronize later in analysis. Blood pressure was taken four times: at the end of the five-minute baseline, the end of neutral questions, and the end of both verbal and arithmetic sections. The button to start measurement was pressed approximately twenty seconds before the end of each aforementioned period because the cuff takes about that long to inflate and deflate enough for measurement. Salivary cortisol was measured twice: after the ten-minute resting period, as mentioned, and also ten minutes following the end of all testing.

![Timeline of sections and measurements taken](image)

Figure 4. Timeline of sections and measurements taken. GSR and pulse oximetry data were also collected continuously throughout the testing session.

**Analysis:** HRV data was imported into and analyzed using VivoSense (Vivonoetics, San Diego, CA) software as per written protocol (Appendix V). Baseline, neutral questions, verbal, and arithmetic section data were annotated in sync with iWorx LabScribe 2 (iWorx Systems, Inc., Dover, NH) markings made during testing. The PPG data was transformed into power spectral frequencies with VivoSense as is a commonly accepted technique in the literature (Aubert et al., 2003). Artifacts due to equipment interference, irregular heartbeats, and movement were removed using the automatic artifact removal function of the software.
Average High frequency (HF), Low Frequency (LF), and LF/HF data for each section was produced and exported into a compiled excel file with all coded subjects. Normalized HF and LF were also produced and exported. Normalized HF was of particular interest because it has been widely accepted to indicate parasympathetic activation (Aubert et al., 2003; Berntson, 1997). Normalized HF was compared using paired T-Tests for baseline versus verbal and arithmetic sections. ANOVA tests would have been ideal for this data but could not be accomplished with unequal sample sizes in Excel. All data will be sent to a professional statistician for more complete analysis. Two exclusions were made from pre-workshop HRV data and one from post-workshop data because of too many artifacts. These may have been caused by interference with equipment, irregular heartbeats, or movement. GSR, cortisol, blood pressure, and pulse data were also analyzed separately (Heuerman, 2013; Ov, 2013; Overton-Harris, 2013; Huerta, 2013).

_Meditation Workshop:_ The one-day (8-hour) meditation workshop was led by professor of religious studies, Dr. Fran Grace, and included introduction to various meditation practices including mindfulness, compassion, loving-kindness, and deep relaxation.

**Compassion Seminar Study**

This portion of the project aimed to assess the effects of an 8-week compassion seminar class on physiological stress response in a college population.

_Recruitment:_ The same procedure from the pilot study was followed for recruitment. Flyers (Appendix I) were posted all over campus and interested students were instructed to contact Dr. Ko. The pre-screening process was the same with an additional exclusion: participants could not have extensive previous meditation experience. Participants were
randomly assigned into a control group or enrolled in the Seminar on Compassion Religion with Dr. Fran Grace. The control group registered for another class of their choice. All participants scheduled times for pre-compassion seminar psychological and physiological assessment as before.

Assessment: The same script was followed from the pilot study with the addition of two more salivary cortisol measurements at t=0 and t=50. A different math problem and different GRE questions in the same format were used.

Analysis: The analysis protocol remained unchanged from the pilot study: the data was imported into VivoSense and was transformed and analyzed as per Appendix V, with normalized HF HRV again of particular interest. Exclusions from analysis included four files: in two the HRV data ended prematurely, one had no HRV data at all due to experimenter error, and one had too many artifacts. GSR, cortisol, BP, and pulse were again analyzed separately (Heuerman, 2013; Ov, 2013; Overton-Harris, 2013; Huerta, 2013).

Compassion seminar: The 8-week compassion course centered on aiding students to live a life of compassion. The course included teachings from world religions and peaceful historical figures such as Gandhi and Mother Teresa. Contemplative meditation practices were also incorporated.
Results

Compassion Workshop

The first phase of the study aimed to assess the impact of a one-day meditation workshop on physiological stress response in a college population. Testing was conducted the day preceding and the day following the workshop (pre- and post-workshop testing days) to measure participants' physiological responses to the modified TSST. The efficacy of the modified TSST in eliciting a stress response was measured based on parasympathetic activation during stressor and baseline intervals as indicated by normalized high frequency heart rate variability.

The subjects' demographic data is displayed in table 2 (Heuerman, 2013). High frequency, low frequency, the ratio of LF/HF and normalized high frequency HRV power data from the workshop testing days are displayed in tables 3 and 4. Normalized high frequency HRV power data is graphed in figures 6-8. Significance is defined as p<0.05 resulting from paired t-tests. No significant differences between baseline and neutral questions were seen in any of the data, thus indicating that subjects talking during the assessment did not confound the data.
Table 2 Demographic data from the meditation workshop (n=8) (Heuerman, 2013).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Birth Control Usage</th>
<th>Caffeine Consumption (Days/Week)</th>
<th>8 oz. Servings of Caffeine/Day</th>
<th>Typical Wake Time (am in hours)</th>
<th>Wake Time Day of Testing (am in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M = 1</td>
<td>19±1.5</td>
<td>M = N/A</td>
<td>3.0±1.9</td>
<td>1.7±2.3</td>
<td>8:38±0:57</td>
<td>8:51±1:09</td>
</tr>
<tr>
<td>F = 7</td>
<td></td>
<td>F = 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Average Normalized HF, HF, LF, and LF/HF HRV power data from pre-meditation workshop testing day

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Neutral Questions</th>
<th>Verbal</th>
<th>Math</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized HF</td>
<td>0.47988</td>
<td>0.42383</td>
<td>0.40632</td>
<td>0.38150</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>1440.7</td>
<td>1701.6</td>
<td>2245.9</td>
<td>3123.2</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>2101.1</td>
<td>1897.8</td>
<td>2383.0</td>
<td>4130.6</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.3402</td>
<td>1.3163</td>
<td>1.0942</td>
<td>1.4163</td>
</tr>
</tbody>
</table>

Table 4 Average Normalized HF, HF, LF, and LF/HF HRV power data from post-meditation workshop testing day

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Neutral Questions</th>
<th>Verbal</th>
<th>Math</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized HF</td>
<td>0.53908</td>
<td>0.40084</td>
<td>0.40109</td>
<td>0.33657</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>872.60</td>
<td>955.52</td>
<td>1823.7</td>
<td>1433.5</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>673.19</td>
<td>1420.67</td>
<td>1812.1</td>
<td>2607.5</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.0253</td>
<td>1.5091</td>
<td>1.2148</td>
<td>1.8665</td>
</tr>
</tbody>
</table>
No significant difference (p=0.285, p=0.192) existed between baseline and verbal/math stressor intervals on the pre-workshop testing day (figure 5); this indicates that a stress response was not elicited.

Figure 5 Parasympathetic activation from pre-workshop testing across baseline, neutral, and stressor intervals as indicated by normalized HF HRV (n=6). p>0.05 for all comparisons and error bars indicate standard deviation.

Post-meditation workshop data showed a significant decrease (p=0.026) in Normalized HF HRV from baseline to math stressor interval (figure 6), indicating that this portion of our modified TSST did work to elicit a stress response because parasympathetic activity decreased.
Figure 6 Parasympathetic activation from post-workshop testing across baseline, neutral, and stressor intervals as indicated by Normalized HF HRV (n=5). Different letters indicate a significant difference (p<.05) and error bars indicate standard deviation.

Baselines from both workshop testing days were not significantly different (p=0.332) as shown in figure 7. This means that the meditation workshop did not increase trait parasympathetic activity and as such did not decrease trait stress.
Figure 7  Comparison of pre- and post-workshop baseline parasympathetic activity as indicated by Normalized HF HRV (n=5). Error bars indicate standard deviation.

Pre-Compassion Seminar Data

The second phase of the study aimed to assess the impact of a semester-long compassion seminar on physiological stress response in a college population. Pre-compassion seminar data was taken in December, 2012. The post-seminar data will be collected in April, 2013. The efficacy of the modified TSST in eliciting a stress response was measured based on parasympathetic activation during stressor and baseline intervals as indicated by normalized high frequency heart rate variability.

The subjects’ demographic data is displayed in table 5 (Heuerman, 2013). High frequency, low frequency, the ratio of LF/HF and normalized high frequency HRV power data from the pre-compassion seminar testing are displayed in table 6.
Table 5 Pre-compassion seminar demographic data from control (n=10) and experimental (n=14) groups (Heuerman, 2013).

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Age (years)</th>
<th>Birth Control Usage</th>
<th>Caffeine Consumption (Days/Week)</th>
<th>8 oz. Servings of Caffeine/Day</th>
<th>Typical Wake Time (am in hours)</th>
<th>Wake Time Day of Testing (am in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>M = 3</td>
<td>19±1</td>
<td>M = N/A</td>
<td>1.8±1.5</td>
<td>2.1±2.9</td>
<td>8:30±0:04</td>
<td>8:53±0:04</td>
</tr>
<tr>
<td>Experiment al Group</td>
<td>M = 6</td>
<td>20±1</td>
<td>M = N/A</td>
<td>4.4±2.7</td>
<td>4.5±5.6</td>
<td>8:01±0:02</td>
<td>8.13±0:04</td>
</tr>
<tr>
<td>F = 7</td>
<td>F = 4</td>
<td>F = 4</td>
<td>F = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6 Average Normalized HF, HF, LF, and LF/HF HRV power data from pre-compassion seminar testing

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Neutral Questions</th>
<th>Verbal</th>
<th>Math</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized HF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>0.50470</td>
<td>0.42730</td>
<td>0.35396</td>
<td>0.39602</td>
</tr>
<tr>
<td>HF(ms²)</td>
<td>1887.32</td>
<td>2635.23</td>
<td>1161.32</td>
<td>3004.82</td>
</tr>
<tr>
<td>LF</td>
<td>2181.42</td>
<td>2552.09</td>
<td>1715.85</td>
<td>3401.71</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.5102</td>
<td>1.5409</td>
<td>2.0262</td>
<td>1.4568</td>
</tr>
</tbody>
</table>

Pre-compassion seminar normalized HF HRV data (Figure 8) shows that the verbal stressor worked to decrease parasympathetic activation and elicit stress response (p=0.014). There was no significant difference between baseline and math stressor interval (p=0.121) normalized high frequency HRV.
Discussion

The results provided evidence to support that our modified Trier Social Stress Test (TSST) partially worked to elicit a stress response. The pre-meditation workshop data showed no significant differences between baseline and the stress intervals' normalized high frequency heart rate variability, which means that the level of parasympathetic activity was not significantly altered by either of the stressors. However, post-meditation workshop data showed that normalized high frequency heart rate variability was significantly less (p=.026) in the math questioning interval than baseline, indicating that parasympathetic activity was significantly lower than baseline during this stressor period and as such the subjects were more stressed on average as compared to baseline. Pre-compassion seminar data showed a significant decrease (p=.014) in normalized high frequency heart rate variability between baseline and the verbal questioning interval, whereas no significant difference was seen from
baseline to math questioning interval. Workshop data appears to indicate that our math 
stressor worked and pre-compassion seminar data seems to indicate that our verbal stressor 
worked, however these findings were not consistent between the two studies.

Although our version of the TSST was only partially effective, a great deal of literature 
exists substantiating the efficacy of the original protocol and other modified forms. Most 
studies are focused primarily on cortisol measurements. The original protocol was developed 
by Kirschbaum et al. (1993) and consistently showed significant increases from baseline in 
salivary cortisol levels during speech and arithmetic periods in six independent studies. Kudielka 
et al (2003) further substantiated the efficacy of the TSST, finding significant increases in 
salivary cortisol during the stressors for 180 adult subjects. Gaab (2003) also showed 
significantly increased salivary cortisol during stressors for a sample of forty-eight healthy male 
students. All of these studies use the original TSST protocol that includes an anticipation period, 
speech, and arithmetic components. Our protocol varied with the speech stressor; instead of 
having subjects prepare and give a speech we tested them on GRE analogies. This eliminates 
the anticipation period which may have contributed to the stress response during subsequent 
stressor intervals, and thus we may want to include some sort of anticipation period in the 
future.

Many different modifications have been made to the TSST in order to make the 
stressors more relevant to the sample population. For example, Jones et al. (2008) used the 
TSST-C when assessing a sample of 140 healthy children, ages 7-9. The subjects told a story for 
the verbal stressor instead of an interview-like speech, and also performed much simpler 
arithmetic calculations. They were given five minutes of rest consisting of calm-video watching
preceding and following the stressors, and also were offered toys as a potential reward for high performance. This modified TSST seems to have been effective in eliciting a stress response, but heart rate and blood pressure were the only physiological measurements used to assess this. Without data on cortisol it is difficult to fully substantiate its efficacy. Hipwell et al. (2009) also found increased HRV in forty-eight girls during the TSST-C. This HRV was indicated by mean RMMSD, an easier method of HRV analysis which will be discussed later. These studies show that modified TSSTs can work to elicit stress response as indicated by HRV data. As such, we must continue to modify our modified TSST slightly in order to make it more effective.

However, other researchers have also faced challenges with ineffective TSSTs, both in original and modified forms. For example, in a master’s thesis at Texas Tech University, Cisneros (2010) found no significant difference in heart rate variability data from baseline during verbal and math intervals. His protocol for the verbal section differed from ours, though, and closely mimicked the original TSST. In his test the subjects had ten minutes to prepare a five-minute speech regarding their qualifications for a hypothetical job in front of a group of “managers.” The subjects were also told that they were being video-taped and analyzed on both non-verbal and verbal behavior. These events seem as though they should evoke a marked stress response, but no significant differences were seen in heart rate, cortisol, or heart rate variability. His study was also very similar to ours in that the overall goal was to assess the impact of an 8-week Mindfulness-Based Exercise Program (MBEP) on stress response. He concluded that his heart rate variability data may be unreliable, though, because errors with the collection equipment led to nine participants’ heart rate variability data during post-testing. He also attributed some of the problems to the small, homogeneous sample; because only eighteen female participants aged 23-31 were tested. This is similar to our study which originally
consisted of twenty-five participants but only seventeen had complete HRV data due to
equipment corruption and experimenter error. His suggestions for TSST improvement mostly
centered on the investigator-subject interactions: limiting social contact between post-test
investigators and subjects during the TSST, a more “strict and commanding authority” from
post-test investigators, and assuring no prior familiarity between subject and “managers”
judging the mock interview speech.

Our investigators upheld professional behavior but may have lacked a commanding,
serious presence. A more stressful environment for our modified TSST could be implemented if
our investigators adapt stricter attitudes in their interactions with participants. This may induce
stress in the subjects, potentially causing them to take the tasks more seriously. Also, an
inclusion of an anticipation period in our TSST might be beneficial to add on to stress by
fluster them before the actual stressor. This would be difficult to add before our verbal
questioning section, but we can tell them that they're about to do something very stressful a
few minutes beforehand and let them sit and anticipate. In addition to this period, the only
other change we made to the TSST was to use GRE questions instead of asking them to prepare
a speech; this was done in the interest of time and to be more relevant to a college student
population. Before changing the stressor protocol back to the original TSST, it is worth
attempting to change the environment and attitudes to reflect a more stressful situation.

It may be necessary to revert back to the original TSST, however. Some researchers
found that modified versions do not work as well. Wallergård et al (2011) did not find a
significant difference in heart rate variability data in a pilot study assessing the efficacy of a
virtual version of the TSST on seven participants. The virtual reality version of the TSST
consisted of three rear projected walls and a floor projection. The subjects had to give their interview and do the arithmetic section in front of a virtual audience consisting of two men and a woman. Jönsson et al (2010) found mixed results in a study of ten men assessing the virtual TSST. High frequency HRV data was not significantly different during the stressors of this modified TSST. However, they used a method of analysis called T-wave amplitude to assess sympathetic activity and found significant increases during the stressor intervals. Cortisol and heart rate data also showed an elicited response during the first session of testing, but this response was not seen the second time participants were exposed to the virtual TSST. Both of these studies attributed the lack of supportive results to the small sample sizes, but perhaps the problem was the modification of the TSST.

While some minor discrepancy exists regarding the true significance of the spectrum frequency ranges of heart rate variability, we used the method of HF HRV because it seems to be most widely accepted as indicating parasympathetic activation in the current literature. For instance, Aubert et al. (2003) showed in their review article that 64% percent of the studies surveyed used an increase in HF HRV to signify increased parasympathetic activity when comparing athletes to a sedentary population. Some did use LF HRV to demonstrate sympathetic activity, but as a whole this method seems to be ill-substantiated. A mix of parasympathetic and sympathetic activation is the most common explanation for LF HRV.

Cisneros' (2010) study differs from ours in the method of heart rate variability analysis. Our study uses normalized high frequency heart rate variability power to signify parasympathetic activity, but he used another method involving standard deviation and root mean squared successive differences (SD/rMSSD) of R-R intervals that has also been shown
effective and possibly easier to calculate. Soller et al. (2007) compared the ratio of low
frequency HRV/ high frequency HRV (LF/HF) to (SD/rMSSD) and found that the latter was a
"good and reliable surrogate" for the LF/HF calculation in the sample of seven healthy controls
and twenty-two patients with pre-frontal damage. This may provide another means of HRV
analysis for our study in the future.

Precise explanation as to why our TSST only partially worked is difficult to pinpoint
because a variety of factors may have contributed. First, it is imperative to consider the
possibility of errors regarding the data, collection and analysis, and sample size. Our workshop
sample size was low to begin with but was further decreased because some participants did not
show up for their post-meditation workshop testing appointments. We only had six subjects for
pre-workshop testing and five for post-workshop testing. The original sample sizes were eight
and six on pre- and post-mediation workshop testing days respectively. Two exclusions had to
be made the pre-meditation workshop data and one exclusion from post-meditation workshop
data. These exclusions were due to the presence of many artifacts in the data. These artifacts
may have been as a result of irregular heartbeats, movement, or interference with the Nonin
Pulse Oximter equipment or Nonin OEM software.

Four subjects from the pre-compassion seminar data collection do not have HRV data
to analyze: two show data that ends abruptly and cannot be analyzed which is most likely due
to a failure in the equipment or software, the third has none at all due to the experimenter not
saving the file correctly, and the fourth had too many artifacts to analyze. An additional two
math stressor data files were excluded and another verbal file because of too many artifacts.
This left us with a sample size of seventeen from our original twenty four for pre-compassion
seminar data. In the future we must work to limit movement and interference with equipment. The small samples make it difficult to infer statistical significance, and we hope to expand the size in the future as we run this project again and accumulate more data.

The project should be run again with an increased sample size and a more stressful environment in order for our modified TSST to work as an effective means to elicit the stress response. The post-compassion seminar data from April 2013 should help to confirm the hypothesis that our modified TSST is effective in eliciting a stress response as indicated by decreased parasympathetic activation during stressor intervals. The post-compassion data analysis work will be left for a future researcher. When our TSST is verified to fully work we can analyze the effects of the compassion meditation course on stress response. Once we gauge if the compassion seminar is effective in improving psychophysiological stress response in a college population we will be able to collaborate with campuses across the country and possibly the world. The body of research in this field will grow so that many college students will experience the positive benefits of compassion and meditation, especially pertaining to stress.
References


Participate in a research study examining the effects of meditation on health and academic performance

Does learning about compassion and meditation affect your view of yourself and the world? Does it help regulate stress? Does it improve academic performance?

We are conducting a research study measuring the psychological and physiological impact of RFIL 250 on stress, well-being, and academic success. RFIL 250, Seminar on Compassion, is a course on exploring what it means to live a life of compassion through the lenses of spiritual teachers and leaders from varying traditions.

If you choose to participate in the study, you will need to commit about 2 hours to complete questionnaires and laboratory tests during the December 2012 and April 2013 finals weeks. You also must be willing to disclose your GPA. You will be compensated $50.00 for completing the study.

For more information, contact: celine_ko@redlands.edu

If you are interested in taking RFIL 250 but not participate in the study, please contact Fran Grace at fran_grace@redlands.edu

Principal Investigators: Lisa Olson, Celine Ko, Fran Grace

IRB Approval # 2012-42-REDLANDS

Mahatma Ghandi  The Dalai Lama  Mother Teresa  Nelson Mandela
Are you interested in learning about meditation and compassion?

We are looking for participants in a research study examining the effects of meditation on health.

Dr. Fran Grace, Professor of Religious Studies and Steward of the Meditation Room is offering:

Meditation and compassion retreat
Saturday, November 3, 2012
9am-5pm

By working with several different meditation practices, you will learn how to cultivate a compassionate presence to yourself and others.

No prior experience necessary.

Research testing (questionnaires and laboratory tests):
2 hours Friday afternoon, Nov. 2 and
2 hours Sunday afternoon, Nov. 4

A $25 stipend will be provided.

Principal Investigators: Lisa Olson, Celine Ko, Fran Grace
For more information, contact: celine_ko@redlands.edu
Appendix II: Informed consent forms

Consent to Participate in a Research Study
(For use with adult subjects only)

What follows is a consent form that explains what will be happening if you choose to participate in this research study. The first section (Investigator Information) should have been completed by the investigator. If this section is incomplete, do not continue with the study. Do not participate if this study has not been assigned an IRB approval number. The information you need to provide begins on Page 2. Please read each section carefully.

Investigator Information (to be completed by Principal Investigator)

IRB approval number: 2012-39-REDLANDS

Title of project: The impact of meditation curriculum on physiological and psychosocial stress, well-being, and correlates of academic success (Phase 2)

Name of principal investigator (PI): Dr. Lisa Olson, Dr. Celine Ko, and Dr. Fran Grace

Email of PI: Lisa_olson@redlands.edu, celine_ko@redlands.edu, fran_grace@redlands.edu

Telephone number of PI: 909-748-8524, 909-748-8669, 909-748-8681

Department or major of PI: Biology, Psychology, and Religious Studies

Position held by PI:
[ ] full-time faculty
[ ] part-time faculty
If PI is a student or staff, complete the remainder of Investigator Information, otherwise go to next page.

Name of faculty or administrator sponsor:

Email of sponsor:

Telephone number of sponsor:

Department or office of sponsor:

Position held by sponsor:

[ ] fulltime faculty
[ ] part-time faculty
[ ] visiting faculty
[ ] adjunct faculty
[ ] administrator
General information about research studies

You are being asked to participate in a research study. Whether you do is entirely up to you. You may refuse to participate, or you may stop participating at any time for any reason without any penalty.

Research studies are designed to gather new information. This new information might help someone in the future. You might not receive any obvious or direct benefit by participating in this study. In fact, there might be risks to being in a research study. If there are, this information and other information about this study are described below so that you can decide whether you want to participate in the study.

You will be given a copy of this consent form. You should ask the investigator(s) named above, or staff members who assist them, any questions you have about this study at any time.

Purpose of this study

The purpose of this study is to test whether meditation courses impact student physiological and psychological well-being, response to stress, and academic performance.

You are being asked to participate in this study because we want to test whether the physiological and psychological measurements we hope to use with students in a future meditation course will work appropriately in a student sample attending a weekend retreat.

Reasons why you should not participate in this study

You should not participate in this study if:
- you are under 18 or over 24 years old
- you have severe mental health issues (such active psychosis)
- you do not read written English well
- you are pregnant
- you take anti-anxiety medication
- you use nicotine (smoking, chewing tobacco, etc.)

Number of people participating in this study
If you decide to participate in this study, you will be one of approximately 10 people who will participate in this study.

**How long this will take (i.e., duration of participation)**

If you choose to participate in this study, your involvement will take about 2 hours on Friday, November 2nd, 8 hours on Saturday, November 3rd, and 2 hours on Sunday, November 4th.

**What will happen if you participate in this study**

If you participate in this study, you will undergo physiological testing on the day before the meditation retreat, and then it will be repeated on the day after the retreat. You will be asked to provide the date of your last menstrual period (if applicable), whether you are taking any hormonal contraceptives, what your pattern of caffeine use is, and what time you woke up that morning. You will provide a saliva sample by placing a cotton swab underneath your tongue for 1 - 2 minutes. You will be asked to sit still while physiological equipment is connected, including several sensors wrapped around your fingers, a wrist-watch type monitor, and a blood pressure cuff on your arm. You will be asked to sit quietly while initial measurements are taken. Then, you will be asked to verbally answer a series of questions, including some academic quiz questions (such as math problems, vocabulary, or grammar questions). The data collected will include blood pressure, heart rate, the amount of oxygen in your body, and your stress response in microscopic sweat production and the secretion of stress hormone in your saliva.

You will also be asked to complete a packet of questionnaires that ask about anxiety, depression, stress, affective states, well-being, personal growth, happiness, compassion, self-compassion, attention and awareness, optimism, gratitude, empathy, and spirituality. You will also be asked about your meditation practice, if applicable.

You will then attend the meditation retreat on Saturday, November 3rd. During the retreat, you will learn about compassion and engage in several different meditation practices. You will then repeat the physiological and psychological testing on Sunday, November 4th.

**Possible benefits of participating in this study**

As mentioned above, research studies are designed to gather new information. This new information might benefit someone in the future. You might also benefit by participating in this study by learning about compassion and meditation techniques that you can use in your daily life.

**Possible risks or discomforts related to participating in this study**

The physiological risks of this study are very minimal. All of the physiological monitoring is external except for providing a saliva sample. You may have discomfort from being asked to sit still or from the pressure of a blood pressure cuff inflating on your arm.
You will be asked questions related to your stress, moods, personal attributes, and behaviors. There is a risk that completing these questionnaires will make you uncomfortable as you examine your attributes and disclose this information. Sometimes, individuals who complete these questionnaires become more aware of possible mental health issues such as depression or anxiety. You may also feel uncomfortable as you engage in the meditation practices. Steps will be taken to ensure confidentiality of your personal information; however, there is also a risk of breach of that confidentiality.

It is possible that there are unknown risks or discomforts. Please report any problems immediately to the researcher.

Videotaping
You will not be videotaped.

Audiotaping
You will not be audiotaped.

Protecting your privacy
Any written private information you will disclose during this study will be kept in a locked file in a private, locked office. Electronic data will be kept in a password-protected file in a password-protected computer. Any personal identifiers will not be stored with the research data.

One of the following researchers may take your physiological measurements and will therefore be aware of the results: Dr. Lisa Olson, Anne Heuerman, Matthew Green, Petter Overton-Harris, Misael Huerta, or Jenny Ov. If you would prefer that a particular individual listed above NOT take your physiological measurements, you may request a different researcher.

People who participate in this study will not be identified in any report or publication about this study. Although every effort will be made to keep the research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is unlikely to happen, but if disclosure is required, the investigator will take whatever steps are allowable by law to protect the privacy of your personal information. In some cases, your information in this research study could be reviewed by representatives of the University of Redlands, research sponsors, or government agencies for purposes such as quality control or safety.
What will happen if you experience any problems or discomforts during or after your participation

Anything you do, including participating in research, carries with it some chance that something problematic or unwanted may happen. This may include risk of personal injury. Despite all of the precautions, you might experience an unwanted reaction or injury related to participating in this study. Although the researcher may direct you to medical, psychological, or other services, any costs related to such problems are your or your insurance company's responsibility. However, by signing this consent form, you are not giving up any of your legal rights.

You may contact the Counseling Center at 909-748-8108 if you are experiencing personal distress or want further evaluation and intervention for any mental health issues that may have been brought to your attentions as a result of participation in this study.

Compensation for participating in this study

If you complete the testing prior to the retreat, you will receive $10. If you attend the retreat and complete the testing the day after the retreat, you will receive an additional $15 ($25 total).

Costs of participating in this study

With the possible exception of any time off from work you choose to take and transportation costs, there are no obvious costs for participating in this study.

Questions about this study

You may ask and have answered any question about the research. If you have questions or concerns or are interested in knowing the results of the study, please contact the Principal Investigators. Their contact information is listed on page 1 of this consent form.

Questions or concerns about the investigators, staff members, and your participation in the study

This study was approved by the University of Redlands Institutional Review Board (IRB). This board tries to ensure that your rights and welfare are protected if you choose to participate in the study. If you have any questions about your role or how you were treated by the research personnel, you may contact the Chair of the IRB at Francisco_silva@redlands.edu or by telephone at 909-748-8673.

Participant's Agreement
I, ___________________________.

Print Name Above

have read the information presented above. I have asked all questions I had at this time. I voluntarily agree to participate in this research study.

<table>
<thead>
<tr>
<th>Signature of Research Participant</th>
<th>Date</th>
</tr>
</thead>
</table>

To be completed by researcher:

________________________________________

Print Name of Person Obtaining Consent

<table>
<thead>
<tr>
<th>Signature of Person Obtaining Consent</th>
<th>Date</th>
</tr>
</thead>
</table>
Consent to Participate in a Research Study
(For use with adult subjects only)

What follows is a consent form that explains what will be happening if you choose to participate in this research study. The first section (Investigator Information) should have been completed by the investigator. If this section is incomplete, do not continue with the study. Do not participate if this study has not been assigned an IRB approval number. The information you need to provide begins on Page 2. Please read each section carefully.

Investigator Information (to be completed by Principal Investigator)

IRB approval number:

Title of project: The impact of meditation curriculum on physiological and psychosocial stress, well-being, and correlates of academic success (Phase 3)

Name of principal investigator (PI): Dr. Lisa Olson, Dr. Celine Ko, and Dr. Fran Grace

Email of PI: Lisa_olson@redlands.edu, celine_ko@redlands.edu, fran_grace@redlands.edu

Telephone number of PI: 909-748-8524, 909-748-8669, 909-748-8681

Department or major of PI: Biology, Psychology, and Religious Studies

Position held by PI:

[x] full-time faculty
[ ] part-time faculty
[ ] visiting faculty
[ ] adjunct faculty
[ ] administrator
[ ] staff
[ ] student

*If PI is a student or staff, complete the remainder of Investigator Information, otherwise go to next page.*

Name of faculty or administrator sponsor:

Email of sponsor:

Telephone number of sponsor:

Department or office of sponsor:

Position held by sponsor:
[ ] fulltime faculty
[ ] part-time faculty
[ ] visiting faculty
[ ] adjunct faculty
[ ] administrator

---

**General information about research studies**

You are being asked to participate in a research study. Whether you do is entirely up to you. You may refuse to participate, or you may stop participating at any time for any reason without any penalty.

Research studies are designed to gather new information. This new information might help someone in the future. You might not receive any obvious or direct benefit by participating in this study.
In fact, there might be risks to being in a research study. If there are, this information and other information about this study are described below so that you can decide whether you want to participate in the study.

You will be given a copy of this consent form. You should ask the investigator(s) named above, or staff members who assist them, any questions you have about this study at any time.

**Purpose of this study**

The purpose of this study is to test whether meditation courses impact student physiological and psychological well-being, response to stress, and academic performance.

You are being asked to participate in this study because you have expressed interest in taking REL 250 (Seminar on Compassion). You may or may not have been selected to take REL 250. We want to compare students who take the class to those students who did not take the class on their physiological stress response, psychological well-being, and correlates of academic success.

**Reasons why you should not participate in this study**

You should not participate in this study if:
- you are under 18 or over 24 years old
- you have severe mental health issues (such as active psychosis)
- you do not read written English well
- you have previously taken REL 250 or have other substantial experience in meditation
- you are pregnant
- you take anti-anxiety medication
- you use nicotine (smoking, chewing tobacco, etc.)

**Number of people participating in this study**

If you decide to participate in this study, you will be one of approximately 40 people who will participate in this study.

**How long this will take (i.e., duration of participation)**

If you choose to participate in this study, your involvement will take about 2 hours during finals week in December 2012, and 2 hours during finals week in April 2013.
What will happen if you participate in this study

You will undergo physiological and psychological testing in December 2012 and in April 2013. For physiological testing, you will be asked to provide the date of your last menstrual period (if applicable), whether you are taking any hormonal contraceptives, what your pattern of caffeine use is, and what time you woke up that morning. You will provide a saliva sample by placing a cotton swab underneath your tongue for 1-2 minutes. You will be asked to sit still while physiological equipment is connected, including several sensors wrapped around your fingers, a wrist-watch type monitor, and a blood pressure cuff on your arm. You will be asked to sit quietly while initial measurements are taken. Then, you will be asked to verbally answer a series of questions, including some academic quiz questions (such as math problems, vocabulary, or grammar questions). The data collected will include blood pressure, heart rate, the amount of oxygen in your body, and your stress response in microscopic sweat production and the secretion of stress hormone in your saliva.

For psychological testing, you will be asked to complete a packet of questionnaires that ask about anxiety, depression, stress, affective states, well-being, personal growth, happiness, compassion, self-compassion, attention and awareness, optimism, gratitude, empathy, and spirituality. You will also be asked about your academic performance and meditation practice, if any.

If you participate in this study, you are allowing the researchers to collect markers of your academic performance. Researchers will collect your grade point average (GPA) every semester from the start of the study until you graduate.

Possible benefits of participating in this study

As mentioned above, research studies are designed to gather new information. This new information might benefit someone in the future. You might also benefit by participating in this study by learning about compassion and meditation techniques that you can use in your daily life.

Possible risks or discomforts related to participating in this study

The physiological risks of this study are very minimal. All of the physiological monitoring is external except for providing a saliva sample. You may have discomfort from being asked to sit still or from the pressure of a blood pressure cuff inflating on your arm.

You will be asked questions related to your stress, moods, personal attributes, and behaviors. There is a risk that completing these questionnaires will make you uncomfortable as you examine your attributes and disclose this information. Sometimes, individuals who complete these questionnaires become more aware of possible mental health issues such as depression or anxiety.
You may feel uncomfortable completing the psychological questionnaires or the physiological testing because you personally know the faculty or student researcher who is doing the testing. Your responses will not affect your current or future relationship with these researchers. You will have the option of requesting a different researcher for testing if one is available.

There is always a risk of breach of confidentiality. We will be taking steps to ensure that your data is kept confidential. It is possible that there are unknown risks or discomforts. Please report any problems immediately to the researcher.

**Videotaping**

You will not be videotaped.

**Audiotaping**

You will not be audiotaped.

**Protecting your privacy**

Any written private information you will disclose during this study will be kept in a locked file in a private, locked office. Electronic data will be kept in a password-protected file in a password-protected computer. Any personal identifiers will not be stored with the research data. Only members of the research team have access to the data; only the PIs will have the ability to connect your personal information to your data.

One of the following researchers may take your physiological measurements and will therefore be aware of the results: Dr. Lisa Olson, Anne Heuerman, Matthew Green, Petter Overton-Harris, Misael Huerta, or Jenny Ov. If you would prefer that a particular individual listed above NOT take your physiological measurements, you may request a different researcher.

In addition, we are not going to examine your individual responses. We are only interested in examining group data. People who participate in this study will not be identified in any report or publication about this study. Although every effort will be made to keep the research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is unlikely to happen, but if disclosure is required, the investigator will take whatever steps are allowable by law to protect the privacy of your personal information. In some cases, your information in this research study could be reviewed by representatives of the University of Redlands, research sponsors, or government agencies for purposes such as quality control or safety.
What will happen if you experience any problems or discomforts during or after your participation

Anything you do, including participating in research, carries with it some chance that something problematic or unwanted may happen. This may include risk of personal injury. Despite all of the precautions, you might experience an unwanted reaction or injury related to participating in this study. Although the researcher may direct you to medical, psychological, or other services, any costs related to such problems are your or your insurance company's responsibility. However, by signing this consent form, you are not giving up any of your legal rights.

You may contact the Counseling Center at 909-748-8108 if you are experiencing personal distress or want further evaluation and intervention for any mental health issues that may have been brought to your attention as a result of participation in this study.

Compensation for participating in this study

You will be compensated $20 for completing the first set of testing in December 2012. You will be compensated $30 for completing the second set of testing in April 2013 (total of $50).

Costs of participating in this study

With the possible exception of any time off from work you choose to take and transportation costs, there are no obvious costs for participating in this study. If you are assigned to take REL 250, you will be charged your normal tuition rate with your normal financial aid package, if any.

Questions about this study

You may ask and have answered any question about the research. If you have questions or concerns or are interested in knowing the results of the study, please contact the Principal Investigators. Their contact information is listed on page 1 of this consent form.

Questions or concerns about the investigators, staff members, and your participation in the study

This study was approved by the University of Redlands Institutional Review Board (IRB). This board tries to ensure that your rights and welfare are protected if you choose to participate in the study. If you have any questions about your role or how you were treated by the research personnel, you may contact the Chair of the IRB at Francisco_silva@redlands.edu or by telephone at 909-748-8673.

Participant's Agreement
I, ____________________________

Print Name Above

have read the information presented above. I have asked all questions I had at this time. I voluntarily agree to participate in this research study.

<table>
<thead>
<tr>
<th>Signature of Research Participant</th>
<th>Date</th>
</tr>
</thead>
</table>

To be completed by researcher:

__________________________________________

Print Name of Person Obtaining Consent

<table>
<thead>
<tr>
<th>Signature of Person Obtaining Consent</th>
<th>Date</th>
</tr>
</thead>
</table>
Instructions for Research Subjects

Please arrive for psychological/physiological testing in Hedco 102 promptly at your appointment time. Testing will take approximately 2 hours. Please follow the below instructions:

- Do not drink alcohol or use any tobacco in the 24 hours prior to your appointment.

- You need to wake up at least six hours prior to your appointment. Please make note of the time you awaken. Do not nap prior to testing.

- Drink caffeinated beverages according to your normal pattern of caffeine intake (for example, if you normally drink coffee, please do so. If you normally do not, please do not.)

- Please wear a short-sleeved or sleeveless shirt to your appointment (or bring one that you can change into when you arrive).

- You cannot participate in the experiment if you have artificial nails (e.g. acrylic, “gel,” etc). If you have natural nails with nail polish on, we will need to remove the nail polish from one finger only at the beginning of your appointment.

- Do not engage in strenuous exercise in the hour prior to your testing appointment.

- Do not eat or drink anything, or brush your teeth, in the 30 minutes prior to your testing appointment.

Thank you for participating in the research study!
Appendix III: Equipment and software protocols

SOFTWARE PROTOCOLS

Note – if you have the cart computer, login with the administrator password (not the one taped on the computer).

Syncing Bluetooth Dongle with Pulse Oximeter

1. Ensure that dongle has been properly installed with the provided software and according to the instruction manual. Also ensure that the Pulse Oximeter is on and in Bluetooth discovery mode. It should be displaying the Bluetooth icon.
2. Perform a search in the windows start menu (bottom left icon on screen) for “Bluetooth” and click on “Add a Bluetooth Device” under Control Panel.
3. Follow the instructions using Microsoft’s “Add a Device” program, and be sure to select that you do have a a pairing code when prompted. This code is found at the end of the devices name. It will look like Nonin_Medical_Inc_[Numbers]. The numbers are the pairing code.
4. If all is successful, the oximeter name should show up in “Devices and Printers” in the control panel and it should be able to connect with the Nonin OEM Evaluation Program. Note: If trouble occurs, try removing the device by right clicking its icon and selecting “remove”. Then restart the computer and the pulse oximeter and try again.

Unlock the meditation research folder:

1. Go into My documents of the user (either research01 or 02 for the laptops, or admin for the cart computer), then Meditation Research. Double click the file “Locker.bat.” Enter the password on the screen (Dr. Olson should have told you this password). “Private” folder will appear in the research folder. Open the private folder and create a subfolder of the date of the experiment (month-day-year). Open the date folder, and create two subfolders named “Pulse Oximetry” and “iWorx.” Make SURE to keep this folder unlocked until the end of the day. Do not relock it until you are ready to go home!

Nonin OEM Software Protocol

1. The oximeter should turn on when the subject’s finger is inserted into the sensor. Make sure that the oximeter is set to communicate via Bluetooth before starting software. The Bluetooth
iWorx Software Protocol

1. Ensure cables are connected correctly to black box before starting software
   - Respiration: Channel 3
   - GSR: Channel 4
   - USB to computer
2. Turn on black box (the power switch is on the opposite side as the channel plugs)
3. Start LabScribe software. It should detect the 214 hardware. Load correct settings by clicking “settings” then “load group.” The file is called “Meditation research.iwxgrp” and can be found at C:\Users\research01(or 02 or admin)\Documents\Meditation Research\iWorx meditation research settings. You may get an error message. Just click OK.
4. Then go back to settings, and choose Meditation research. You should now see two channels (respiration and GSR).
5. Proceed to the next steps after the subject is hooked up to all equipment.
6. The bottom left corner of the screen should have a floppy disk with a green arrow. Click to change it to a red X to put the program in preview mode.
7. Click “preview” at the top right of the graphs. Your subject’s respirations and GSR should be displayed and be variable. Once you have confirmed that the equipment is detecting the signal, click stop.
8. **Very important** Go back to the bottom left corner of the screen and click the red X to make it back into a floppy disk icon with a green arrow.
9. When ready to begin experiment, click the green “record” button on top right.
10. Marks can be made during data collection at specific times by typing in the top center box next to “mark” and pressing enter.
11. When finished recording, click the red stop button.
12. To save, click file→"save as." Title the file with the researcher’s name and the subject’s appointment time as in step 10 of the Nonin protocol. Save it to C:\Users\research01 (or 02 or admin)\Documents\Meditation research\Private\(experiment date\)\iWorx\Private (you made this file above).

13. Click File – New on iWorx to clear the old subject’s recording and you are now ready for a new subject. Start with a new subject at step 5.

Blood pressure monitor - make sure it is plugged in and air tube is securely pressed into machine. Keep track of which monitor is in which room with which computer. Check the date/time on the monitor to make sure it is correct, and that xxx3 is OFF.

At the end of the research day:

Move the file with the experiment date onto Dr. Olson’s USB drive to put it onto the laptops. If you are on the cart computer, after you have confirmed that it is saved on the laptops, DELETE THE FOLDER WITH THE EXPERIMENT DATE OFF THE CART COMPUTER.

Upload the blood pressure data:

Login to healthvault.com with lisa_olson@redlands.edu and the laptop password. Click "switch person" to select the computer that you are on (Research01 Laptop, Research02 Laptop, or Cart Computer), and then upload the blood pressure data from the monitor. Export the data with researcherA’s name and the date in the file name into the private folder.

Then, relock the meditation research folder:

1. Before you can relock the folder, make sure any files within the folder are closed. Then go into My documents, then Meditation Research. Double click the file “Locker.bat.” When prompted “do you want to lock the file?” type Y. “Private” folder will disappear in the research folder.
Appendix IV: Script

DATA SHEETS: Pre-Meditation Workshop

Subject's appt time: _______ Researcher A: _______ Researcher B: _______

Subject ID (to be filled in by Dr. Olson after testing): __________

<table>
<thead>
<tr>
<th>Researcher A</th>
<th>Researcher B – setup software in advance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greet the subject and introduce yourself, have them place belongings somewhere near where they will exit.</td>
<td>Greet subject.</td>
</tr>
<tr>
<td>I will be speaking with you and taking some measurements of your physiological responses. Please take off all metal jewelry on your hands or wrists, take out any electronics in your pockets. Do you have any nail polish on? (Remove if necessary). Please wash your hands before we begin and there is also a cup over by the sink for you to wash out your mouth with water. Swish for a few seconds and spit out the water into the sink.</td>
<td></td>
</tr>
<tr>
<td>(Attach the respiration monitor with sensor facing in, ear buds, blood pressure machine to the left arm, the GSR to the left ring and forefingers, and the pulse oximeter to the right forefinger.)</td>
<td></td>
</tr>
<tr>
<td>First, I am going to take some baseline measurements, and then I will ask you some</td>
<td>Put GSR into preview mode to check that it is</td>
</tr>
</tbody>
</table>
questions. Please let me know if you feel uncomfortable at any time. Please remain as still as possible during the testing. In particular, I need you to NOT move your hands at all. At various points during the next half hour your blood pressure will be taken and we will collect a saliva sample. When it's time to take a saliva sample, I will hand you a cotton swab like this. You can pick up the swab with your right middle finger and thumb. Please place it underneath your tongue perpendicularly and allow your saliva to saturate it for a couple of minutes. I will prompt you to then place the swab into this tube when the swab is saturated.

Do you have any questions? Once we start I can’t really chit chat with you, because I have to stick to a script.

Please relax. You may close your eyes if you like, and I am going to play some calming music for you. We don’t want you to fall asleep, though, so if you need to open your eyes to stay awake, please to so. You will stay like this for about 5 minutes.

Clock time:_____

OK, now we are going to leave the room for 10 minutes. Please just sit and relax, and keep your hands as still as you can.
T=0. Start Nonin and GSR simultaneously. Mark BLS. Start timing 5 minutes.

T=5. Take blood pressure. Mark BPS. Mark BPF. Mark BLF. Start timing 10 minutes.

Give saliva swab to subject. Time 1-2 minutes. Ask if it is saturated, and hold tube open for them to place it in. Put on ice.

Clock time:_______

Now, I am going to ask you a variety of types of
questions. For this initial group of questions, your answers will not be part of the data analysis. However, please let us know if for any reason one of the following questions is distressing.

(Remember to go slowly and say "#1...")

1. Do you live in a dormitory?
2. How many pets do you have?
3. How many siblings do you have?
4. Were you born in the United States?
5. What year are you?
6. What is your major?
7. Do you like chocolate?
8. Do you like broccoli?
9. Do you have a car?
10. Do you like the beach?
11. Where are you from?
12. What's your favorite food?
13. What's your favorite animal?
14. How old are you?
15. Do you play a sport?
16. Do you play an instrument?
17. Do you have a roommate?
18. Have you traveled outside of the country?
19. What is your favorite color?
20. Do you have a bicycle here on campus?
21. Do you prefer more dressy or more casual clothes?

T=15.

Mark NQS. Start timing 5 minutes.

Mark NQ1, NQ2, etc. as researcher A begins each question. When five minutes have elapsed, take blood pressure. Mark BPS and
[22] Have you ever been to Texas?

[23] What day of the week is today?

[24] Have you gone to the movies in the last month?

[25] Are you right or left handed?

Clock time:_________

Now, I am going to ask you some analogies taken from the verbal section of a previous GRE exam. If you would like me to repeat a question, please say so, and I will repeat once only. I will not be able to give you any hints to the questions. After you answer, I will tell you if you were correct or incorrect. (Circle subject's answers below).

1. MASON :: STONE
   A. soldier : weapon
   B. lawyer : law
   C. blacksmith : forge
   D. carpenter : wood XX

2. ARTICULATE :: SPEECH
   A. predictable : event
   B. coordinated : movement XX
   C. dangerous : disease
   D. active : thought

3. INCEPTION :: CONCLUSION
   A. departure : arrival XX
   B. culmination : upshot
   C. refutation : approval
   D. approach : return

4. SCINTILLATING :: DULLNESS
   A. erudite : wisdom
B. desultory : error  
C. boisterous : calm XX  
D. cautious : restraint 

5. ELUCIDATE :: CLARITY  
A. envision : memory  
B. aggragate : problem  
C. conceal : oblivion  
D. illuminate : light XX 

6. SHARD :: POTTERY  
A. seed : flower  
B. smoke : fire  
C. dish : menu  
D. splinter : wood XX 

7. REPEL :: LURE  
A. dismount : devolve  
B. abrogate : deny  
C. abridge : shorten  
D. miscarry : succeed XX 

8. PENURY :: MONEY  
A. starvation : sustenance XX  
B. independence : freedom  
C. infirmity : illness  
D. reality : foresight 

9. ARABLE :: LAND  
A. impenetrable : jungle  
B. navigable : waterway XX  
C. fertile : fertilizer  
D. shallow : pond 

10. ATTENUATE :: SIGNAL  
A. exacerbate : problem  
B. modify : accent  
C. dampen : enthusiasm XX  
D. elongate : line 

11. FURNACE :: SLAG  
A. vegetable : garbage  
B. factory : goods  
C. fire : ashes XX  
D. automobile : gasoline 

12. PROSAIC :: MUNDANE  
A. obdurate : foolish  
B. ascetic : austere XX 

T = 20. Mark VQS. Start timing 5 minutes. 

Mark VQ1, VQ2, etc. as researcher A begins each question. When five minutes have elapsed, take blood pressure. Mark BPS and BPF. Mark VQF.
C. clamorous : captive
D. loquacious : taciturn

13. SALACIOUS :: WHOLESOME
A. religious : private
B. expensive : profligate
C. conservative : stoic
D. mendacious : truthful XX

14. PONDER :: PROBLEM
A. remove : doubt
B. capture : runaway
C. seize : time
D. ruminate : idea XX

15. HACKNEYED :: FRESHNESS
A. stale : porosity
B. facile : delicacy
C. ponderous : lightness XX
D. central : vitality

Clock time:___________

Now I would like you to continually subtract the number 13 from 1,022 as fast and as accurately as possible. On every failure to calculate correctly, I will stop you and ask you to start over. (Allow to continue for 5 minutes. If subject gets an incorrect answer, say “Stop, go back to 1,022,” and circle below and mark as mistake 1, 2, etc.)

1,022 1,009 996 983
970 957 944 931
918 905 892 879
OK, we're finished with this part. I'm going to unhook all the equipment and I'll ask you to wait in the room where you took your questionnaires. In 10 minutes, I'll come take one more saliva sample, and then we'll be finished.

(Detach all equipment)

Take saliva sample #1 to freezer in Rm 120.

Clock time: 

(Collect saliva sample and put on ice, then transfer to freezer)

Thank you so much for coming. We appreciate you taking the time to help us with our study.

(When five minutes have elapsed, take blood pressure. Mark BPS and BPF. Mark VQF.)
\[ T = 25. \text{ Mark MQS. Start timing 5 minutes.} \]

Mark any incorrect answer with MQI. When five minutes have elapsed, take blood pressure. Mark BPS and BPF. Mark MQF.

Set up software for next subject.

T=40
Appendix V: Vivosense software data analysis protocol

Importing a Nonin .csv file into Vivosense for HRV analysis

Note: make sure the “private” folder is unlocked before attempting

1. Open Vivosense and select “File” at the top left and then “Import Hardware File” from the dropdown menu. Find and click on “Nonin 3150 Csv File.”

2. It will ask you to locate the file on the hard drive, go into the location where the file is saved (C:\Users\research\computer number\Documents\Meditation Research/Private\[Date of testing]\Pulse Oximetry) and click on the .csv file you want to analyze.

3. You may remove any charts that initially appear by right-clicking in the middle of the chart, selecting “chart” and then “remove.” Also, to clear the screen you can click the “empty” module under “layouts” at the bottom left of the screen.

4. Make sure that the “Layout Start Time” and “Duration” at the top-middle of the screen are the times you want to analyze.

5. Before beginning analysis, you’ll want to remove artifacts. To do so, find “HRV analysis” blue folder under “Modules” in the “Layouts” section at the bottom –left portion of the screen. Click “PPG” and then “Artifact Management.”

6. Click the “Session” drop down menu at the top left of the screen. Then go “Artifact Management”→ “Automatic”→ “Automatic Artifact Marking (P-P)”

7. Leave the settings as they are and click “ok.” A statistics pop up window will appear, click “ok.”

8. Under the “view” drop down at the top of the screen, find “Annotation Manager (F7).” Upon clicking, a window will appear at the bottom of the screen showing all the artifacts and interpolations. They discrepancies have already been removed from the data as you can see on the P-P chart.

9. After removing artifact, click the X to close annotations manager and remove any unnecessary charts on the screen.

10. To view raw pulse rate data click on the blue folder “Pulse Oximeter” under “Sessions” in the data explorer at the top left of the screen. Click “measures” and then under “Channels” select “Pulse Rate.” You may also select “SPO2” and “P-P” under “Channels.”

11. To select the time regions (Baseline, NQ, VQ, MQ) based on the markings from iWorx data for the same file (C:\Users\research\computer number\Documents\Meditation Research\Private\[Date of testing]\iWorx), have the P-P chart up (and pulse rate if you want) and then bring up the HRV channels (HF, LF, and LF/HF) by going to HRV(PPG)→Frequency Domain→Regions→Channels under “Sessions” in the data explorer at the left of the screen.

12. Once these are open, place cursor on the desired start time for a region (this must be done on either blank HF, LF, or LF/HF charts, not on P-P or Pulse rate) and click and drag, releasing the click at the desired end time. Upon released a drop-down menu will appear, click “Select HRV
Region" at the bottom of it. Since the regions are 5 minutes, it is usually easiest to type in the marking start time in "Layout Start Time" at the top of the screen, and make the duration 5 minutes. This way you can just select the whole region easily. Note: Baseline should be marked from 1:00 to 5:00 min unless the data is erratic, if so use best judgment. Also, regions must be long enough to analyze, so if a region is too short or has too many artifacts, nothing will show up on the charts. If all goes correctly, a single solid line should show up at some value in HF, LF, and LF/HF charts within the region you selected.

13. Once the HRV regions are marked, you can label them individually by again going to the annotation manager under "view," clicking the desired HRV mark, and typing in the label in the top right of the screen under "appearance" where it says "name." You can also remove the regions by right-clicking and selecting "remove."

14. To export, click the "session" dropdown menu at the top of the screen, then export->data by annotation and ensure the right file path (C:\Users\research[#]\Documents\Meditation Research\Private\[date]\Vivosense\[Subject ID] HRV Annotations.csv) and the configuration should be "Meditation HRV" which includes HF, LF, and LF/HF channels. This configuration should already be setup on your computer but if not, go to "view" dropdown at the top of the screen, then find "export manager." Right click in the export manager window and select "new configuration," name it and right click to "add channel." Do this for the three desired channels (HF, LF, and LF/HF channels). Now it will become available when exporting. If all is done correctly you should end up with a .csv that opens in excel showing the different labeled regions and their HF, LF, and LF/HF data.