Stress response to Stroop color test following a meditative practice, measured with Heart Rate, Blood Pressure, and Respiratory Rates

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Introduction

The human body is physiologically capable of responding to several stressors simultaneously. From waking up late for class to traumatic life experiences, our bodies experience different types of stress and reacts accordingly. The way our bodies react to these stressors is by increasing physiological functions including heart rate, breathing rate, and the liver's release of glucose. (McLeod)

The physiological mechanism that the body goes through to respond to some of these stressors is through the hypothalamic-pituitary axis. The hypothalamus releases CRH (Corticotrophin-Releasing Hormone) which goes through the portal system, which is a series of blood vessels, to the anterior pituitary and causes the release of ACTH (Adrenocorticotropic Hormone) which then flows in the blood plasma to the Adrenal cortex, causing the release of Cortisol, from the Zona fasciculate, throughout the body. Cortisol causes effects on organic metabolism including stimulation of protein catabolism, liver uptake of amino acids, enhanced vascular reactivity, etc. (Widmaier et. al 2016). The hypothalamus also stimulates the adrenal medulla which causes the secretion of epinephrine otherwise known as adrenaline. The sympathetic effects from epi-secretions include increased cardiac function, increased lung ventilation
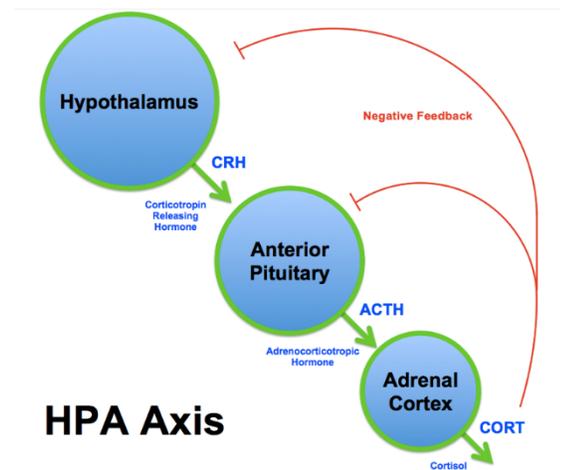

Figure 1. Shows the Cortisol axis where the Hypothalamus releases CRH, the Anterior Pituitary which releases ACTH, and finally the Adrenal Cortex which releases Cortisol, which then negatively feeds back onto the release of CRH and ACTH.

by dilating the airways, as well as vasoconstriction in the viscera and vasodilation in the skeletal muscles. These effects from the increased cortisol help mediate the stress the body feels from different stimuli. Not only do many everyday activities cause humans stress, but there are even times when we knowingly induce stress in ourselves or our peers.

One test that has been shown to induce a stress response in participants is the Stroop test. Participants are shown a page with the names of colors on them, but the text color of each word is in a different color than the word itself (Figure 2). The participants are then asked to read not the word, but the color the word is filled in by. Participants take longer to respond because the word and the font color do not match; reading the word interferes with identifying the color (Stroop 1992). Barbosa et al. (2010) found that the Stroop test was a useful instrument to induce cardiovascular reactivity in women. There are many other stimulants in life that can influence our physiological functions.

RED	GREEN	BLUE	YELLOW	PINK
ORANGE	BLUE	GREEN	BLUE	WHITE
GREEN	YELLOW	ORANGE	BLUE	WHITE
BROWN	RED	BLUE	YELLOW	GREEN
PINK	YELLOW	GREEN	BLUE	RED

Figure 2. an example of a Stroop test, showing the format and dynamic of trying to read the color filling in the name of the color.

One stimulus that has been shown to have an effect on the body's physiological response to stress is meditation. Delmonte (1994) found that meditative states in humans are found to lower physiological activation, even more than when participants are in an eyes-closed resting state. Mohan et al. 2011 also concluded that meditation had physiologic and psychologic effect opposite the effect of stress, but then effects differed when meditation preceded or followed a stress.

In real life, you will usually not just have one stimulus affecting you at a time. The question is, does multiple stimuli show different effects on physiological function, or does the newest stimulus always control the bodies functions. In the case of our experiment, those two stimuli in question are first a guided meditation and second, a Stroop test. We hypothesized that subjects exposed to a 5-minute guided meditation practice will illustrate a decreased stress response following a Stroop test to induce stress on the subject. Stress response was measured via heart rate, blood pressure and respiration rates. We chose to measure these three functions because stress is supposed to affect each of them as well as with our limited resources, these measurements were the most efficient to take in terms of time and resources. The purpose of the study was to assess the impact of acute meditative practices on stress response, as evaluated by the previously stated measurements.

It was anticipated the experimental group would show decreased rates/levels of these three physiological responses. From this test, quantitative data values were gathered, analyzed, and compared to a control group that experienced the same procedure but without the guided meditation.

Methods and Materials

Participants

   Twelve total male and female participants from the University of Wisconsin - Madison (ages 18-22) were recruited from the undergraduate Physiology 435 course. All 12 subjects signed and agreed to a written consent form laying out the experimental procedure. All 12 participants were ensured anonymity in representation of their recorded data and informed of the study's demands prior to participation.

Materials

   Physiological measurements were collected using a pulse oximeter, a respiration belt, and automatic blood pressure detector. Measurements of the participants' heart rates were recorded in beats per minute (BPM) using a Nonin Medical Inc. Pulse Oximeter and CO_2 Detector (Model 9843, SN# 118103091, Plymouth, MN). An Omron Healthcare 10 Series+ Upper Arm Blood Pressure Monitor (Model BP791IT (HEM-72220-ITZ), SN#20141004369LG, Lake Forest, IL) was used to measure the participants' blood pressure in mmHg. Using the Biopac Student Lab System MP 36 hardware, 4.0 software, respiration rate was recorded in breaths per minute with a BIOPAC Respiratory Transducer SS5LB.

Experimental Design

After a brief introduction to the procedure, researchers then connected the BIOPAC Pressure Cuff, BIOPAC Respiratory Transducer, and BIOPAC Pulse Transducer to subjects (Figure 3. Shows a timeline of events). Baseline data for both experimental and the control group's heart rate, blood pressure and respiration rates were gathered over a 1-minute period. During this time, the subject rested without talking in a seated position. Researchers recorded heart rate at 5-second intervals for 1-minute using the BIOPAC Pulse Transducer. Systolic and diastolic blood pressure was taken and recorded during this 1-minute period with the BIOPAC Pressure Cuff. Finally, respiration rate was continuously recorded utilizing the BIOPAC 4.0 software at the beginning of this 1-minute period and throughout the entire experiment marking transitions between recording stages.

Subjects from the experimental and control groups were then told to place headphones over their ears. The experimental group then listened to a 5:30-minute long guided breathing meditation from an online recording produced by UCLA's Mindful Awareness Research Center. Control

subjects wore headphones but no audio was played. Subjects were instructed to sit upright in their chair, with their feet on the floor following the audio instructions to the best of their ability. Heart rate data was collected again at 5-second intervals throughout this 5:30-minute period from the BIOPAC Pulse Transducer. Respiration rate data continued to be recorded via the BIOPAC Respiratory Transducer. Upon completion of this listening session blood pressure was taken and record using the BIOPAC Pressure Cuff.

After this listening session was completed for both groups, two researchers administered the Stroop Test via paper format. Subjects were instructed to read a grid of colored words, reporting what the color of the word is to the researcher as opposed to the actual word (color names). This test was intended to induce stress in the participant. Researchers also induced stress by emphasizing to the subjects that they were being scored for time and accuracy. Participant heart rate was again recorded at 5-second intervals during the Stroop Test. Respiration rate data was continuously recorded, but will not be used in data analysis due to conflict with the requirement for subjects to verbally report answers. The test was run until completion for all subjects.

Following completion of the test, the subject's blood pressure was taken and recorded immediately. This began a 1-minute post-stressor period of recording conducted after the test to gather respiration rate data that was compromised during the verbal test. During this time, the subject again sat resting in the chair, while heart rate data was recorded at 5-second intervals. Respiration rate was collected throughout this 1-minute period. Finally, the recording instruments were removed and participants were excused.

Positive Controls:

In order to ensure positive controls, we tested our three metrics of heart rate, blood pressure and respiration rates. This was done by running a baseline assessment of these metrics while subjects were at rest, seated in a chair. Data was collected on all three metrics over a 1-minute period. Then subjects were instructed to stand and perform aerobic jumping exercises for 30 seconds. Following this exercise, the subjects were

Figure 3:

Figure 3. Shows a visual progression of events during the experiment.
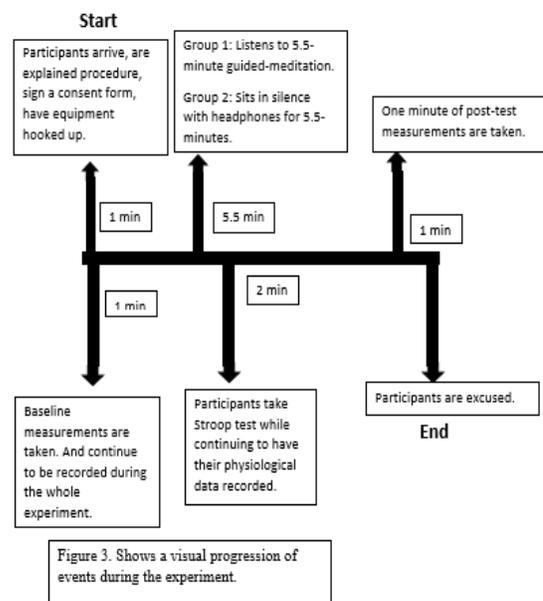
again seated, and assessed for their heart rate, blood pressure and respiration rates using the same metrics as before.

Table 1: Blood Pressure Data

Subject	Pre/Post-Exercise	Systolic/Diastolic
Subject #1	Pre-Exercise	115/76
	Post-Exercise	145/113
Subject #2	Pre-Exerciser	105/66
	Post-Exercise	131/73

Figure 6:

— Figure 7:

Positive Control of Heart Rate vs. Time Pre and Post-Exercise

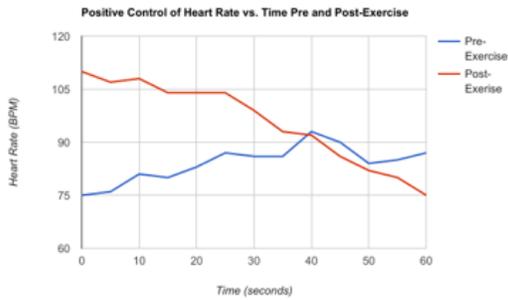Positive Control of Heart Rate vs. Time Pre and Post-Exercise
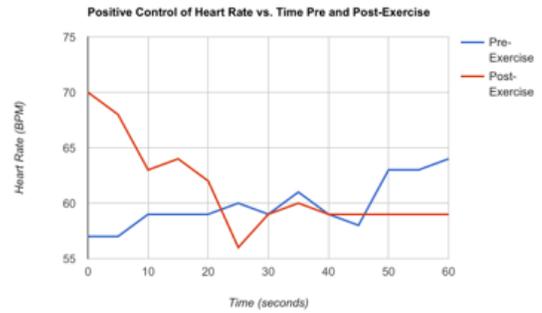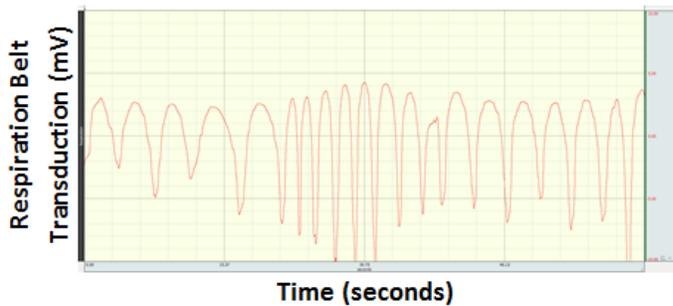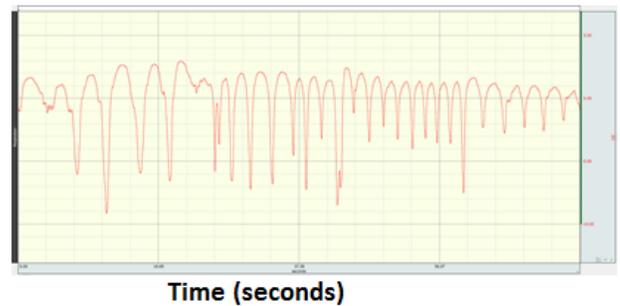
Figure 4:

Figure 5:

Figure 4 and Figure 5: These figures show the overall respiration rate data for the positive control subjects taken using a BIOPAC Respiratory Transducer. The x-axis shows time in seconds, and y-axis shows the transduction levels of the respiratory band as the subjects breathed normally for 20 seconds, followed by 20 seconds of breathing while performing aerobic exercise, and 20 seconds while recovering.

Results

Heart Rate

Each participant's heart rate data points collected in beats per minute (BPM) before the experiment, used as their baseline data, during treatment, during stress, and post stress were all averaged. The baseline data was used as a reference point to view any changes that occurred during and after each session. Average change in heart rate during the treatment period was determined by subtracting the baseline averaged heart rate from the averaged heart rate during treatment. The changes in heart rate during stress and post stress were also found in this way. The average change for each individual was compiled to calculate the average group change in both the control and experimental group. For the control group, the average change of heart rate during treatment was 1.36 ± 2.77 BPM, average change during stress was 18.80 ± 6.98 BPM, and the average change post stress was -1.75 ± 3.71 BPM. For the experimental group, the average change of heart rate during treatment was -2.06 ± 2.40 BPM, average change during stress was 9.02 ± 1.95 BPM, and average change post stress was -5.56 ± 2.88 BPM (**Figure 8**). An unpaired t-test was conducted and it was determined that there was no significant statistical difference between the control and experimental groups during treatment ($p = 0.3723$), during stress ($p = 0.2072$) or post stress ($p = 0.4365$).

Blood Pressure

Systolic pressure (SP) and diastolic pressure (DP) were measured and recorded before the experiment (baseline), post treatment, and post stress. The mean arterial pressure (MAP) was calculated for each different time points using the formula: $MAP = DP + \frac{1}{3}(SP - DP)$. The baseline measurements were again used as a reference point to the rest of the data to view any change. The average change in MAP of the post treatment and post stress from the baseline was taken and used to compare each group. The average change in MAP for the group that did not meditate was 0.28 ± 8.13 mmHg post treatment and -0.67 ± 8.22 mmHg post stress. For the group that did go through the meditation exercise, the average change in MAP was -4.72 ± 2.32 mmHg post treatment and -2.33 ± 2.55 mmHg post stress (**Figure 9**). The unpaired t-test that was performed on this data found there to be no significant statistical difference between the two groups for both post treatment ($p = 0.9336$) and post stress ($p = 0.6475$).

Respiration Rate

Respiration rates were taken constantly throughout the experiment, but respiration per minute (RPM) measured and recorded for further analysis was taken before the experiment (baseline), post treatment, and post stress. The data points obtained were then averaged for each time specific category. The change in average respiration rate for post treatment was calculated by subtracting the baseline respiration rate from the post treatment respiration. The average respiration rates were then used to compare each group with each other. For the control group, the average change in respiration during treatment was -3.40 ± 1.29 RPM and -2.71 ± 0.92 RPM post stress. For the experimental group, the average change during treatment was -2.29 ± 0.87 RPM and 1.331 ± 1.66 RPM post stress (**Figure 10**). Another unpaired t-test was conducted for the respiration rate data and it was concluded that there were no significant statistical differences between those that did not meditate versus those that did for both post treatment ($p = 0.9491$) and post stress ($p = 0.1478$).

Performance

During the stress induced test, each participant was timed and scored based on accuracy out of a total of 100 points. The average time and score for the control and experimental groups were calculated by taking the mean of all the individual data points of that group. The average

score by the control group was 99.5 ± 0.23 with an average time of 91.67 ± 5.11 seconds. For the experimental group, the average score was 99.5 ± 0.34 with an average time of 90.0 ± 5.00 seconds (**Figure 11**). A final unpaired t-test was conducted and found no statistical significance between the two groups for their time ($p = 0.8203$) nor their scores ($p = 1$).
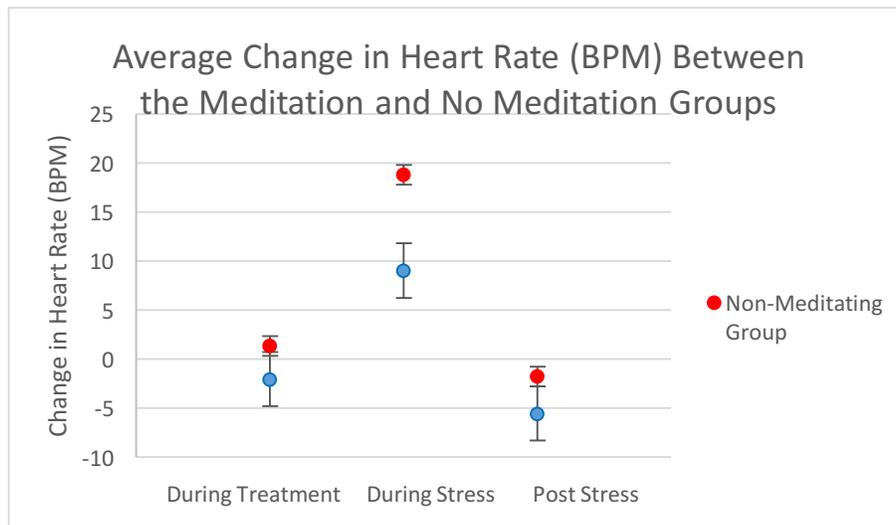

Figure 8. The average change in heart rate compared between the non-meditating (1.36 ± 2.77 BPM, 18.80 ± 6.98 BPM, -1.75 ± 3.71 BPM) and the meditating (-2.06 ± 2.40 BPM, 9.02 ± 1.95 BPM, 5.56 ± 2.86 BP) was not shown to have been statistically significant ($p = 0.21$, $p = 0.44$).
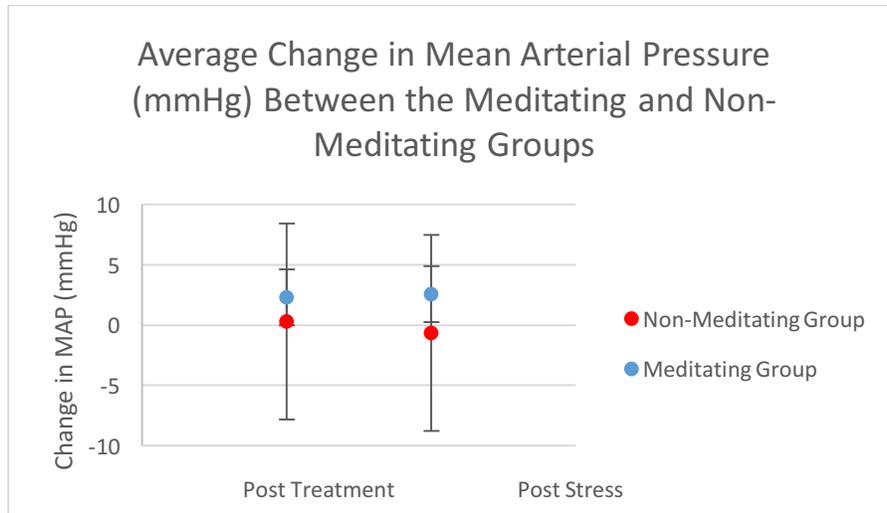
Figure 9. The average difference in mean arterial pressure (MAP) in mmHg compared between the non-meditating group (0.28 ± 8.13 mmHg, -0.67 ± 8.22 mmHg) and the meditating group (-4.72 ± 2.32 mmHg, -2.33 ± 2.55 mmHg) showed no significant statistical difference (*p* = 0.94, *p* = 0.65).
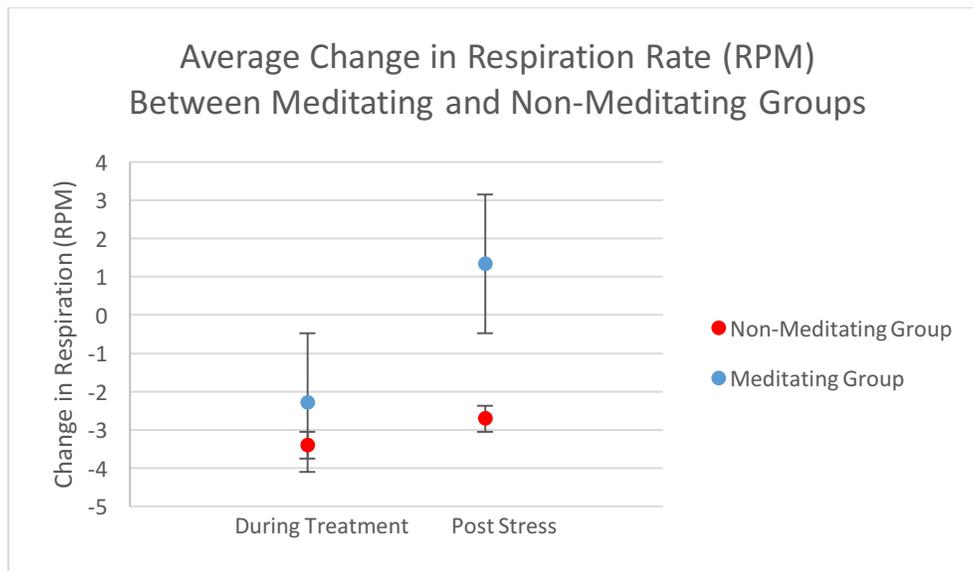

Figure 10. The average difference in respiration rate in respirations per minute (RPM) compared between the non-meditating group (-3.40 ± 1.29 RPM, -2.71 ± 0.92 RPM) and the meditating group   (-2.29 ± 0.87 RPM, 1.33 ± 1.66 RPM) was concluded to have not been statistically significant (*p* = 0.95, *p* = 0.15).
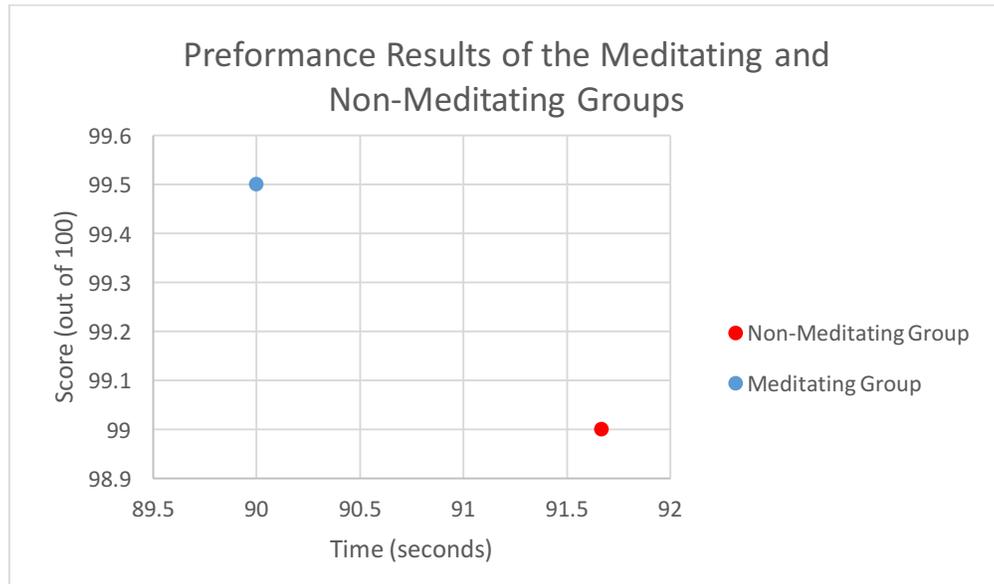
Figure 11. The average performance scored on the Stroop Test and the average time it took the non-meditating group (99.5 ± 0.23, 91.67 ± 5.11 seconds) and the meditating group (99.5 ± 0.34, 90.0 ± 5.00 seconds) did not prove any significant statistical difference ($p = 0.82$, $p = 1.00$)

Discussion

In this experiment, we examined the effect of a 5-minute guided meditation session on stress that was induced using a Stroop test. Stress responses were measured using the physiological variables of heart rate, blood pressure and respiratory rate. We hypothesized that the participants who meditated (experimental group) would show a decreased stress response to stroop test, with decreased blood pressure, heart rate and respiratory rate compared to the control group. However, based on the results of our study, our hypothesis was not largely supported as there were no significant differences in the variable. Nevertheless, while comparing individual data and variables, a slight difference is observed in blood pressure and heart rate especially between the experimental and control group.

The guided meditation audio used was from UCLA's mindfulness meditation program. The audio entails a set of instructions that allows the participants to relax and feel the sensation running through their body for the first 2 minutes. The following 3 minutes consist of breathing

exercises, were the participants are asked to keep a track of their breath and breathe normally. This meditation audio is solely based on finding a sense of ease and well being within oneself (Free guided meditation, n.d.). This particular mindfulness meditation audio was used because from a meta analysis conducted it was found that series of UCLA mindfulness audios were shown to be effective in stress reduction and cognitive therapy in adolescents and young adults (Black et al., 2009). This experiment also uses stroop task as a way to induce stress because of literature review done by psychophysiology journal that stated that stroop task was seen to be an efficient laboratory stress inducer. This experiment measured the emotional and physiological responses to stroop test and it was seen that there was an increase in response during the test compared to the baseline (Renaud et al., 1997). Therefore, we felt that stroop task would be a good stress inducer in a class laboratory set up compared to other inducers.

Our experiment was based on the assumption that 5 minutes of acute meditation could help reduce stress. However, in comparison to literature, previous studies on meditation and stress releases were more focused on long term meditation. For example, according to a meta analysis most experiments were conducted over a longer duration, i.e for at least more than 6 weeks and approximately 2.5 hours of intensive meditation per week. These experiments showed positive results in stress reduction and other disabilities (Grossman et al., 2004). Another study was conducted on medical students who went through a meditation course that lasted for about 8 weeks and the results were shown to reduce stress, depression and anxiety (Shapiro et. al, 1998). In an another meta analysis it was found that long term mediation helps reduce MAP, blood pressure and improves cardiovascular efficiency and respiratory performance (Woodyard, 2011). All these experiments were involved with a longer period of mediation compared to our experiment that was an acute session of a one-time 5.5-minute long meditation. This made it particularly challenging for us to come to a conclusion about whether or not mediation helps stress responses, with only such a short exposure to the practice. Also, our data only showed a slight difference in blood pressure and MAP between the experimental and control groups. However, it is possible that increasing the duration of this study could help us acquire better data and come to a better conclusion about how meditation impacts the heart rate, MAP, and respiratory rate.

There are caveats to the study that could have impacted the results as well. For example, the respiration rate and blood pressure were measured after the stress response rather than during the stroop test as the test involves talking which could impact the respiration data, and the blood pressure monitor would have interrupted the subjects during the task which could have also affected the data. Due to these reasons there is no data that could strongly support the increase in stress during the stroop test. In addition, during the post treatment phase, a lot of our subjects were talking and this could have had an absolute effect on the recording of respiration rate. There were other factors as well that could have affected the data such as the subject's past mediation experience and how focused they were during the meditation could have also impacted the results. In addition, different individual's mental and physical fitness could have also attributed to the variations in data. Finally, our sample size was relatively small, with only a total of 12 participants, 6 participants in control and 6 experimental. This led to difficulty in being able to generalize the impact of meditation on human stress response for the general population. All of our participants were college aged students, and results might be different for a wider age representation and with a bigger sample size.

If this experiment were to be reproduced to minimize these caveats a few changes would be done to the methods and data collection techniques. First, the respiratory rate parameter would be changed to a better stress response that is not easily affected by movement or talking for example the galvanic skin conductance. Second, the stress inducer could be changes as well, stroop test is effective but it is hard to accommodate people who are color blind, and the physiological responses were not as high as one would expect it to be. This could be improvised by using forced treadmill exercise which could possibly have a higher physiological response. Another factor that would be changed is increasing the duration of study and bringing in a larger population and bigger sample size. These possible changes could help produce more accurate results that could help reach a better conclusion about this experiment.

Although our experiment did show a slight significance in the MAP and heart rate between control and experimental, there is still great room for further study. Based on all the research and meta-analysis, it can be concluded that increasing the duration of study could induce better results that show the effectiveness of meditation. This data could be useful for

psychologists and psychiatrists in creating a mindfulness based stress release program for their patients coping with anxiety disorders.

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