Effect of Rose Scent on Physiological Signifiers of Anxiety

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Abstract: This study was designed to determine the efficacy of using aromatherapy to treat the physiological symptoms of stress. While taking a high school level math skills test, experimental participants were exposed to aromatherapy via the test proctor applying lotion with rose scent and allowing the scent to diffuse throughout the room. An unscented lotion was applied during the control trials. Respiratory rate (RR), electrodermal activity (EDA), and heart rate (HR) were the three physiological indicators of stress measured. The results of the EDA & HR tests showed no significant differences before and after lotion application in both the treatment and control groups. Both of the RR groups showed significant differences after lotion treatment. The control group showed a decrease in RR after treatment ($p < .05$). The experimental group showed an increase in RR after treatment (p-value < 0.05). The aromatherapy was not found to be an effective treatment for the physiological symptoms of stress.

Introduction

The efficacy of administering aromatherapy in order to elicit anxiolytic effects has been under scientific investigation for some time. Aromatherapy is the use of certain plant extracts or essential oils to alleviate symptoms such as pain and anxiety (Tugut et al., 2017). It is considered to be a complementary and alternative medicine (CAM) therapy. To elaborate, aromatherapy is used to supplement allopathic medical care. Research findings on the anxiolytic effectiveness of aromatherapy are mixed (Cooke, Ernst, 2000).

The circuitry of the brain's olfactory pathway associates scents with emotion. The first cells in the olfactory pathway are olfactory receptor neurons, which lie in the olfactory epithelium in the upper part of the nasal cavity. Receptors on the epithelium have specificity for certain odors. Olfactory receptor cells send afferent input to the olfactory bulb. The olfactory bulb then bypasses the thalamus and transmits sensory input directly to the olfactory cortex and limbic system. The limbic system is highly associated with emotional behaviors and memory.

Because the olfactory bulb synapses directly onto the limbic system, scents often have strong associations with memory and emotion (Widmaier, Raff, and Strang, 2019). Anxiety, being an emotional state, has the potential to be influenced by scent.

Researchers have treated participants with aromatherapy in a variety of ways, making it difficult to determine the most effective route of administration (American College of Healthcare Sciences, 2019). Although aromatherapy implies inhalation of odors, these substances can also be applied topically and absorbed through the skin (Sánchez-Vidaña et al., 2016). One study evaluated the efficacy of aromatherapy to reduce the stress of burn patients before and after receiving medical treatment. Researchers used both inhalation and massage (absorption) of aromatherapy substances. The study found that inhalation of rose oil is the most effective to treat and reduce anxiety. The findings suggest aromatherapy via inhalation has a greater effect than through skin absorption (Seyyed-Rasooli et al., 2016). This research supported inhalation as the optimal administration method of aromatherapy and was the methodology used in the present study.

Lavender is the aroma most commonly associated with anxiety reducing effects. Rose is thought to have similar effects, but has not been studied as extensively (Robbins, 1997 - 2019). Igarsahi (2014) showed rose fragrance to have relaxing effects both physiologically and psychologically in participants. Furthermore, Seyyed-Rasooli et al. supplemented the therapy of burn patients with rose aromatherapy. This was done prior to and during their medical treatments. They found aromatherapy assisted in increasing circulation, which in turn, aided in promoting deep breathing and relaxation. Additionally, it was noted that rose fragrance helped stabilize vital functions and reduce anxiety (Seyyed-Rasooli et al., 2016). Further research indicated that the inhalation of rose fragrance decreased sympathetic nervous system activity and adrenaline concentration, both signifiers of stress reduction (Fazlollahpour-Roknia et al., 2018). These findings justified further exploration of rose as an anxiety reducing aromatherapy option.

In the current study, three anxiety-related variables: electrodermal activity (EDA), respiratory rate (RR), and heart rate (HR) were measured while participants took a difficult math test. These physiological measurements were chosen because they increased in response to psychological stress in previous studies (Nogueria-Ferreria, *et. al*). Under physiologically arousing situations, the skin becomes a better conductor of electricity which is measured by the EDA electrodes (Biopac, 2013). It was predicted that in participants not exposed to rose, indicators of anxiety would increase as they progressed, and remain elevated until the end of the test. In the experimental group, it was predicted that when the participant began the test their indicators of anxiety would increase similar to the control. However, when exposed to the rose scent, their indicators of anxiety would decrease. To determine if rose had an anxiolytic effect, the change in EDA, RR, and HR during the test were compared between the control and experimental groups.

Materials

Respiratory rate (RR), heart rate (HR), and electrodermal activity (EDA) were the three variables measured during this experiment. These physiological measurements gave insight into how stress affected participants during a High School Math Skills Test (HSMST). The measurement devices were applied to participants before the start of the introductory questionnaire in order to capture measurements both before and after aromatherapy exposure. RR was measured using the BIOPAC Respiratory Transducer SS5LB (Manufacturer: BIOPAC Systems, Inc. in 42 Aero Camino, Goleta, CA 93117). HR was measured using a Nonin Medical Inc, Pulse Oximeter 9843 (Plymouth, MN, USA), and recorded manually. EDA was measured using the BIOPAC Electrode Lead Set SS3LA (Manufacturer: BIOPAC Systems, Inc. in 42 Aero Camino, Goleta, CA 93117) and the BIOPAC system GEL 101 Isotonic Recording Electric Gel. The gel was applied into the groove of the EDA sensor. Both RR and EDA were measured and analyzed using the BIOPAC Student Lab System (BSL 4 Software, MP 36). The HSMST was created with problems from www.varsitytutors.com. Problems (**figure 1 and 2**) were taken from multiple tests with varying levels of difficulty and then condensed into this experiment's HSMST. The HSMST question difficulty was evenly distributed. Researchers used rose perfume fragrance as the independent variable. The fragrance was purchased from The Soap Opera in Madison, WI, USA. 13 drops of the perfume was mixed with 100 mL of unscented lotion and placed in an unlabeled, clear bottle. This concentration was used because it allowed the aroma to remain in the room for the intended final 5 minutes of the test. Additionally, this concentration created a subtle scent that did not distract from the experiment. An unlabeled, clear bottle was used to avoid biases from participants. The test proctor applied a dime sized amount of lotion to their hands 5 minutes after participants began the HSMST, which allowed physiological measurements to be taken before and after the rose perfume fragrance permeated the room.

Methods

Participants: Screening and Consent

Participants (n=50), ages 18-25 were recruited on a voluntary basis from Physiology 435 classes at the University of Wisconsin-Madison. Physiological measurements were collected at the UW-Madison Medical Sciences Center. Each participant was given a consent form prior to participating in this study in order to ensure they were informed about the logistics of the experiment, confidentiality measures, and researcher contact information. Participants were eligible to participate in the study as long as they did not have an allergy to the rose scent.

Procedure

At the beginning of the experiment, participants received a demographic questionnaire to fill out while the experimenter set up the measurement devices. This equipment consisted of the BIOPAC Respiratory Transducer SS5LB, Nonin Pulse Oximeter 9843, and the the BIOPAC Electrode Lead Set SS3LA and the BIOPAC system GEL 101 Isotonic Recording Electric Gel. These devices measured participants' Respiratory Rate (RR), Heart Rate (HR), and Electrodermal Activity (EDA), respectively. The respiratory transducer (SS5LB) and BSL EDA finger electrodes were attached to the Biopac Student Lab System via channels 1 and 2, respectively. In the BIOPAC software, "Respiration" (SS5LB) was selected for RR, measured in millivolts (mV) for channel 1, and "Electrodermal activity" (EDA, SS3L, SS3LA, SS57L, 0-35 Hz) was selected for skin conductance, measured in microsiemens (μS) in channel 2. Before the participant filled out the demographic questionnaire, the respiratory transducer (SS5LB) was wrapped around the participant's upper chest, with the monitor placed on the sternum. The pulse oximeter was placed on the ring finger of the participant's nondominant hand to gather heart rate data in beats per minute. Oxygen saturation data was not collected in this experiment. The BSL EDA finger electrodes were strapped to the participant's index and middle fingers on their non dominant hand with the electrode facing the finger pads. Once this equipment had been properly set up, baseline measurements were gathered for all three metrics while the participants filled out the questionnaire. Experiments were conducted in a small room to allow for efficient diffusion and to ensure minimum dissipation of the smell. Twenty-six of the participants were exposed to lotion with rose perfume, and twenty-four of the participants were exposed to an unscented control lotion. At least two researchers were present for the entire duration of the study to ensure accurate measurements were collected, and to administer the aromatherapy or control lotion. The researchers did not wear any scented personal hygiene products that could have been detected by the participant. The participant was asked to sit down at a desk and allow an experimenter to set up the aforementioned devices. Then, the participant was given a demographic questionnaire to fill out while baseline measurements were gathered. The time allotted for this was 2 minutes. After collecting the demographic form, they were given the HSMST exam. The participant was told they would have ten minutes to complete the test. Five minutes into the test, a dime sized amount of rose scented lotion or the control lotion was administered by one of the proctors to their own hands. After ten minutes the experimenter notified the participant that the time was up, and assisted the participant in removing the monitoring devices.

Data Analysis

Three measures were taken for data analysis: change in RR, change in HR, and change in EDA. Delta values were used to account for participant's individual baseline values. RR was measured in breaths per minute, and this was calculated by counting the number of breaths (peak to peak on the BIOPAC monitor) within a 60 second period (**figure 3**). HR was measured in beats per minute and was recorded manually every 30 seconds. Electrodermal Activity (EDA) measured participants skin conductance in microsiemens (mS) using the BIOPAC Electrode Lead Set SS3LA and the BIOPAC system GEL 101 Isotonic Recording Electric Gel. The mean skin conductance was calculated over a 150 second time interval (**figure 4**).

In order to analyze EDA and HR, the data was sectioned off into strategic time intervals (**table 1**). Baseline was determined by taking the average EDA measurement from 60-120 seconds, before the math test was given at 120 seconds. Time range 0-60 s was not considered as part of baseline to allow participants to acclimate to the testing environment. According to non-reported data, this was more than enough time to become acclimated to the environment. First, the baseline value was subtracted from each time interval to compute a relative value to account for individual differences. The time intervals used were 270-420 seconds (before treatment) and 570-720 seconds (after treatment). The 270-420 second interval should reflect a period of maximum stress for participants, and the 570-720 second interval is when the aromatherapy should have had enough time to impact the level of stress in participants, if they received the treatment. Next, the mean change in EDA between 270-420 seconds was compared to the mean change in EDA between 570-720 seconds for each treatment independently. Two independent samples t-tests was used in order to compare the two means, one for the control group and one for the experimental group. Additionally, the mean change in physiological response from before to after the application of the treatment was compared between the control and experimental group. The same intervals for each of the measures used above were used for this test.

Similar to EDA and HR, the baseline for RR was taken during the 60 to 120 second interval. The first interval compared to the baseline was the 300 to 420 second interval. This was deemed to be the highest stress interval in the study for this measurement. This varied slightly from the time increment we chose for EDA because the RR measurement was recorded in full minute increments. The next interval chosen was 600-720 seconds. At this point in the study, the effects of the aromatherapy should have materialized. The same statistical tests ran for EDA and HR were ran for RR.

Positive Controls

Positive Controls were conducted for this experiment in order to establish our research group was able to produce physiological changes in test participants with the equipment the researchers chose to use. The three experimental parameters measured during our experiment were RR, HR, and EDA. These parameters allowed us to see how aromatherapy affects stress levels in students while taking an HSMST. Two group members performed the positive control while they wore the equipment used for the tests. The average change in rates was recorded. This was measured by having individuals take a baseline test in which they rested for 30 seconds, followed by one minute of running in place and 30 seconds of rest afterwards. During this control an average change of +35 beats per minute was recorded while measuring the HR during the activity. RR values were found to increase by +71 breaths per 30 seconds. Change in EDA was recorded to be 3.271 microsiemens. The values recorded from the positive controls confirmed the equipment was effective in the physiological parameters being tested.

Negative Controls

Baseline measurements were taken during the first two minutes of the study while the participant filled out the questionnaire. The data for breaths per minute and skin conductance were measured using the BIOPAC system. Beats per minute was measured using the pulse oximeter. This data shows the equipment was accurately measuring the physiological parameters when the High School Math Skills test was not being administered. The baseline measurements will be used to quantify the magnitude of change in response to treatment.

Results

50 people participated in our research study. 26 participants were exposed to aromatherapy treatment and 24 participants were exposed to the unscented control lotion. The experiment was conducted as a randomized single blind study. The experimenter who administered the treatment was not blind to the experimental conditions; however, the participants were not aware if they had received the treatment or control. We conducted two-tailed single variable t-tests for each measurement comparing change from baseline measurements to see if there were differences between control and experimental groups. All tests were ran with 95% confidence intervals.

Electrodermal Activity

No significant differences were found before and after treatment for both the experimental and control groups when compared to baseline (experimental p-value = 0.226899 , control p-value = 0.652356) (**figure 5**). EDA data from 9 of 50 participants was excluded due to poor readings from the electrodes. The baseline values for these participants were negative values, and the values did not change for the duration of the experiment, suggesting the electrodes were not reading properly. Participants who received aromatherapy treatment showed greater EDA activity both before and after treatment. The control group showed increased EDA activity after treatment, whereas the experimental group showed decreased EDA activity after treatment. The mean EDA value (Siemens) for the control group was 0.4218 before treatment with a standard deviation (SD) of 1.0003 and 0.5844 after treatment with a SD of 0.5878. The mean EDA value for the experimental group was 1.1333 before treatment with a SD of 1.2126 and 1.0731 after treatment with a SD of 1.4700. The mean change for the control group before and after treatment was 0.1596 with a SD of 0.3717 (**figure 6**). The mean change for the experimental group before and after treatment was 0.4853 with a SD of 0.3376. When comparing mean change before and after treatment between experimental and control groups, no significance was found (p value = 0.0710).

Respiratory Rate

Significant differences were found before and after treatment in the experimental and control groups when compared to baseline for RR (experimental p-value = 0.0179, control p-value = 0.0058) (**figure 7**). Due to improper placement and tightness of the respiratory belt, readings for 15 participants were irregular, and thus excluded. Participants in both the experimental and control group showed a decrease in respiratory rate both before and after treatment. The control group saw a larger drop in RR values than the experimental group did post treatment. The mean RR values for the control group were 2.8765 before treatment with a SD of 1.2941 and 2.5143 after treatment with a SD of -0.5588. The experimental treatment mean RR values were 2.6253 before with an SD of 1.3235 and 3.1432 after the treatment with an SD of -0.3824. The mean change for the control group before and after treatment was -1.8529 breaths per minute with a SD of 2.7258 (**figure 8**). The mean change for the experimental group before and after treatment was -1.7059 breaths per minute with a SD of 3.3310. When comparing mean change before and after treatment in the control group to the mean change before and after treatment in the experimental group, no significance was found (p-value = 0.8889).

Heart Rate

No significant HR differences were found in either the experimental or control groups when comparing the pre and post treatment mean values to baseline (experimental p-value = 0.4789 , control p-value = 0.08458) (**figure 9**). All 50 participants' data was used for HR calculations. When looking at differences in heart rate between the stress induced period and after the scented or unscented lotion was used, there was a greater change in the control group relative to the experimental group (**figure 10**). However, the mean change in HR before treatment in the control group was larger compared to the experimental. The mean change in the experimental group before treatment was 0.5962 with a SD of 9.1452 and the after treatment mean was -0.9936 with a SD of 6.7428. The mean change in the control group before treatment was 2.0694 with a SD of 5.7781 and the after treatment mean was -0.6667 with a SD of 4.9434. The mean change for the control group before and after treatment was -2.7361 with a SD of -3.1027 (**figure 10**). The mean change for the experimental group before and after treatment was -1.5897 with a SD of 5.1847. When comparing mean change before and after treatment between experimental and control groups, no significance was found (p value = 0.3612).

Discussion

The present study aimed to investigate the potential anxiolytic effects of aromatherapy, specifically rose. The short period of onset relative to pharmaceutical anxiolytic drugs made aromatherapy an appealing solution to situational stresses such as preoperative stress. Our results that pertained to EDA, HR, and RR suggested that aromatherapy does not combat these measures of stress - there was no difference between the control and experimental mean changes before and after treatment for any of these physiological indicators of stress.

Our findings were inconsistent with the findings of Diego, et. al, who saw significant decreases in physiological measures of stress in their subjects when exposing them to aromatherapy. Once reason for this difference may be their method of aromatherapy administration. They put three drops of their essential oil onto a cotton swab, put that swab in a vile, and then held the vile 3 inches under the subject's nose for 3 minutes. One big difference in their administration method is their aromatherapy potency was highly controlled, whereas the present study was variable. Another important difference is that Diego, et. al's test subjects knew they were being tested on how aromatherapy affected their stress levels, whereas the present study's test subjects were blinded to the independent variable.

The study conducted by Ahmad, et al. (2019) investigated the effects of aromatherapy on stress. Although these researchers used lavender oil instead of rose, they were also studying its potential anxiolytic effects on academic stress. The placebo and experimental groups showed a significant difference in a self-reported stress score and heart rate compared to the control group, therefore justifying the single blind nature of the present study.

Limitations and future directions:

There were a number of limitations with the present study. Once the experimental treatment was administered to a participant, there was not a sufficient amount of time allocated to allow for the room to air out before the next participant began the study. The average time between participants was 0-5 minutes. Some ways to prevent this from happening would be conducting this test in a room with better ventilation, providing more time between trials or having an unacclimated researcher confirm the rose scent was undetectable.

In addition, there was a loud banging noise in the room, which could have thrown off the participants while taking the exam. Having a more test-friendly environment would be important if this study were to be repeated. The present study also used a perfume instead of an essential oil. The perfume was a more cost effective alternative but it could have affected the results. The route of administration could have been more direct than relying on the scent of the perfume (in a lotion) to diffuse enough. A patch with the scent could have been placed on the participant's chest to ensure that the treatment was more potent. However, this methodology would have eliminated the single blind nature of the experiment. There was also a lack of incentive to perform well on the exam, which could mean that the test may not have been an effective way to elicit a stress response.

In a future study it might be beneficial to include a self report survey of the anxiety experienced during the exam. Many of the researchers reported a verbal response from participants expressing the stress they felt during the exam.

Final Paragraph

Although many stress inducing studies have been conducted in order to assess the effectiveness of aromatherapy on reducing stress, no decisive conclusions have been determined. With this in mind, our group elected to take on this topic as it is pertinent to topics studied in Physiology 435. We have discussed the connection between scents and emotion. The olfactory bulbs are continuous with the hippocampus which is a high memory association area. Additionally, prolonged periods of stress (increased cortisol levels) had negative effects on the human body. For example, when cortisol levels are high, this increases bone reabsorption, leads to high blood pressure, and stunts growth.

In our study, we expected that an increase in stress levels would lead to an increased heart rate, increased electrodermal activity, and respiratory rate. In our results, these expectations were met. However, we also hypothesized that by using aromatherapy, this would decrease the stress levels in our participants, thus decreasing the physiological parameters that we tested. The results did not follow our hypothesis. This study was designed to account for non-desirable variables that would influence the outcome. Even with this consideration, there were still confounding variables we could not control for. We learned there are always going to be pathways, processes, and variance that you cannot account for within the human body.

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Tables

	Baseline	Before Treatment	After Treatment
EDA	60 - 120 seconds	270-420 seconds	570-720 seconds
RR	60 - 120 seconds	300-420 seconds	600-720 seconds
HR	60-120 seconds	270 - 420 seconds	570-720 seconds

Table 1: Time intervals used for data analysis.

Figures

1. You have 6 apples and 8 bananas. Each apple weighs 0.25 pounds and each banana weighs 0.15 pounds. What is the total weight of the fruit you have?

Figure 1: Sample question from HSMST with a low difficulty.

13. Solve for x:

$$3^{2x+1} = 9^{2x-4}$$

Figure 2: Sample question from HSMST with a high difficulty.

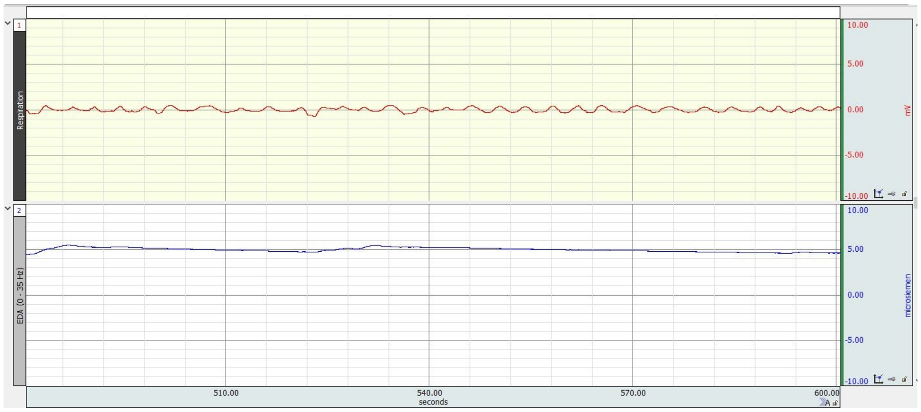

Figure 3: Respiratory rate (RR) was calculated by manually counting the number of peaks per minute interval.

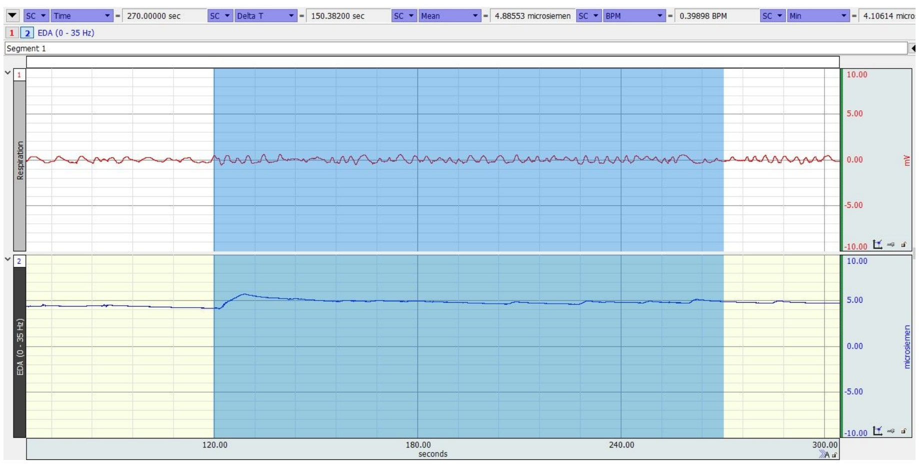

Figure 4: The mean skin conductance (EDA) was calculated by highlighting specific 150 second intervals.

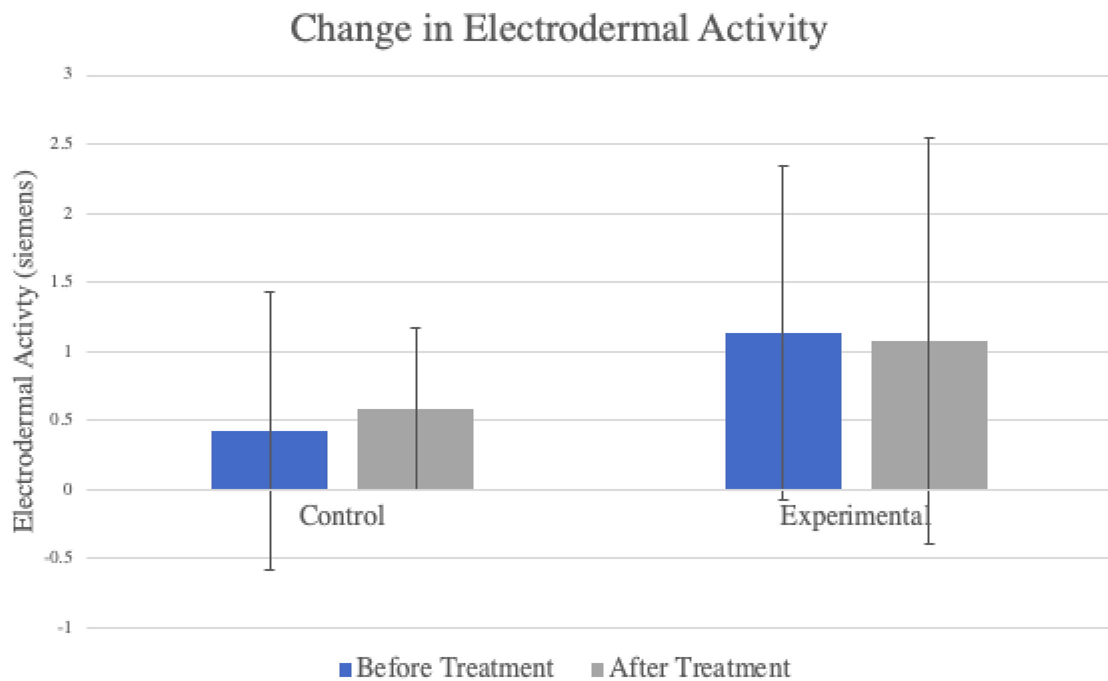

Figure 5: During the math test there was no change in electrodermal activity before and after treatment for both the control and experimental groups. Control: n = 22 p = 0.652356 | Experimental: n = 20 p = 0.226899

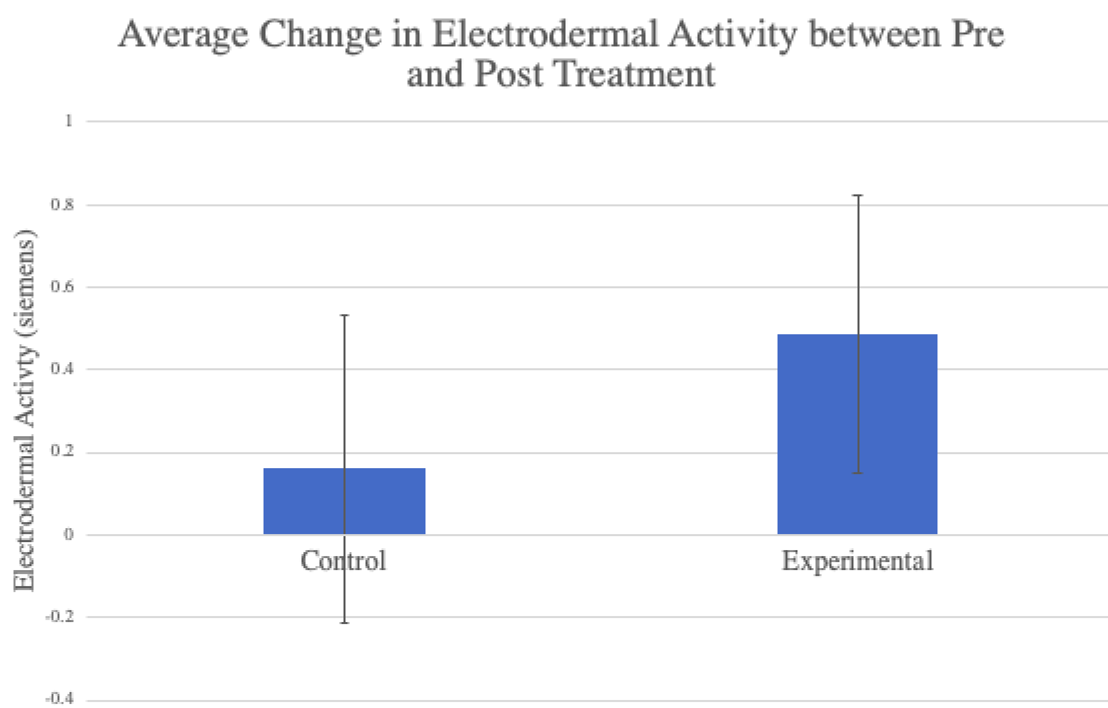

Figure 6: There was no difference between the change in electrodermal activity between the experimental and control group. n = 42 p = .07101

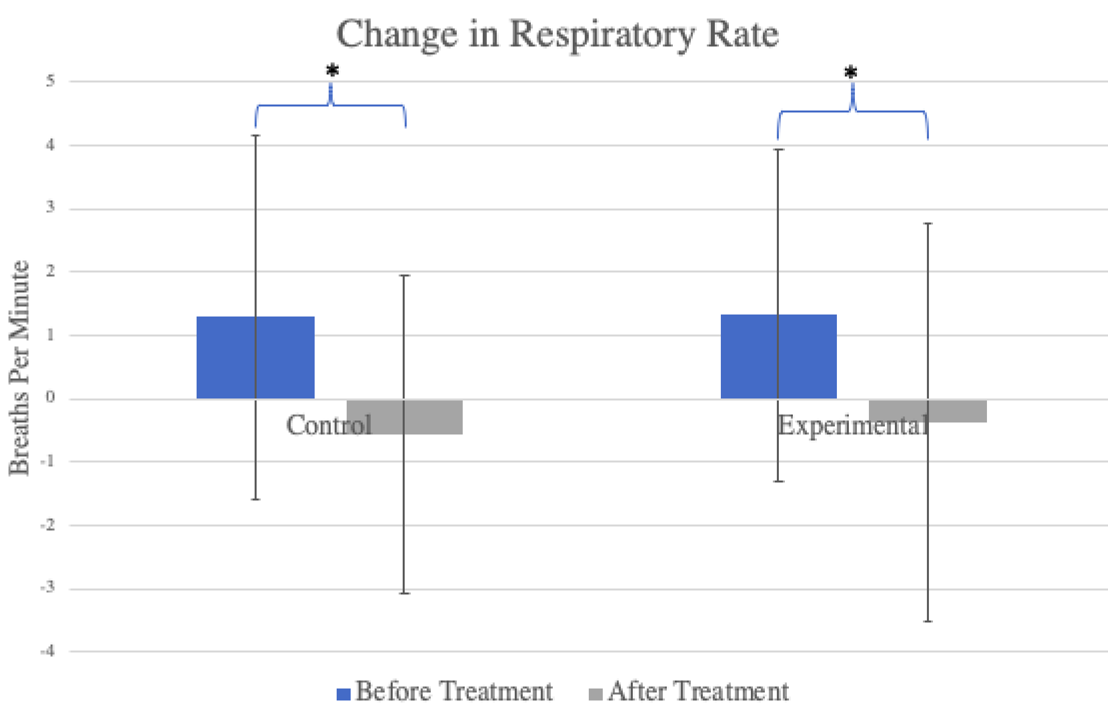

Figure 7: During the math test there was a decrease in respiratory rate before and after treatment for both the control and experimental groups. Control: n = 17 p = 0.05787 | Treatment: n = 18 p = 0.017879

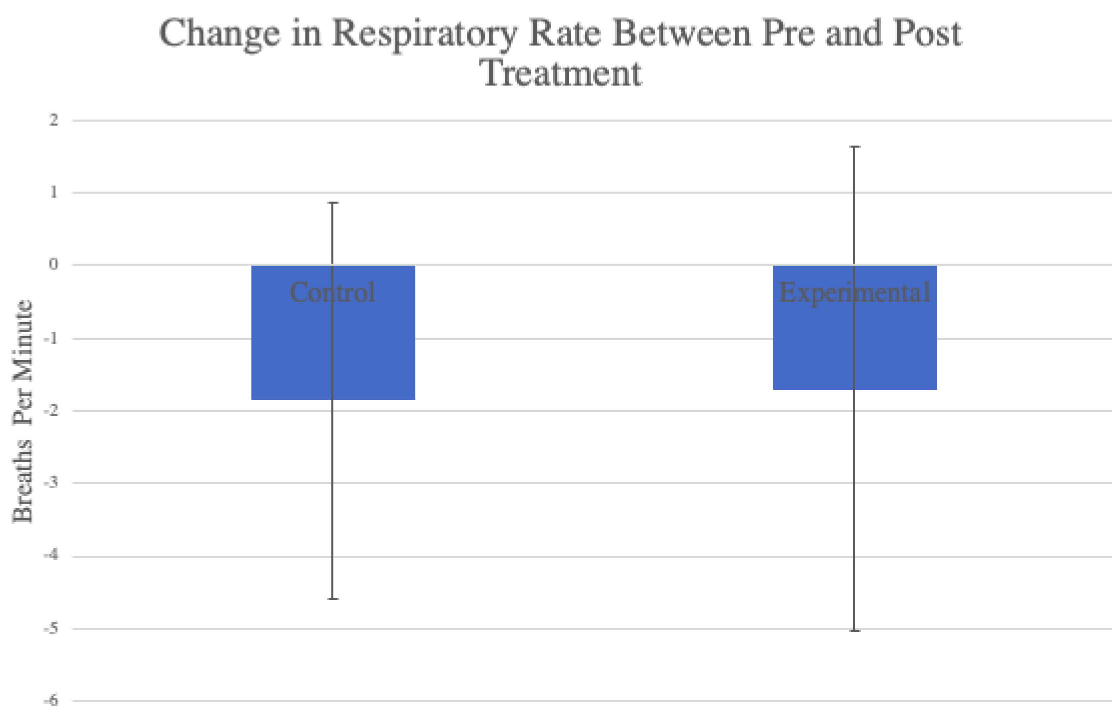

Figure 8: There was no difference between the change in electrodermal activity between the experimental and control group. n = 35 p = 0.888853

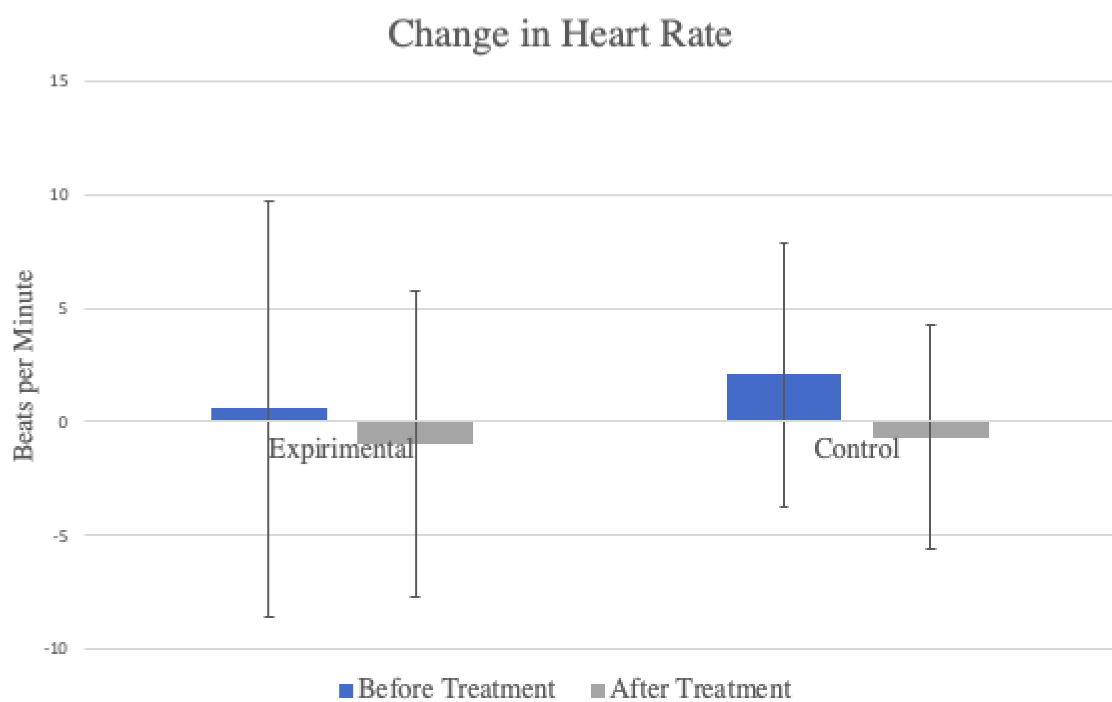

Figure 9: During the math test there was no change in heart rate before and after treatment for both the control and experimental groups. Control: n = 26 p = 0.084588 | Treatment: n = 24 p = 0.478904

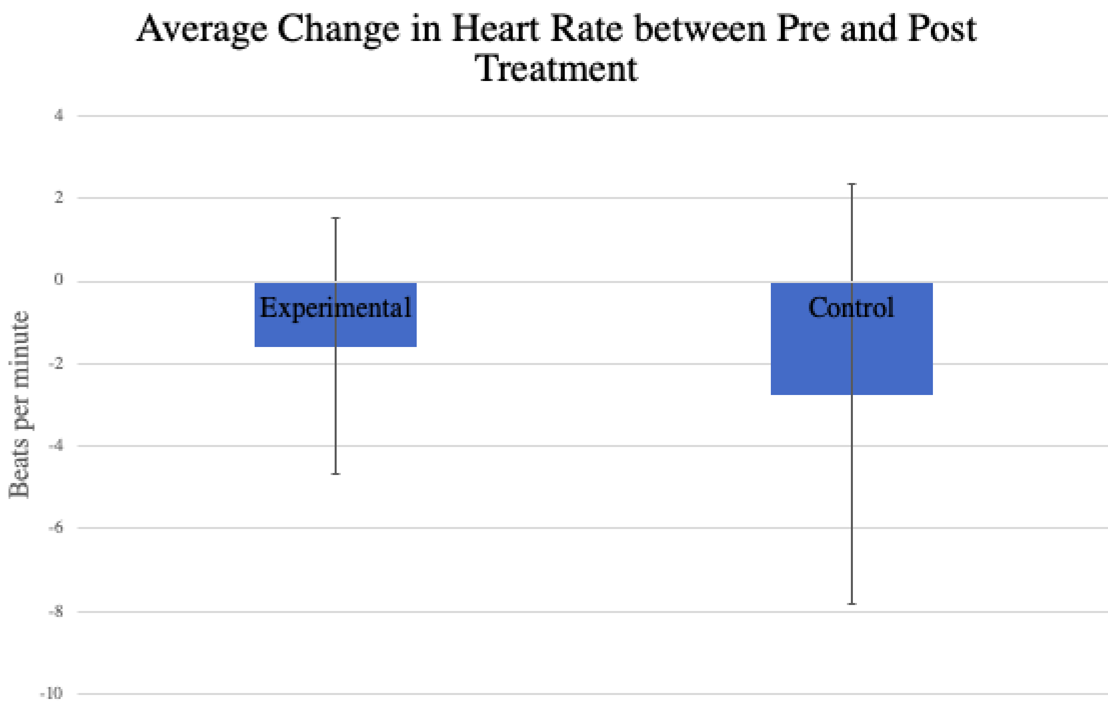

Figure 10: There was no difference between the change in electrodermal activity between the experimental and control group. n = 50 p = 0.361234