

Physiological Effects of Stress Response on Working Memory

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Abstract:

There is currently little scientific consensus on the effects of stress on working memory. This paper sought to assess the immediate impact of a stressful startle stimulus on subjects' working memory. While monitoring heart rate, respiration, and skin conductance, subjects in the treatment group (n=21) performed a working memory test that was followed by an unexpected scream and frightening image. Control subjects (n=18) did not receive the startle stimulus, but a "thank you" message. After receiving the startle stimulus or "thank you" message, subjects completed the working memory test again. The physiological metrics revealed a significant difference in heart rate ($p=0.0002$) and respiratory rate ($p=0.0001$) between treatment and control groups. However, there was no significant difference between skin conductance and memory test performance, with both control and treatment groups showing an average score increase of 2.72 and 2.33 in test scores respectively. While the startle stimulus appeared to evoke a stress response (seen by an increase in heart rate and respiratory rate), the similar performance of control and treatment memory tests suggests no relationship between stress and working memory.

Introduction:

A stressful situation, such as losing a job, approaching deadlines, or financial hardship, can lead to a variety of physiological responses. Breathing rate may increase, the body may tense up, and sweat may begin to appear on the skin. Stress is defined as a state of threatened homeostasis caused by internal or external forces referred to as stressors. These stressors cause various intricate physiological responses in order to restore the body back to a state of equilibrium (Tsigos, 2016). These are part of the body's natural response to stress through the activation the sympathetic division of the autonomic nervous system, often referred to as the fight-or-flight response system. This system evolved as a way for the body to quickly respond to threatening events. However, long-term exposure to stressors can also have detrimental effects on the body. Chronic stress can contribute to high blood pressure, cardiovascular disease, and obesity (Harvard Health Publishing, 2018). Almost 50% of adults routinely experience stress and 75-90% of physician appointments are related to stress. Chronic stress not only leads to an increased risk of heart disease and high blood pressure, but it can also lead to an increased risk for depression, decreased immune function and even reproduction challenges (2015 Stress in America).

If a stimulus is perceived as threatening, a cascade of physiological and endocrine changes occur in order to reestablish homeostasis (Vogel and Schwabe, 2016). When a stressor is sensed by the body, signals are sent to the amygdala. The amygdala is the emotional processing area of the brain and sends signals to the hypothalamus that the body is in danger. The hypothalamus is the master command center of the brain and coordinates neural and endocrine function. After the hypothalamus receives distress signals from the amygdala and other parts of the body, it causes the adrenal gland to release significant amounts of cortisol, a glucocorticoid,

into the bloodstream to have widespread effects on the body. Some of these effects include the regulation of metabolism, inflammatory response, and memory formation (Zandara et. al., 2016). Additionally, the hypothalamus can activate spinal nerves of the sympathetic nervous system which synapse on the adrenal gland, causing the secretion of epinephrine, also known as adrenaline. Epinephrine binds to various adrenergic receptors throughout the body to cause a variety of physiological effects. Some of these effects include constriction of blood vessels, increased glycogen breakdown, and increased heart rate. These responses all prepare the body to fight or flee a potential threatening situation (Tsigos, 2016).

Research has identified hormones and neurotransmitters released as a result of stressful events as major modulators of learning and memory processes. These processes are at the heart of the educational system, thus stress can have critical implications in educational contexts. Studies have shown that moderate to high levels of stress induced in students before an exam decreased their performance (Roozendaal, 2003). There been several studies conducted that have investigated the effects of glucocorticoids on long-term memory, however, there have been few studies that have investigated the effects of stress hormone elevation on working memory. In one study conducted by Luthi et. al., 35 adult subjects were randomly assigned to either a stress or control group. The stress group completed the Trier Social Stress Test (TSST) which consisted of a stress anticipation period followed by a speech and arithmetic test performed in front of an audience. Working memory was assessed before and after the TSST using a reading span test. Results showed that subjects exposed to the stress test had a lower reading span and lower total correct scores on the working memory test than control subjects, showing working memory deficits in association with increased stress levels (Lüthi et. al., 2008). It is known that critical brain areas for cognition and emotion, such as the amygdala, hippocampus, and the prefrontal cortex (PFC), have a high density of glucocorticoid receptors.

However, there are conflicting findings on the effects of various stressors on these regions of the brain and the specific memory processes they modulate (Zandara et. al., 2016). A literature review by cognitive neuroscientist Elizabeth Goldfarb compiles a number of studies which serve as prime examples of research in support of the opposite hypothesis, more specifically the idea that stress can serve as a tool to *enhance* one's memory. Goldfarb references a study in which humans who encoded information in a stressful context showed improved declarative memory as well as enhanced amygdala-hippocampal connectivity (de Voogd et. al., 2017), as well as another in which rats who learned a water maze during a stressor had better memory for the maze than rats who learned in the absence of the stressor (Sandi et. al., 1997). Goldfarb uses these studies and several others to support the inference that the glucocorticoid release induced by stressful stimuli has indirect effects on hippocampal function. By primarily stimulating the basolateral nucleus (BLA) of the amygdala which in turn broadly modulates hippocampal plasticity and signaling, stress hormones can ultimately enhance spatial and declarative memory (Goldfarb, 2018).

Although there is a vast range of existing studies regarding the effects of stress on memory formation, many focus on either how stressors experienced prior to learning negatively affect long term memory, or how stressors experienced during learning positively affect short term memory. There is still varying consensus on the role stress has on working memory. The goal of this study was to analyze how the induction of glucocorticoid release immediately prior to memory formation would affect working memory in human subjects.

In this study we investigated the physiological effects of a startle stimulus on working memory. The three physiological variables measured in this study were heart rate (HR), respiratory rate (RR) and electrodermal activity (EDA). Heart rate has long been understood as a natural response to stress due to the activation of the sympathetic nervous system and the subsequent release of epinephrine. Thus, it is expected that HR will increase when stressed. RR is also affected by stimulation of your sympathetic nervous system. During a threatening situation, there is an increasing demand for oxygen in order for muscle contraction and energy production to occur. Therefore, it is expected that RR will increase when faced with stress (Widmaier et. al., 2015). EDA can be a measure of physiological arousal. The skin temporarily acts as a conductor of electricity due to sweat gland activity when the sympathetic nervous system is activated by an external stimulus. The conductance between electrodes placed on the skin can be quantified and used as a measure of sympathetic activity. It is expected that conductance will increase with stress due to the activation of the sympathetic nervous system (Beissner et. al., 2013). Additionally, this study also assessed working memory (WM). WM has been defined as the PFC's ability to retain temporary information and manipulate and process that information in order to carry out complex cognitive tasks (Zandara et. al., 2016).

We hypothesized that exposure to a startle stimulus will increase heart rate, respiratory rate and skin conductance and decrease performance on a working memory test. Investigating the impacts of a startle stimulus on working memory could help further our understanding on how stress impacts various memory and learning processes and could potentially lead to changes in educational systems.

Materials:

In this experiment, three variables were measured in order to determine the effects of a startle stimulus on working memory. Respiratory rate (RR), skin conductance (EDA), and heart rate (HR) were measured using three Biopac devices. Respiration, in breaths per minute, was measured using a Biopac Respiratory Transducer attached directly across the chest of the participant (Model: SS5LB, Biopac Systems, Inc. Goleta, CA). Skin conductance, in microsiemens (μS), was measured using BSL EDA finger electrodes in conjunction with a Xdcr lead set placed on the index finger and middle finger of the participant's non-dominant hand (Model: SS3LA, Biopac Systems, Inc. Goleta, CA). Heart rate was measured in beats per minute (BPM) using a Biopac Electrode Lead Set (Model: SS2LB, Biopac Systems, Inc. Goleta CA) and Biopac Disposable Electrodes (Model: EL503, Biopac Systems, Inc. Goleta CA) placed at both

ankles and the right forearm of the participant. Using Biopac Student Lab System (BSL 4 Software, MP 36) HR, RR and skin conductance were analyzed. The Biopac Systems, Inc. Student Manual (ISO 9001: 2008, Biopac Systems, Inc.) was used as a reference for analysis. Working memory was tested before and after the participant completed a simple startle game. The startle game is a program that randomly assigned subjects to control or treatment. Subjects pushed indicated directional keys that continuously decrease in size throughout the trial. After 30 seconds, subjects assigned to the control group received a thank you message, and the treatment group received a loud female scream and flashed image of a clown. Working memory was tested before and after the startle game using an online working memory test hosted at Open Cognition Labs (<http://opencoglab.org/memtest1/>). Two standard Dell computer desktops were used for the study. One computer was used to monitor all Biopac data and the other computer was used by the participant to complete the working memory tests and the startle game.

Methods:

Participants

Students from the University of Wisconsin-Madison Anatomy and Physiology 435 lab course were asked to participate in this experiment. All data was collected at the university's Medical Sciences Center. Before any experimentation was performed or any equipment was set up, participants were given a consent form stating the potential risks. Once the consent form was signed and filed, participants were able to participate in a double blind study where the participants first completed a working memory test and then a simple startle game. The game randomly selected which participants were given the startle stimulus and which participants were given a thank you message. Participants were then asked to complete the same working memory game they encountered before the startle game. All students only participated once to prevent previous knowledge of the working memory test and the startle stimulus.

Procedure

Prior to testing, Biopac equipment was set up as follows: the ECG electrode leads were connected to the Biopac Student Lab System on the first computer, the respiratory transducer was connected, and the BSL EDA finger electrodes were connected. Computer one was positioned out of the visual field of the participant to prevent them from altering their respiratory rate during testing. The Biopac program was opened on the computer one. In the program, Electrocardiogram (ECG) 0.5-150 Hz was selected for channel 1, Respiration SS5LB was selected for channel 2, and Electrodermal Activity (EDA) SS3LA 0-35 Hz was selected for channel 3. The respiratory transducer monitor was attached to the middle of the upper chest of the participant. BSL EDA finger electrodes were placed on the index finger and middle finger of the participant's non-dominant hand. Electrode patches from the ECG Biopac Electrode lead set were placed on the ankles and right wrist of the participant. The first electrode was placed on the medial surface of the right leg just above the ankle with the black ECG attached, the second

electrode was placed on the medial surface of the left leg just above the ankle with the red lead attached, and the third electrode was placed on the right anterior forearm at the wrist with the white lead attached. All Biopac equipment was calibrated according to the Biopac Student Manual after the participant was connected to all Biopac devices and before initial readings were conducted. The experiment took place in a private room in order to minimize distractions. Two researchers remained in the room during the experiment. One researcher was responsible for ensuring measurements were being recorded on computer one for the duration of the study and had the ability to stop the experiment if the participant did not feel comfortable continuing. The other researcher was responsible for instructing the participant of the steps of each test and helping the participant navigate from test to test. The participant was instructed to sit on a chair directly in front of computer two with both feet on the floor. The participant was informed of the instructions of the working memory test (**Figure 1**). An initial recording of HR, RR and skin conductance was conducted for 30 seconds after instructions were given in order to ensure the Biopac equipment was working properly. Data collection began when the participant hit the start button on the working memory test. After the working memory test was completed, the participant was instructed to navigate to the startle game by explaining they were going to be completing another memory test. Data collection continued as the participant completed the startle game, where the participant clicks different arrows presented on the screen and after 30 seconds the program displays a “startle”. The startle is randomized and is either a sudden 1 second long female scream with an image of a clown flashed on the screen or a “thank you for your participation” message, which was used as a negative control. The participant was then instructed to navigate to the last memory test, which is the same test they took prior to the startle game. Upon completion of the last memory test, data collection was stopped and the participant was assisted with the removal of all Biopac equipment. A post-experiment questionnaire was completed in order to determine participants’ previous exposure level to startling stimuli, as well as how they typically respond to stimuli that may evoke fear. A more detailed representation of the experimental time frame can be seen in **Figure 2**.

Data Analysis

Following the experimental period, participant data for the three variables (HR, RR, and skin conductance) were logged for analysis from the Biopac program in order to compare the differences between baseline and post-startle stimulus measurements between the group of participants that received the startle image and the group of participants who received the “thank you” message. In order to analyze the HR of each participant, the average heart rate during the first minute of the first memory test was recorded as the baseline measurement. The average heart rate during the first minute of the final memory test was recorded the post-startle measurement. The RR baseline measurement was recorded as the total number of breaths taken in the first minute of the first memory test by counting the number of peak-trough pairs. The RR post-startle measurement was analyzed in the first minute of the final memory test. The skin

conductance baseline measurement was recorded as the maximum EDA value in the first minute of the first memory test. The skin conductance post-startle measurement was recorded as the maximum EDA value in the first minute of the final memory test. The absolute changes between baseline and post-startle measurements were calculated. A sample biopac screen showing HR, RR, and EDA before and after the startle stimulus is shown in **Figure 3**. In addition, the participants' scores in the working memory test both before and after the startle game were logged in order to compare potential differences. An unpaired t-test was used to determine if the scores between the group that were exposed to the startle image and those who received the "thank you" message were significant. An additional unpaired t-test for was used to determine the difference in baseline and post-startle measurements for HR, RR and skin conductance between the two groups to analyze if there was a significant difference in physiological parameters between the two groups. *Positive Controls*

To determine that the Biopac equipment was recording accurate measurements, positive control tests were performed by the researchers. First, baseline measurements for RR, HR and skin conductance were taken at rest for two minutes. These resting averages were found to be 13.67 ± 0.88 breaths per minute, 62.221 ± 6.108 beats per minute (BPM), and 2.560 ± 0.12 microsiemens (μS), respectively. The second set of measurements were taken after 30 seconds of running in place. These averages were 20.33 ± 1.45 breaths per minute, 88.938 ± 14.177 BPM, and 3.71 ± 0.70 μS . All of the measurements recorded increased after the short period of exercise as expected. This demonstrated that the equipment was functioning properly and the measurements of the various physiological parameters tested in this experiment are present.

Negative Controls

The researcher-designed startle game randomly assigns subjects to control and treatment groups. After 30 seconds of active game time, the program will display a "thank you for your participation" message for negative control subjects, and they can proceed to the last memory test. The message is meant to not invoke any physiological response in the participant, unlike the startle stimulus the treatment group receives.

Results:

A total of 39 participants completed the study. 21 participants received the startle stimulus (treatment group) and 18 participants received the "thank you" message (control group). A series of two-tailed T-tests were used to compare the differences between baseline and post-startle stimulus of each of the three physiological parameters and memory tests scores between treatment and control groups. A p-value 0.05 or less was considered significant when testing parameters of this experiment. Change in heart rate (after treatment - baseline) for each of the participants is shown in **Figure 4**. The average change in heart rate for the treatment group was 10.25 beats per minute (BPM) (SD=9.97). The average change in heart rate for the control group was -0.02 BPM (SD=3.59). The average change in heart rate over the course of the

experiment for treatment and control groups can be seen in **Figure 5**. There was a significant difference in change in heart rate between treatment and control groups ($p=0.0002$). Change in respiratory rate (breaths per minute) for each of the participants is shown in **Figure 6**. The average change in respiratory rate for the treatment group was 3.67 breaths per minute ($SD=2.99$). The average change in respiratory rate for the control group was 0.17 breaths per minute ($SD=1.82$). There was a significant difference in change in respiratory rate between treatment and control groups ($p=0.0001$). Change in electrodermal activity (EDA) in μS for each of the participants is shown in **Figure 7**. The average change in EDA for the treatment group was 0.49 μS ($SD=0.59$). The average change in EDA for the control group was 0.20 μS ($SD=0.61$). There was no significant difference in change in EDA between treatment and control groups ($p=0.1504$). Change in working memory test scores for each of the participants is shown in **Figure 8**. The average change in test scores for the treatment group was 2.33 points ($SD=5.08$). The average change in test scores for the control group was 2.72 points ($SD=3.79$). There was no significant difference in working memory test scores between the treatment and control group ($p=0.7909$).

Discussion:

Research has identified hormones and neurotransmitters released as a result of stressful events as major modulators of learning and memory processes. However, there are varying conclusions in scientific literature about the effects of these stress modulators on working memory. This experiment tested the effects that stress has on working memory by measuring the change in scores on a working memory test after participants were exposed to a startle stimulus. Heart rate, respiratory rate and electrodermal activity were also measured. It was hypothesized that HR, RR and EDA would increase, and performance on the working memory test would decrease after being exposed to a startle stimulus.

The results showed that there was a significant difference in change in heart rate between the group who received the startle stimulus (treatment) and the group who received the thank you message (control) as seen in **Figure 4**. The group that received the startle stimulus had a significantly higher change in heart rate than the group that did not. Similarly, the change in respiratory rate between the two groups was also significant as seen in **Figure 6**. These findings support the hypothesis that the sympathetic nervous system is activated in the presence of a startle stimulus when the body perceives that there is a threat to homeostasis. This is shown as an increase in HR and RR due to the activation of β_1 -adrenergic receptors in the SA node by norepinephrine from the sympathetic postganglionic fibers.

There was no significant difference between control and treatment groups when electrodermal activity was analyzed. Although it was hypothesized that EDA would increase in participants subjected to a startle stimulus due to the activation of the sympathetic nervous system, the lack of significant results could be attributed to experimental setup error. Skin conductance gel was placed on participant's fingers prior to attaching EDA sensors. However,

there was no set amount of gel that was consistently used among participants. Additionally, there was no set tightness of the sensor bands that was consistently used between participants. Variability within either of these factors could have led to the change in EDA between groups being insignificant. The amount of gel used may have been excessive or insufficient to properly sense the change in electrodermal activity among test subjects. Or, in the second case, the variability in sensor bands tightness may have led to improper recording of EDA. Future studies should attempt to standardize the amount of gel used and the tightness of sensor bands in order to reduce variability and ensure accurate data collection.

Additionally, there was no significant difference in the change of memory test scores between treatment and control groups. **Figure 8** shows that the average change in test scores between treatment and control groups was greater than 1, showing that on average most participants scored better on the second working memory test. The insignificant difference in change in test scores between the two groups does not allow us to conclude that working memory is affected by the induction of stress. Additionally, the results show the change in test scores are relatively equivalent and leads us to believe that there were several limitations present in the experimental procedure.

One such limitation was the working memory tests that were administered to each participant. This experiment required participants to complete the same working memory test two times, which could have influenced the difference in test scores between the first and second attempt. Scores could have increased from the first to the second test since participants had more familiarity with the test. They could have had a better understanding of the directions and possibly learned some of the patterns within the test, raising their scores. In order to further this research to see if working memory is truly not influenced by stress induction, different types of working memory tests should be utilized.

Additionally, with a large number of participants comes a large range of preferences towards or against horror media. Some people find horror films more enjoyable than others. In the experimental post survey, which asked each participant how much they enjoy horror media on a scale of 1 to 5, 25.7% of participants expressed enjoyment of horror media (rating enjoyment as a 4 or a 5), while 54.3% did not (rating enjoyment as a 1 or a 2). A preference or enjoyment of scary media could factor into an individual's physiological response to the startle stimulus presented in the experiment. If a participant found the startle stimulus to be more enjoyable than startling, their stress levels (and thus their heart rate, respiration rate, and electrodermal activity) may not have increased significantly enough to cause an impact on their working memory test performance. To improve upon this and ensure more consistency amongst test subjects, a survey could be done before experimentation, and those who reported that they enjoyed horror media would then be excluded or used as a control group for the experiment. Alternately or in addition to limiting test subject fear preference variability, a more drastic startle with louder sounds and the utilization of headphones to limit distractions from the surrounding

environment is needed in order to maintain consistency and ensure a startle response in every participant regardless of how much they enjoy horror media.

Lastly, the diversity of experimental participants presented a limitation to our experimental findings. The sample group used for this experiment was exclusively chosen from the UW-Madison's Physiology 435 class list. This resulted in very uniform demographic of participants, where most were 21 year-old, college students. The external validity of the results of this study may have been compromised by this fact, meaning the data presented above cannot be considered highly representative of a wider population. Further experimentation using a more diverse demographic of test subjects would be necessary in order to make inferences regarding effects of stress on working memory in the broader population. Additionally, a larger sample size is necessary in order to reduce standard deviation of physiological variables tested within treatment and control groups.

The results in this research show that inducing stress through the administration of a startle stimulus increases respiratory rate and heart rate. However, further research is still necessary in order to conclude whether working memory is affected by the induction of stress and if so, whether this induced stress has positive or negative impacts on working memory capacity in human subjects. Experimentation with a larger, more diverse sample and a more stressful startle stimulus could help determine whether stress has an impact on working memory. Further research is essential, as it could help us better understand implications of stress in situations where working memory is utilized and lead us to provide a more suitable environment in these situations, such as in educational settings.

This research can expand into the broader context of physiology because it is crucial for us to understand the exact effects of stress on our memory function. This is important since stress has many different implications in learning processes, and thus can have large impacts in educational settings. Research on how stress affects memory functioning can help us better understand the best environment for students to learn in. In understanding how and to what extent stress impacts our working memory, we can develop new goals to optimize learning environments and teaching strategies to maximize students' abilities to retain information. This is especially crucial for children and young adults whose brains are still developing and can be highly influenced by stressors. Overall, this research could ultimately help us change the educational system in order to benefit students in the best possible way.

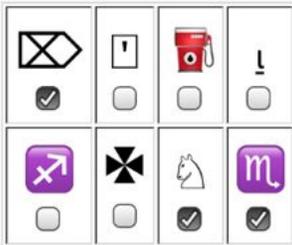
Appendix

Test procedure

In the test you will be shown a series of symbols and have to remember them. For example, one series might consist of the following four items:



These items would be shown to you one at a time, for about one second each, then you would be shown a group of eight items and asked to remember which of those eight you just already saw in the sequence. For example, here are possible answers for the example sequence (the correct ones have already been marked):



It is important that you mark down items that were in the series, but it is also important not to mistakenly say you saw an item when you did not. Each item you correctly identify as having seen in the series is worth one point, for each item you claim to have seen but did not actually, you lose a point.

In the full test you will do this (see a series, then have to remember what items were in it) 21 times. It should take most people 4 to 8 minutes to complete the test.

Interactive test

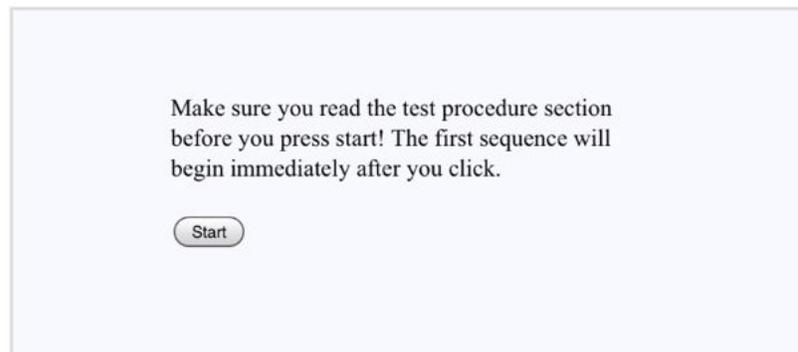


Figure 1. Directions for the working memory test.

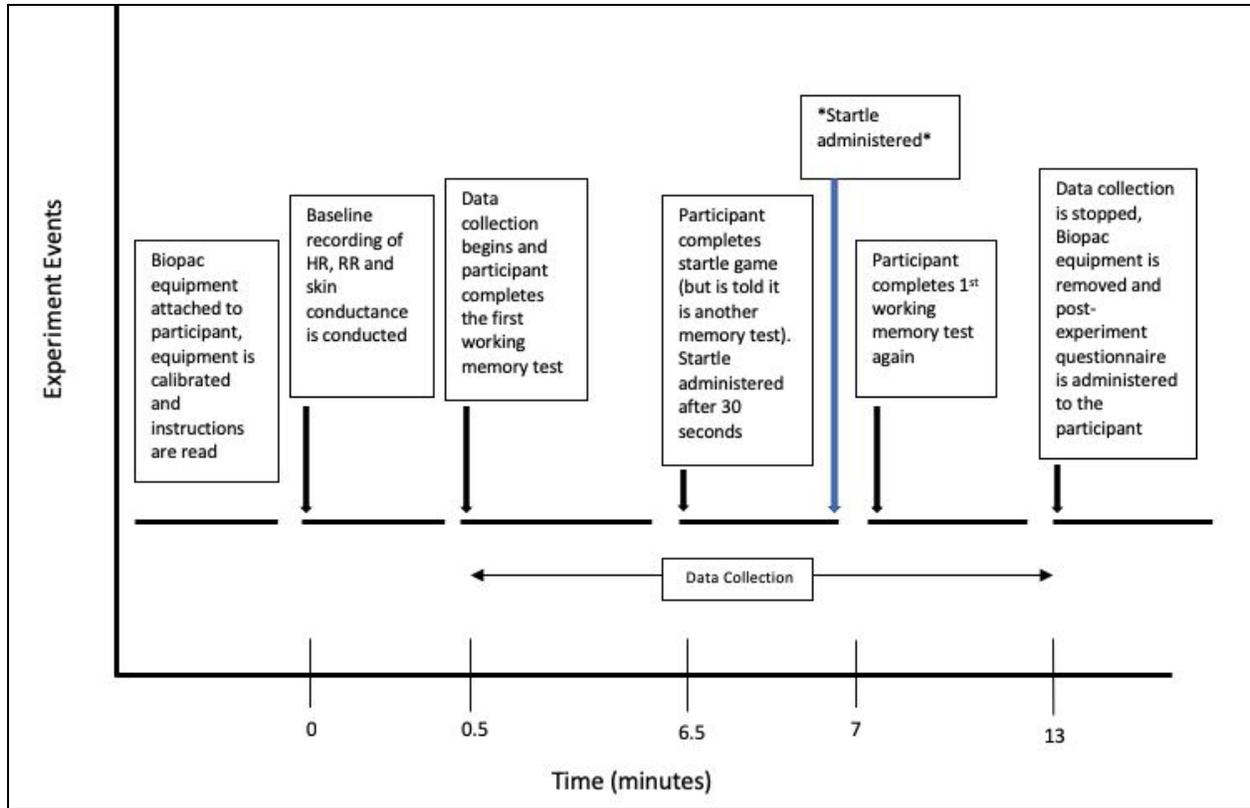


Figure 2. Experimental timeline.

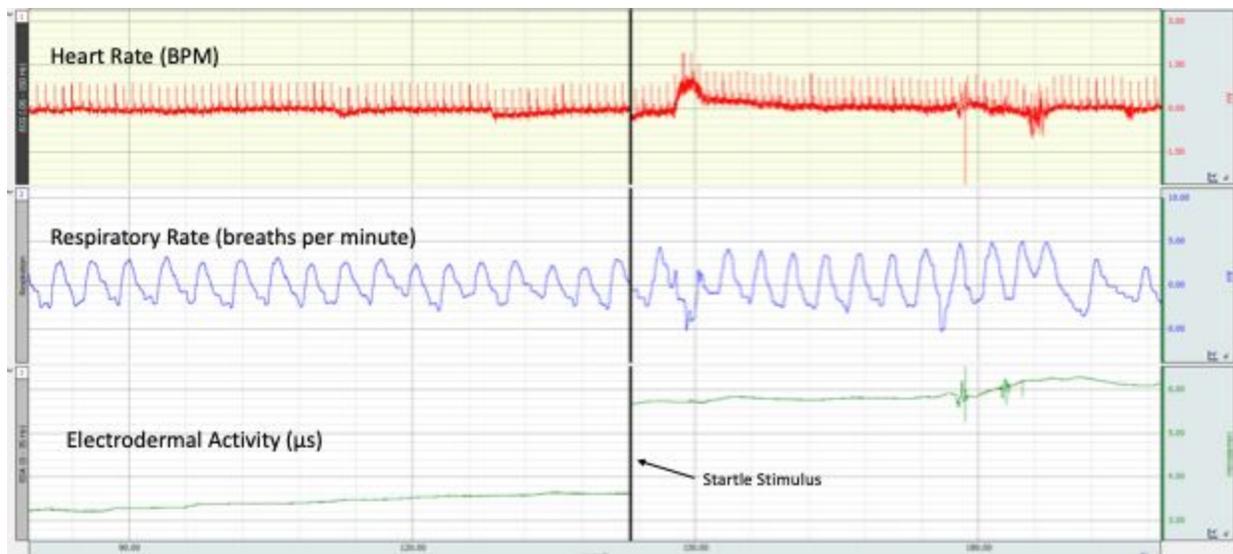


Figure 3. Example of one trial from the Biopac program that was analyzed for HR, RR and EDA before and after startle stimulus (differentiation between before and after indicated by the black line).

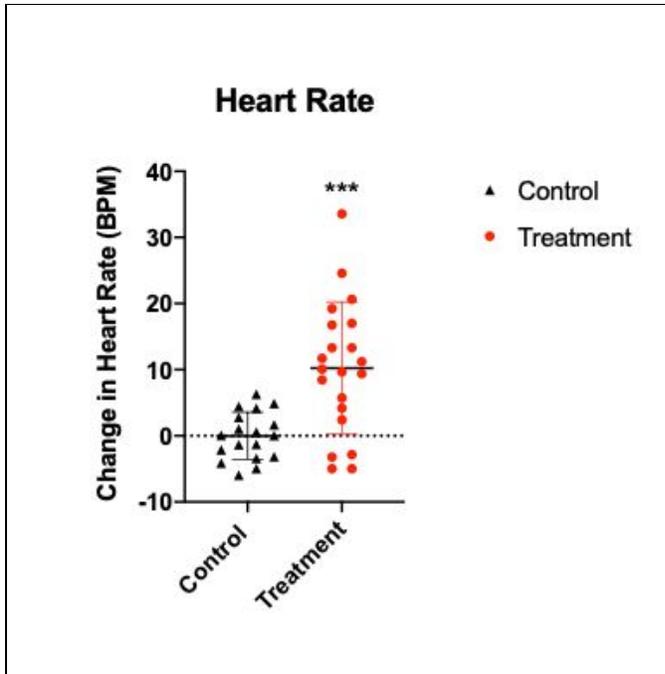


Figure 4. Change in heart rate from baseline to after startle stimulus or thank you message was administered. Change was calculated by subtracting the baseline heart rate from the heart rate after the startle stimulus or thank you message. Difference between treatment and control groups was significant ($p=0.0002$).

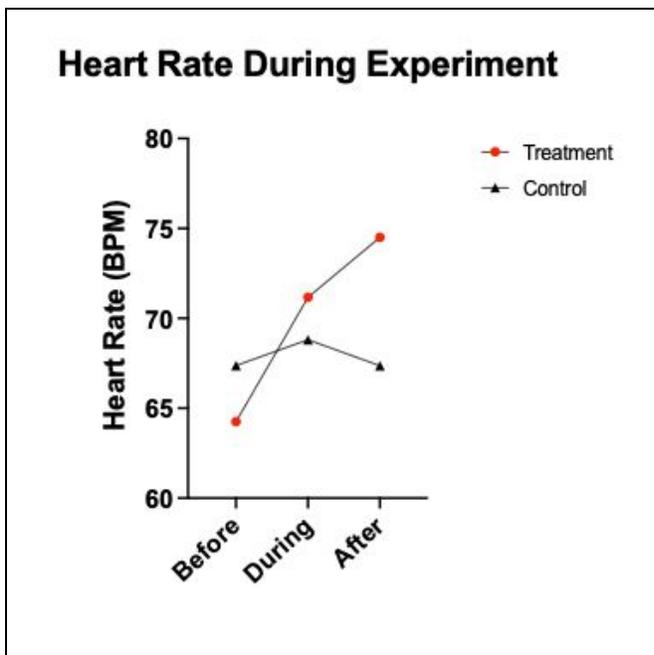


Figure 5. Change in heart rate for treatment and control groups over the course of the experiment.

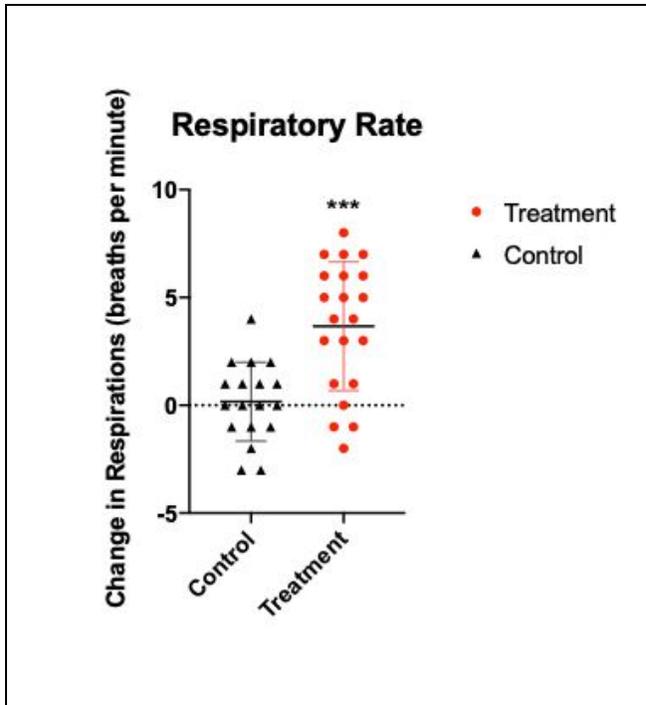


Figure 6. Change in the number of respirations from baseline to after startle stimulus or thank you message was administered. Change was calculated by subtracting the baseline respiratory rate from the respiratory rate after the startle stimulus or thank you message. Difference between treatment and control groups was significant ($p=0.0001$).

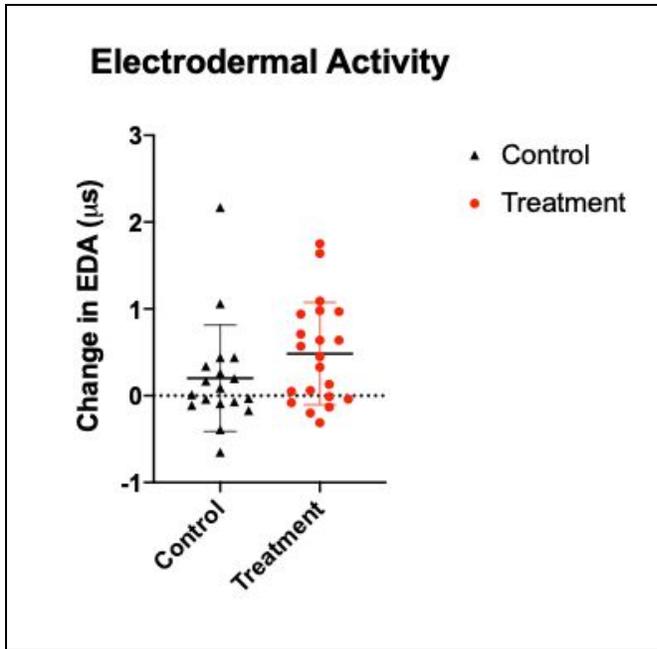


Figure 7. Change in the electrodermal activity from baseline to after startle stimulus or thank you message was administered. Change was calculated by subtracting the baseline EDA from the EDA after the startle stimulus or thank you message. Difference between treatment and control groups was not significant ($p=0.1504$).

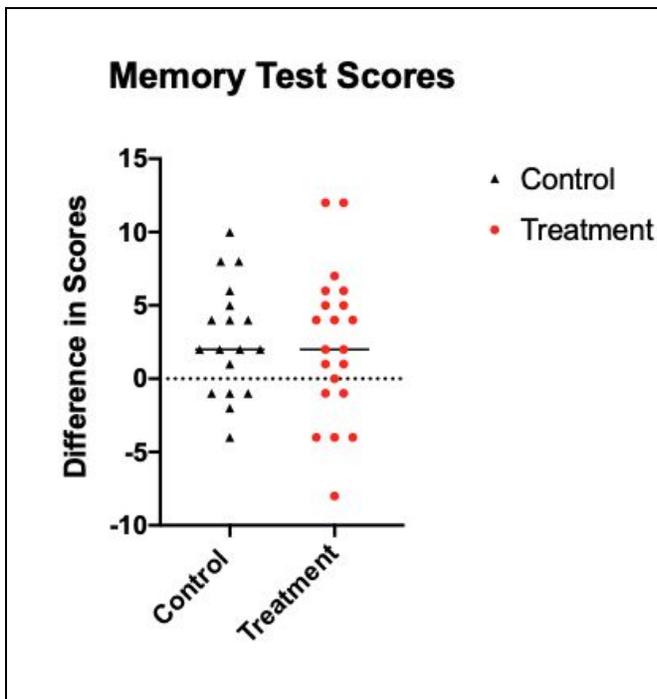


Figure 8. Change in test scores from first working memory test to second memory test (taken after the startle stimulus or thank you message was administered). Change was calculated by subtracting the score of the first working memory test from the score of the second working memory test. Difference between treatment and control groups was not significant ($p=0.7909$).

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