Physiological Startle Response to Frightening Stimuli in Light and Dark Environment

Breanna Adkins, Elise Brennan, Hailee Hoffmann, Krishna Patel and Collin Schmit

KEYWORDS: Startle Response, Fear, Dark, Heart Rate, Masseter EMG (electromyography), IAPS Images (International Affective Picture System), Electrodermal Activity (EDA)

Abstract

Fear is an emotional response that humans experience in situations that are perceived as dangerous and is accompanied by physiological responses that would protect the body from potential harm. Different environments, such as darkness, can elicit altered responses in this physiological change because of the vulnerability and insecurity humans experience when in that situation. The purpose of our experiment was to determine if there is a significant difference in the physiological startle response in a dark environment compared to a light environment. We had 50 students from the UW-Madison Physiology 435 course participate in the study in which their heart rate (HR), electrodermal activity (EDA), and electromyography (EMG) were analyzed. They were shown a 2 minute video of ten cards being flipped over one-by-one, and were told they were performing a memory test. At the 1 minute mark, an emotionally provocative International Affective Picture System (IAPS) image appeared while paired with a high-pitched shriek. Participants were randomly selected to watch the video in the dark or in the light. Since it has been found that humans feel increasingly vulnerable around potentially threatening stimuli in dark environments, it was hypothesized that the participants who watched the video in the dark would have a greater startle response than those who watched in the light. Our results showed that there was no significant difference in heart rate, EDA, and EMG for participants startled in the dark and light conditions. However, we did find an unexpected significant difference in masseter EMG between female participants in the light and dark conditions. Future studies could investigate gender differences as well as a wider, more randomized population in order to explore different demographics and maintain research confidentiality. Lastly, our methods could be improved in the future by not allowing subjects to see the testing room before conducting the study and having the lights off when subjects tested in the dark enter the room.

Introduction

It is a basic characteristic of humans to react fearfully in conditions that are perceived as dangerous. Humans feel increasingly vulnerable in dark environments because they are unable to detect who or what is around them, thus enhancing their startle response to a stimulus perceived as frightening (Grillon, 1998). Fear functions as a signal of threat, which triggers adaptive

responses in the body to protect us from potential harm (Steimer, 2002) There are many frightening stimuli one can experience, but exposure to both audio and visual stimuli together induce a larger startle response than when exposed to a single stimulus (Taffou et al. 2013).

The adaptive responses in the body due to a frightening stimulus can be summarized as a rapid activation of the sympathetic nervous system by the amygdala, the area of the brain that perceives fear (Holand et al., 1999). The activation of the sympathetic nervous system is often termed the 'fight or flight' response, and causes physiological changes in the body that would be advantageous in a challenging or startling situation such as increases in heart rate (HR) and electrodermal activity (EDA) (Steimer, 2002). The sympathetic nervous system has been shown to directly impact muscle spindle activity (Jull, 2008), which would be expected to cause an increase in the clench force of the masseter electromyography (EMG) when startled. Furthermore, a study conducted by Valls-Solé *et al.* demonstrated it is difficult for subjects exposed to startling stimuli to avoid masseter activity (1995), thus suggesting that this EMG is expected to increase during a fear response. Increased heart rate, another indicator of an active sympathetic nervous system, is also expected to occur after a subject is exposed to a sudden, startling sound (Holand et al., 1999). Finally, it has been shown that electrodermal activity increases involuntarily in response to startling stimuli (D'Hondt et al., 2010). In our study, we elicited a startle response using an audiovisual stimulus of a screaming woman, which has been used by similar studies to elicit a startle response (Klumpers et al., 2015). The visual component of this stimulus was a negatively valenced high arousal image from the International Affective Picture System (IAPS), which is a standardized and validated set of images available for researchers that have been shown to elicit specific emotional responses (Lang & Bradley, 1997).

We hypothesized that there would be a greater increase in HR, EDA, and masseter EMG on subjects tested in the dark condition over those in the light when the negatively valenced high arousal IAPS image and audio were presented on the screen in the middle of the video. By analyzing these three physiological variables, we were able to determine whether darkness impacted the startle response of subjects exposed to the frightening audiovisual stimulus.

Materials

Heart rate (HR), electrodermal activity (EDA), and electromyography (EMG) of the masseter were simultaneously measured using three separate quantifying systems. Heart rate was measured using BSL electrodes to record the electrocardiogram (Model number: SS2L; Biopac Systems, Inc. Goleta, CA). Masseter EMG was also measured using BSL electrodes to record millivolts (mV) (Model number: SS2LA; Biopac Systems, Inc. Goleta, CA). Vinyl, 1⅜" electrode disks (Model number: EL503, Biopac Systems, Inc. Goleta, CA) were used in tandem with the above systems. BSL EDA finger electrodes (Model number: SS3LA; Biopac Systems, Inc. Goleta, CA) were used to measure skin conductance with Isotonic Recording Electrode Gel (Model #:101, Biopac Systems, Inc. Goleta, CA) to record the data in microsiemens. The Biopac Student Lab System (BSL 4 software, MP36) and the Biopac Systems, Inc. Student Manual (Biopac Systems, Inc. ISO 9001:2008) were used for data analysis, recording, and equipment set-up.

A two minute, twelve second video was presented during the experiment in order to test the startle response. The video was created using iMovie (Version 10.1.1, Apple Software, Inc. 2011) and contained a recording of playing cards being flipped over slowly and an approved IAPS image 1300 (Lang et al. 2008). First, a black screen was presented for roughly ten seconds,

then ten cards were placed face down on an empty table. One-by-one, they were slowly flipped face up and then flipped again to face down. The IAPS emotionally evocative image then appeared for two seconds before the card sequence was continued. Audio used in the video was provided by iMovie as well as a YouTube video titled "Scary Maze Game" (ryanjyn2008, 2007), and presented to subjects using a pair of Beats Solo3 Wireless headphones. This video was presented in order to capture the participant's attention, obtain their baseline measurements, and measure their physiological response when startled.

Methods

Participants and Consent

Participants were recruited from the University of Wisconsin-Madison Physiology 435 class. Before participation, the students were required to read and sign a consent form which described the confidential nature of the study and the potential risks for participants with heart problems, claustrophobia, and fear of the dark. This consent was obtained prior to participants entering the testing room. The participants were then divided by ABAB randomization into groups: those tested in a light environment and those tested in a dark environment. Each participant completed the experiment only once.

Experimental Set-Up and Data Acquisition

Participants were escorted into a small, windowless room containing the necessary Biopac instruments, desktop computer, and laptop with the experiment video. The Biopac instruments were adjusted prior to each experiment as follows: "Electromyogram" (EMG, 5-500 Hz) was selected for masseter movement, measured in millivolts (mV), on channel 1, "Electrocardiogram" (ECG, 0.5-35 Hz) for heart rate, measured in beats per minute (BPM),

using channel 2, and "Electrodermal Activity" for skin conductance, measured in microsiemens (μS), on channel 3. In addition to this setup, each instrument was single-point calibrated prior to collection of data to ensure proper measurements. Subjects were assisted in attaching six Biopac electrode disks: one on the right posterior side of the neck, two along the side of the right masseter, one on the inside of the left wrist, and one on each side of the inner ankles (**Figure 1**). Two EDA finger electrodes were placed on the participants right index and middle fingers with Isotonic Recording Electrode Gel. Participants were instructed to sit still with their feet flat on the floor and palms resting on their thighs facing upwards, and the correct wires were connected to their respective electrode disks. Finally, a pair of Beats Solo3 Wireless headphones were placed on the participant and were set to a predetermined volume of approximately ½ strength.

Participants were instructed to watch a two-minute video that would test their working memory. The experimenter informed the participant whether they were going to be tested in the dark or the light, and started the video and recording physiological data simultaneously. As the experimenter exited, they would either leave the lights on or turn them off. The video had a length of two minutes and twelve seconds, with the first ten seconds being a black screen so the experimenter could start the video and Biopac recording and have time to exit the room. For one minute after the black screen, a video of an experimenter flipping over ten cards to the sound of iMovie piano music was shown. Then, a startling audiovisual stimulus of a screaming woman (ryanjyn2008, 2007) as well as IAPS image 1300 were presented for two seconds at the 69 second mark. The last minute of the video was similar to the previously described card flipping video, but this time with different cards. An experimenter remained by the door with a timer, and returned to the testing room as soon as the experiment concluded. Immediately upon returning to the testing room, Biopac recordings were stopped. The experimenters then helped

the participant disconnect the physiological equipment, and debriefed them. Participants were administered a short follow up survey that asked them to indicate their age and gender. Participants were thanked for their participation, were reminded to maintain confidentiality, and were escorted out of the testing room. A timeline of the experiment can be seen in **Figure 2.**

Data Analysis

Each participant's test measurements were organized and analyzed in Google Sheets. The Google Sheets document contained subject number, experimental condition, gender, age, and physiological data. Continuous physiological data recordings were taken during the whole duration of the video. The physiological data collected for the baseline condition were average heart rate (BPM), average peak to peak EMG amplitude (mV), and average maximum EDA (μS).

The experimental data collected were measurements from when the startle stimulus occurred to ten seconds after; data collected were heart rate (BPM) (**Figures 3A and 3B**), maximum peak to peak EMG amplitude (mV) (**Figure 4**), and maximum EDA amplitude (μS) (**Figure 5**). This ten second window, from seconds 70 to 80, was chosen because it most reliably contained the peak reaction to the startling stimulus without outside noise. The baseline data collected were similar measurements but were taken from seconds 15 to 45 to acquire average resting EMG signal, average resting heart rate, and average resting EDA. Change in physiological data was calculated by taking the difference between the experimental recording and the baseline recording, and the standard errors were calculated based on these changes. Two sample t-tests were conducted on the calculated differences between baseline and experimental values by comparing the dark condition to the light condition. P-values $\leq .05$ were considered significant for this study. All statistical analyses were completed in R (R Core Team, 2016) and RStudio (RSTudio Team, 2012). Linear regression models (using R's lm function) were used to

identify main condition interactions. Subsequent analyses were conducted using linear regression models which controlled for age and sex.

Positive Control

The study team conducted a brief pilot study to ensure that EMG, EDA, and ECG changes could be adequately recorded. To accomplish this, each study member had resting measurements taken for one minute. Then, the researcher performed jumping-jacks for thirty seconds to increase heart rate and skin conductance. The researcher was quickly reconnected to the biopac equipment and measurements were again taken for one minute. Once recording was restarted, the researcher was instructed to briefly clench their jaw to check the masseter EMG. To compare baseline and experimental results, heart rate (BPM), EDA (µS), and EMG (mV) from the baseline were compared to the maximum values from the experimental condition. Mean change in heart rate was 25.15 ±2.308 BPM, mean change in skin conductance was 1.32 ±0.245 µS, and mean change in masseter EMG was 0.65 ±0.228 mV. Complete data from this experiment can be found in **Figure 6**. These results indicated that the study team was able to record changes in EMG, EDA, and ECG.

Negative Control

The initial minute before the frightening audiovisual stimulus occurred was used as a negative control, and was performed in the light or dark environment to which they were assigned. These measurements were collected without experimental manipulation. These data showed that the Biopac system was able to collect data accurately when there was no stimulus present.

Results

During the startle task, average change in peak to peak EMG amplitude was 0.239±0.048 for subjects viewing the task video in the light, and 0.289±0.164 for subjects viewing the task in the dark (**Figure 7A)**. Average change in heart rate was 3.2±2.069 beats per minute for subjects viewing the task video in the light, and 1.6±1.399 beats per minute for subjects viewing the task in the dark **(Figure 7B)**. Average change in EDA was 0.675±0.111μS for subjects viewing the task video in the light, and 0.551±0.092 μS for subjects viewing the task in the dark **(Figure 7C)**. There was no significant difference in change in EMG, change in heart rate, or change in EDA between subjects in the light condition compared to the dark condition (p=0.767, p=0.522, p=0.397). In addition to our primary analysis, secondary analyses were conducted to identify any condition-by-sex interactions. While no correlations were identified within the heart rate or EDA data, there was a significant condition-by-sex interaction for EMG **(Figure 7D)**. Females in the light had an average EMG change of 0.289±0.076 while females in the dark had an average EMG change of 0.052±016 (p=0.005). EMG change for males in the light was 0.169±0.040 compared with EMG change for males in the dark 0.655±0.391, but this finding was not significant (p=0.208).

Discussion

This experiment resulted in p-values that were not significant for any of the three physiological responses measured for participants in the dark and light condition. Therefore, the hypothesis that there would be a greater increase in HR, EDA, and masseter EMG on subjects tested in the dark condition over those in the light was not supported. Based on the previous notion that humans feel increased vulnerability and insecurity in dark environments, we believed

that participants would have had a greater startle response in the dark condition. However, our results did not reflect this notion.

One result that did arise from the experiment was that female participants in the light condition had a greater masseter EMG clench response to the startle stimulus than female participants in the dark condition. This was the only statistically significant result, and was unexpected because the clench was greater in the light environment, and it was only seen in females and not males. Therefore, it cannot be said for certain whether or not gender was an important factor in startle response based on this study. Further experiments could examine this difference in startle responses between men and women while in dark conditions. Studies have shown that men have a greater 'fight-or-flight' response whereas women have a 'tend-and-befriend' response; this indicates that men are more likely to utilize their heightened sympathetic nervous system for confrontation in high stress situations, while women are more likely to utilize their heightened stress to deescalate the situation (Balhara, 2012).

There are several possible outlets where error played a role in confounding our results. One of these errors may be attributed to feedforward regulation. Fearful stimuli can be learned from previous experiences or memories. Because of this, the body is then expected to produce a feedforward response to prepare for a fear-inducing stimulus (Geangu, 2016). Our study had a restricted population of 50 students, all of whom were enrolled in Physiology 435 during the spring 2019 semester. Due to the restricted participant group, research confidentiality was a complication for our experiment as participants could have discussed the experiment with one another. This could have affected our results as the participants anticipated the startling stimulus, which in turn could have caused feedforward regulation to alter their physiological response in preparation for the stimuli.

The negative results we obtained could be explained by other mitigating factors such as the testing room and previous exposure to the test environment. Because of the small participant population, many of the participants had been familiar with the testing room due to frequent usage over the course. Participants tested in the dark condition were also exposed to the testing room before the lights were turned off. The exposure to the room could have allowed the subjects to have enhanced feelings of security even when the startling stimulus was presented. The video itself may have also provided enough light in the room for the subject to feel more comfortable during the experiment. The subject could have also felt familiar with the room, so that being enclosed in it may have dampened any effect of darkness on their startle response.

Our results could also be skewed due to the environmental factors the subjects were exposed to, including the stress of a memory test, sitting in dark conditions, and being alone in a small room. We also experienced technical difficulties during data collection due to inconsistent Biopac recordings. The EMG results could have been affected by sudden head movements and constant jaw tension in some participants. Both EMG and ECG measurements could have been altered by participants jumping or moving rapidly after the startling stimulus. The EDA results could have been skewed due to an inadequate amount of electrode gel or not being securely fastened. Future studies could investigate a wider, more randomized population in order to explore different demographics and maintain research confidentiality. Our methods could be improved by not allowing subjects to see the testing room before conducting the study and having the lights off when subjects tested in the dark enter the room.

Conclusion

Our study examined the sympathetic nervous system and its 'fight-or-flight' response when exposed to a startling stimulus. Throughout the entire physiology 435 course we discussed the mechanisms and effects of the sympathetic nervous system and how it is activated in life-threatening situations. The 'fight-or-flight' response releases norepinephrine and epinephrine which can result in increased heart rate, clenching of the masseter (EMG), and electrodermal activity. Norepinephrine and epinephrine have an effect on heart rate by increasing sinoatrial node frequency and the conduction velocity of all cardiac cells. Both of these substances change the ion permeability in cardiac cells allowing for this increase. In unit one, we learned that the sympathetic nervous system activates processes in order to prepare for physical exertion. Epinephrine is a neurohormone released by the adrenal medulla and circulates through the vascular system, which would suggest that it would have an effect on skeletal muscle cells causing an increase in EMG. The sweat glands are exclusively innervated by the sympathetic nervous system, so when a subject is startled they will have increased EDA as a response due to increased signaling of the sympathetic chemical messengers.

Our study attempted to determine if the sympathetic nervous system would release more epinephrine and norepinephrine when startled in the dark condition than the light which we believed would result in greater changes in heart rate, EDA, and EMG of the masseter. Our conclusions showed that this was not the case, and that there was no significant difference in the sympathetic nervous system response to a startling stimulus. This research helped further develop our understanding of human physiology by allowing us to directly witness the changes expected when the sympathetic nervous system is activated. We were also able to observe just

how quickly your nervous system adapts to certain situations to induce the necessary response in order to protect the body.

References

Balhara, Yatanpal Singh, et al. “Gender Differences in Stress Response: Role of Developmental and Biological Determinants.” Industrial Psychiatry Journal, vol. 20, no. 1, 2012, p. 4., doi:10.4103/0972-6748.98407.

D'Hondt, F., Lassonde, M., Collignon, O., Dubarry, A., Robert, M., Rigoulot, S., . . . Sequeira, H. (2010). Early brain-body impact of emotional arousal. Frontiers in Human Neuroscience. doi:10.3389/fnhum.2010.00033

Geangu, Elena, et al. “Three-Year-Olds’ Rapid Facial Electromyographic Responses to Emotional Facial Expressions and Body Postures.” Journal of Experimental Child Psychology, vol. 144, 2016, pp. 1–14., doi:10.1016/j.jecp.2015.11.001.

Grillon, C., & Ameli, R. (1998). Effects of threat of shock, shock electrode placement and darkness on startle. *International Journal of Psychophysiology,28*(3), 223-231. doi:10.1016/s0167-8760(97)00072-x

Grillon, C., Heller, R., Hirschhorn, E., Kling, M. A., Pine, D. S., Schulkin, J., & Vythilingam, M. (2011). Acute Hydrocortisone Treatment Increases Anxiety but Not Fear in Healthy Volunteers: A Fear-Potentiated Startle Study. *Biological Psychiatry,69*(6), 549-555. doi:10.1016/j.biopsych.2010.12.013

Holand, S., Girard, A., Laude, D., Meyer-Bisch, C., & Elghozi, J. (1999). Effects of an auditory startle stimulus on blood pressure and heart rate in humans. *Journal of Hypertension,17*(Supplement), 1893-1897. doi:10.1097/00004872-199917121-00018

Jull, G., Sterling, M., Falla, D., Treleaven, J., & Oleary, S. (2008). The Cervical Spine and Sensorimotor Control. *Whiplash, Headache, and Neck Pain,*59-71. doi:10.1016/b978-0-443-10047-5.50009-6

Klumpers, F., Morgan, B., Terburg, D., Stein, D. J., & Honk, J. V. (2014). Impaired acquisition of classically conditioned fear-potentiated startle reflexes in humans with focal bilateral basolateral amygdala damage. *Social Cognitive and Affective Neuroscience,10*(9), 1161-1168. doi:10.1093/scan/nsu164

Lang, P., Bradley, M., & Cuthbert, B. (1997). International Affective Picture System (IAPS): Technical Manual and Affective Ratings. *NIMH Center for the Study of Emotion and Attention*.

Ryanjyn2008. (2007, July 7). *Scary Maze Game* [Video File]. Retrieved from https://www.youtube.com/watch?v=0WjDrQ0IhBw&t=2s

Steimer, T., PhD. (2002). The biology of fear- and anxiety-related behaviors. *United States National Library of Medicine*. PMC ID: 3181681

Taffou, M., Guerchouche, R., Drettakis, G., & Viaud-Delmon, I. (2013). Auditory–Visual Aversive Stimuli Modulate the Conscious Experience of Fear. *Multisensory Research*,*26*(4), 347-370. doi:10.1163/22134808-00002424

Valls-Solé, J., Solé, A., Valldeoriola, F., Muñoz, E., Gonzalez, L., & Tolosa, E. (1995). Reaction time and acoustic startle in normal human subjects. *Neuroscience Letters*,*195*(2), 97-100. doi:10.1016/0304-3940(94)11790-p

Figures

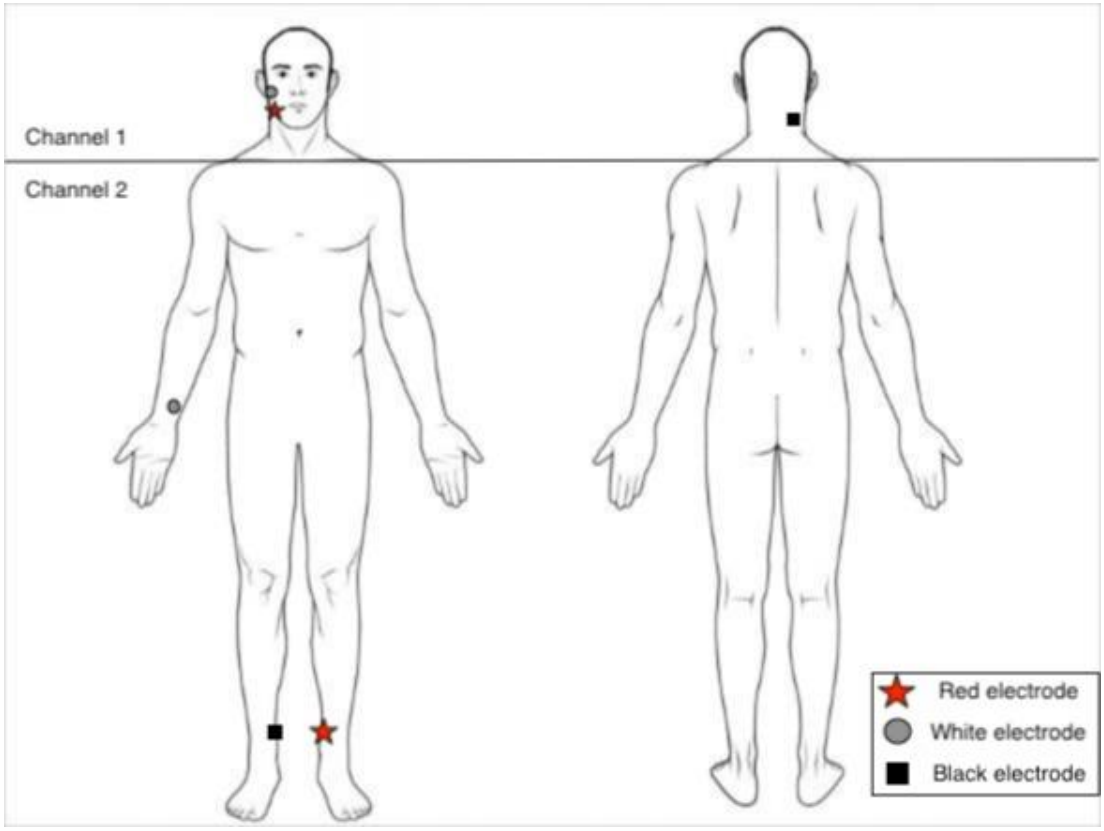

Figure 1. A depiction of the correct placement of electrode disks and their corresponding electrode connections. Channel 1 is related to the EMG measurements, while channel 2 is related to ECG measurements.

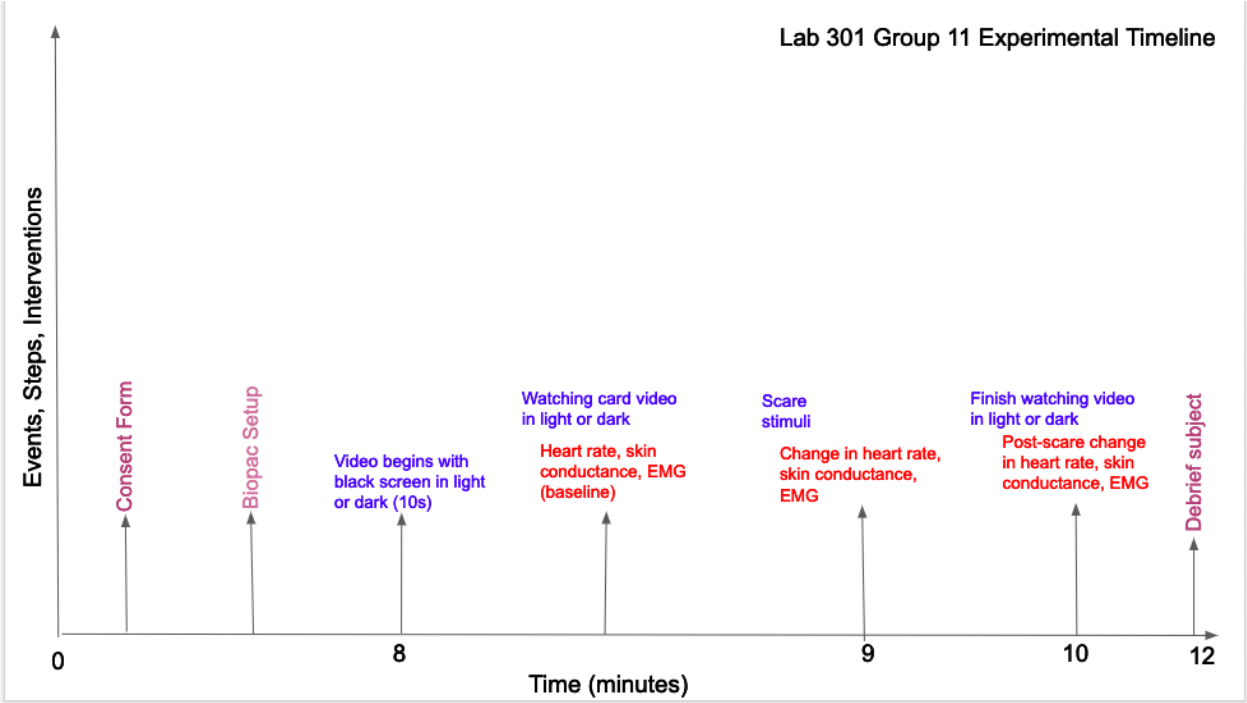

Figure 2. This figure shows a timeline of events that take place during the setup and data acquisition of the experiment.

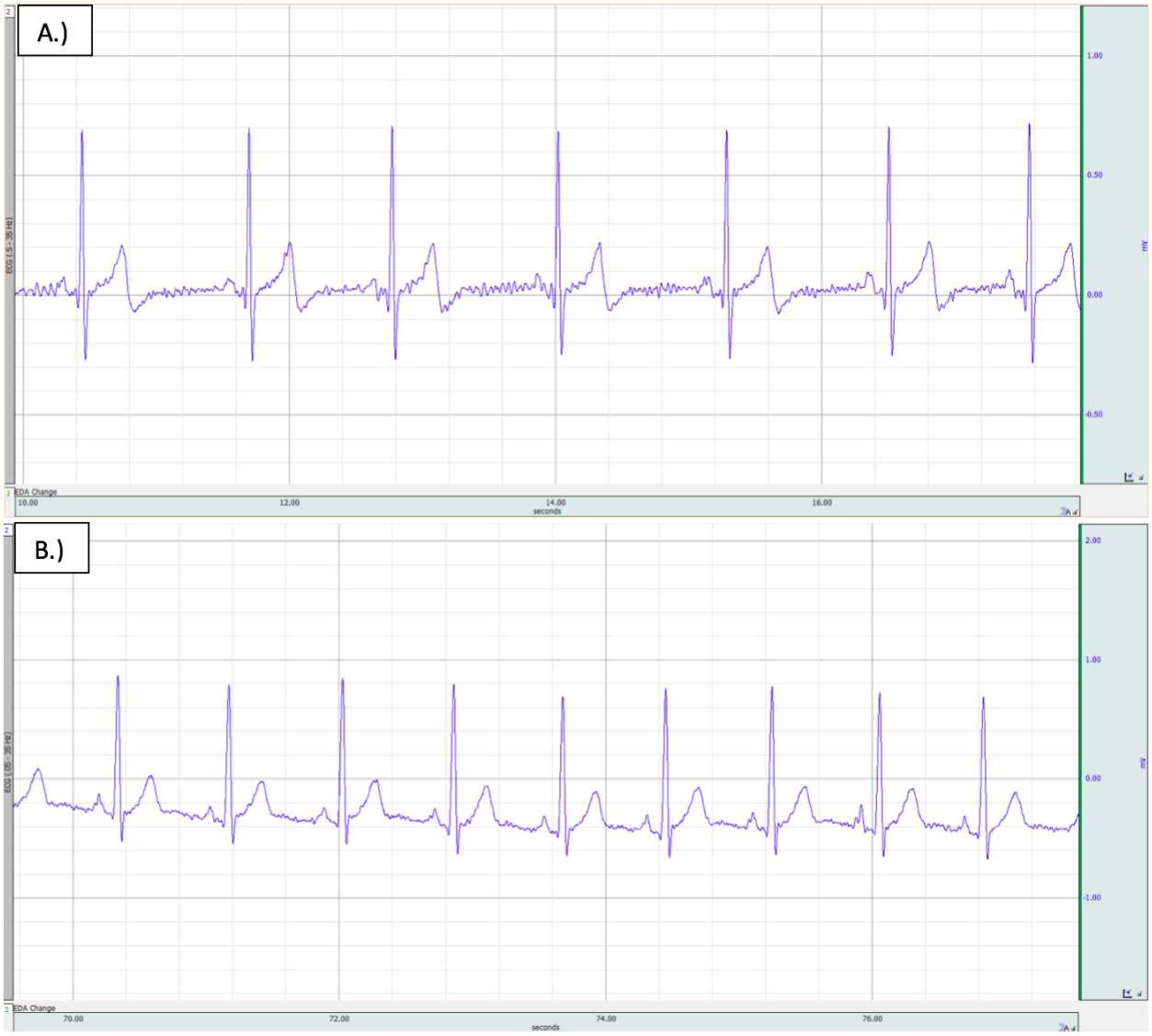

Figure 3. This figure depicts a typical example of the BIOPAC HR program. This participant was from the dark environment group. The image A selected is the heart rate before the IAPS audiovisual stimulus. The image B selected is the heart rate following the IAPS audiovisual stimulus presented at the 69 second mark. Y axis measured in mV and X axis in seconds.

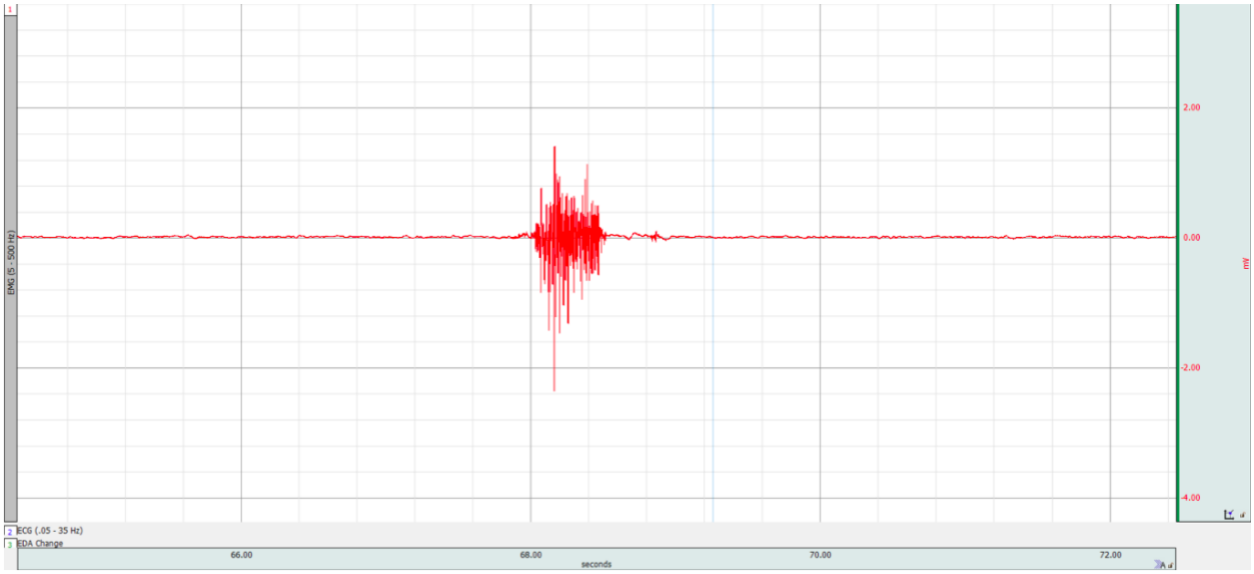

Figure 4. This figure depicts a typical example of the BIOPAC EMG program. This participant was from the dark environment group. The image depicts an 8 second clip of before, during, and after the IAPS audiovisual stimulus. As can be seen, there is minimal contraction in the beginning of the recording prior to the startle and moments after. The large amplitude in the middle of the graph shows the participant's response to the startle stimulus presented at the 69 second mark. Y axis measured in mV and X axis measured in seconds.

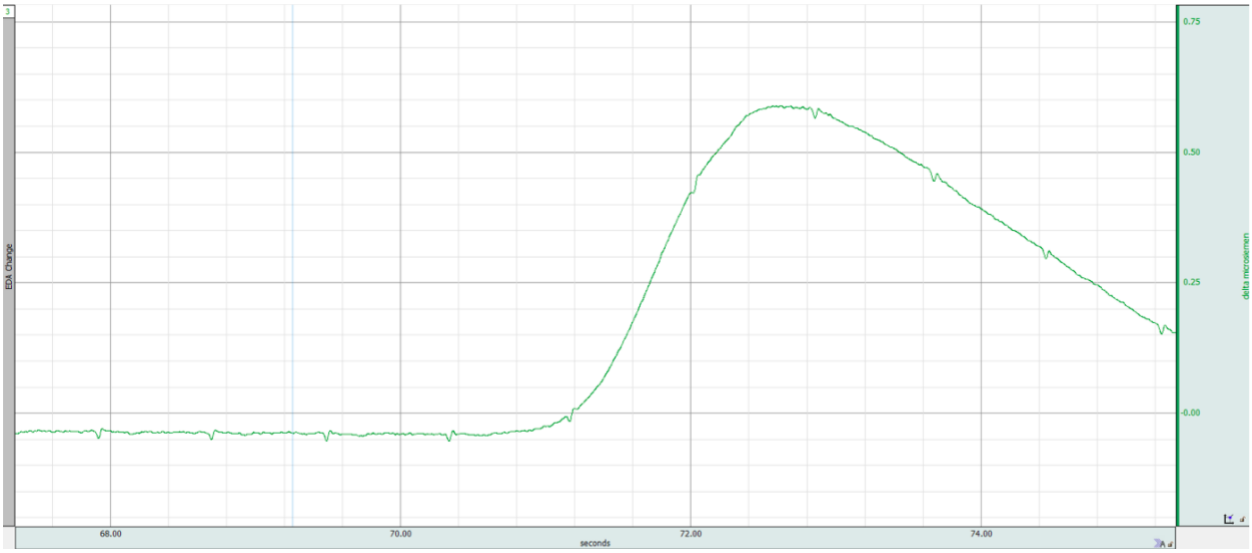

Figure 5. This figure depicts a typical example of the BIOPAC EDA program. This participant was from the light environment group. The image depicts an 8 second clip of before, during, and after the IAPS audiovisual stimulus. As can be seen, there is minimal conductance in the beginning of the recording prior to the startle and moments after. The sharp incline in the middle of the graph shows the participant's immediate response to the startle stimulus presented at the 69 second mark. Y axis measured in μS and X axis measured in seconds.

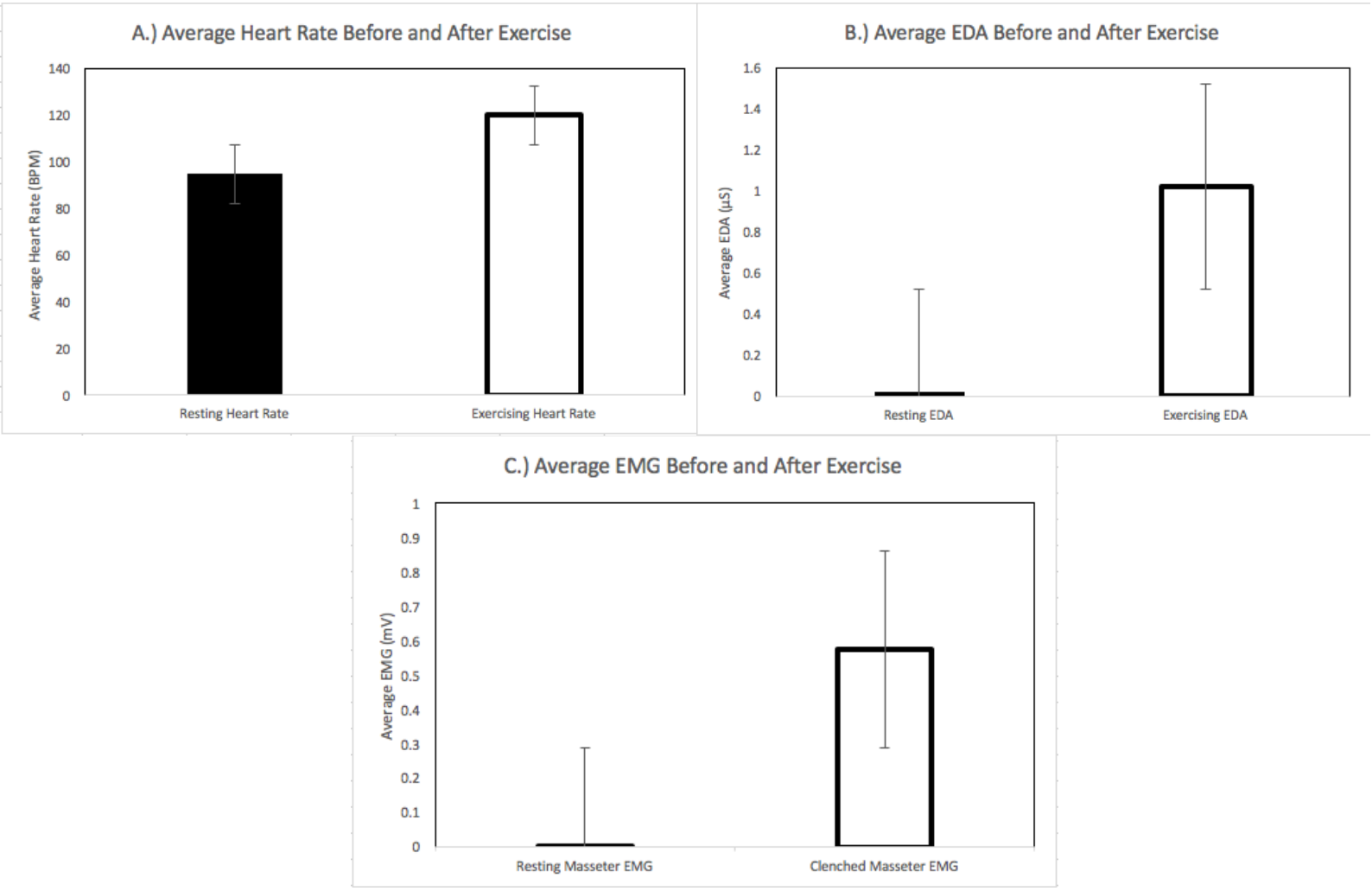

Figure 6. The graphs are showing the results of the positive control measured for this study as mean ±SE. With N=5, experimenters measured resting and running A.) ECG (BPM), B.) EDA (µS). Experimenters measured C.) EMG (mV) when the masseter muscle was relaxed and when it was clenched after instruction to do so.

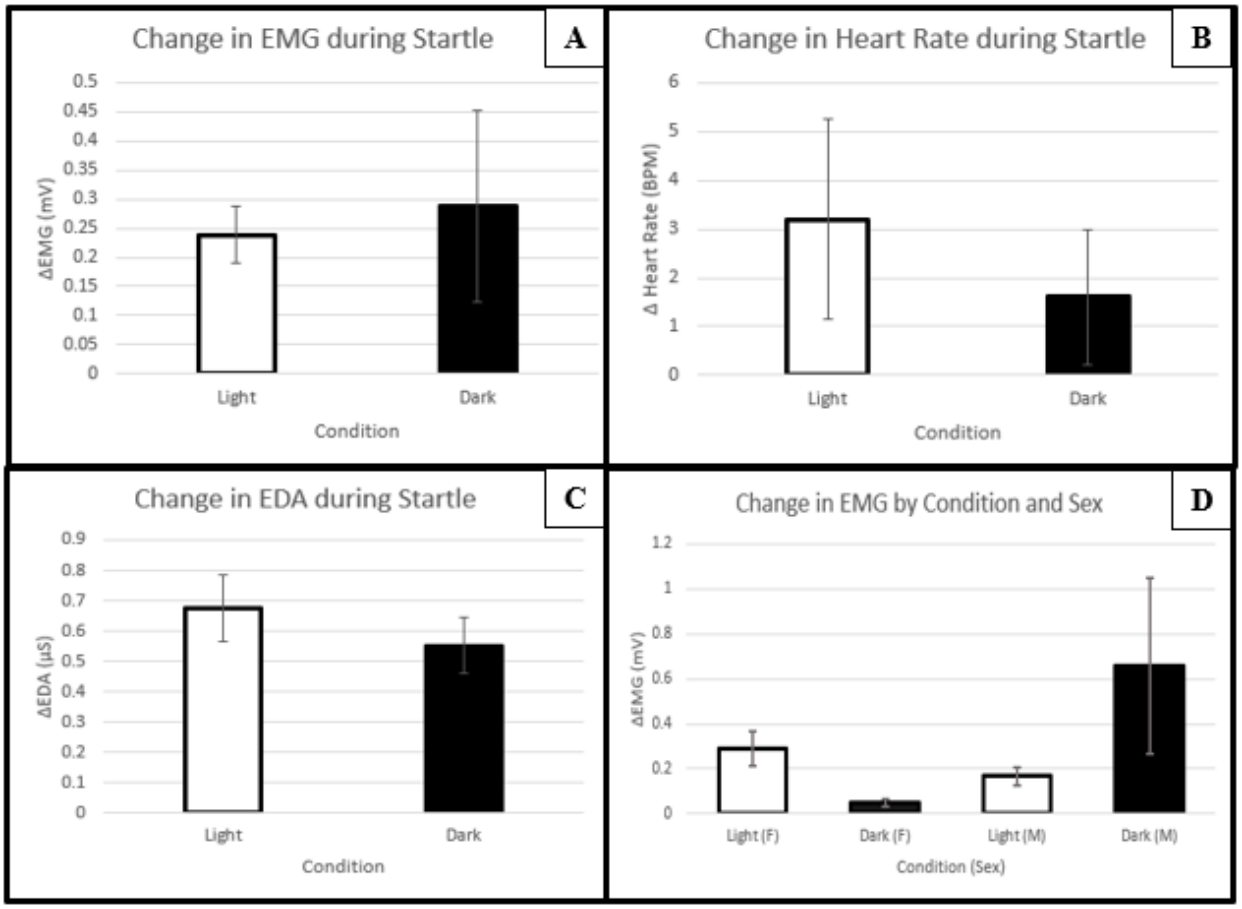

Figure 7. The graphs are showing the results of the control group (N=25 in light) and experimental group (N=25 in dark) during the startle period measured for this study as average change ±SE. Experimenters calculated A.) average change in EMG (mV), B.) average change in heart rate (BPM), C.) average change in EDA (μS), and D.) average change in EMG by condition and sex.