Feed-forward response of three physiological factors in men and women due to a startle stimulus

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Abstract

A feed-forward response is an unconscious anticipatory response that humans have in preparation to respond to a stimulus before it actually occurs. A fear-inducing stimulus may induce an anticipatory physiological feed-forward response at a later time. Physiological responses to fear include increased facial muscle activity, brain activity, or heart rate, which were measured throughout the experiment. Feed-forward response was tested by having a participant view a calming video clip followed by a fear inducing stimulus. The same calming video was then played again in attempt to generate a feed-forward anticipation of the same fear stimulus. The differences in the physiological values were taken between the time periods of Anticipated Scare and Control 2 and compared to the difference found between Control 2 and Control 1. The differences in these values were analyzed using a paired t-test. The results of this experiment did not support our hypothesis that feed-forward response to a fear stimulus increased facial muscle activity, brain activity, or heart rate in both men and women.

Introduction

The human body can predict and then prepare for stimuli before a person is even conscious of the new stimulus. This anticipation is possible through a feed-forward response. Feed-forward response is the body's ability to change and prepare regulated variables for an anticipated change in homeostasis (Widmaier, 2015). Due to the generalization of stimuli, the ability to anticipate these changes in homeostasis can be learned, whether from experience or from the perception of a similar, previous experience. This leads to optimal physiological behavior (Büchel, 2015). Studies show that in response to threatening stimuli, increased brain activity can be measured in the insula, thalamus, and amygdala, implying the importance of these

brain regions in fight-or-flight situations (Lindner, 2015). Other physiological variables associated with fear include increased heart rate and increased skin conductance, suggesting their involvement in an anticipated fight-or-flight situation (Casteneda, 2004). Previous research has also evaluated the association of muscle contraction in the face with emotions, including fear. Geangu, et al (2016) measured facial electromyographic (EMG) activation of two facial muscles in response to videos that elicited particular emotions in children. When shown a threatening video, there was activation of the frontalis medialis associated with the fear response of the child. Because fearful stimuli can be learned from fear memories and experiences, it is expected that the body will produce a feed-forward fear response in preparation for a fear inducing stimulus (Geangu, 2016).

It is also relevant to note the sex differences of a fear response. Thunberg, M., & Dimberg, U. (2000) measured facial muscle reactivity in males and females using EMG and found that females are more facially reactive to a fear-relevant stimulus. Additionally, in a study comparing responses to an auditory stimulus, females showed more physiological signs of fear including greater heart rate and skin conductance as compared to men (Ruhd, L. & Meagher, M., 2001).

It is hypothesized that if subjects are exposed to a calm stimulus, followed by an abrupt fear inducing stimulus, the facial muscle activity, brain wave, heart rate activity of the subject will react similarly to a repeated exposure to the same calm stimulus in due to feed-forward mechanisms of homeostasis.

Methods

Participants:

Participants selected were students from the University of Wisconsin-Madison Physiology 435 class in Spring 2016. There were a total of 24 participants, 10 males and 14 females, who completed the study. All participants were given a consent form that allowed them to stop during the experiment if they felt uncomfortable at any point.

Materials

Facial muscle activity, brain wave activity, and heart rate were recorded while participants sat and watched a 6 minute video in a secluded room with over-the-ear headphones. An EMG (Model: SS2LB, Biopac Systems, Inc., Goleta, CA) with disposable electrodes (Part EL503, Biopac Systems, Inc., Goleta, CA) was used to measure the muscle activity of their forehead. An electroencephalograph (EEG) (Model: SS1LA, Biopac Systems, Inc., Goleta, CA) was used to record brain activity with two shielded electrode leads (Model: LEAD110S-R/W, Biopac Systems, Inc., Goleta, CA), one unshielded ground lead (Model: LEAD110, Biopac Systems, Inc., Goleta, CA), and disposable electrodes (Part: ADD204, Biopac Systems, Inc., Goleta, CA). Both the EMG and EEG were connected to Biopac Systems MP36 Acquisition Unit for data collection and analysis. Finally, a pulse oximeter (Model 9843 Nonin Medical, Inc., Plymouth, MN) was used to measure heart rate.

Experimental Design

The experiment was run in accordance to the timeline displayed in Figure 1. The participant entered the room and filled out the consent form. The participant was then seated in front of a computer with the loaded video. Then the three recording devices described in the materials section were hooked up to the participant. The pulse oximeter was placed on the participant's left index finger and pulse rate was taken at different intervals throughout the video.

The EMG was used to measure muscle activity by recording electrical activity in the muscle, more specifically the frontalis medialis muscles. Guidelines were followed provided by Fridlund & Cacioppo (1986), where the ground lead was placed on the right side of the neck just below the jaw, the vin+ lead was placed on the left side of the participant's forehead, and the vin- lead was placed on the right side of the participant's forehead. All leads were attached to disposable electrodes. An EEG was used to measure the current flow within the cerebral cortex (Teplan, 2002) with the vin+ lead placed approximately a centimeter above the left ear, the vin- lead placed directly behind the left ear, and the ground lead placed directly below the left ear, with all leads attached to disposable electrodes.

Before the video began, we gave a brief explanation to the participant that they would be watching a 6 minute video while we recorded data. The EMG and EEG data was recorded immediately as the video started and continuously throughout the 6 minute video. The layout of the video including analyzed intervals of the video are presented in Figure 2. This figure also illustrates which portions of the video were calm and which were fear inducing. The heart rate was taken either every 10 seconds or 5 seconds during the video depending on where we anticipated the most change as seen in Figure 3.

Following the video, the participants took a brief survey about their experiences with fear during the experiment and in real life.

Data and Statistical Analysis

Data from the EMG, measured in mV, and EEG, measured in μV, was collected by the Biopac Systems MP36 Acquisition Unit and displayed using the Biopac MP36 program as shown in Figure 4, representing the negative control with no startle stimulus and Figure 5,

representing the positive control in response to a random startle stimulus. Heart rate data was collected manually, by taking values from the pulse oximeter at the intervals previously mentioned. EEG and EMG data was extracted from the Biopac MP36 program, exported to Microsoft Excel, and intervals were chosen to correspond with different sections of the video, resulting in Control, Actual Scare, and Anticipated Scare intervals (Figure 2).

The Control 1 interval was determined to be and measured during the first calm video from 0:00-2:00 minutes. The Actual Scare interval was measured directly after the two second scare stimulus from 3:00-3:10 minutes. The Control 2 interval occurred from 3:25-5:00 minutes. The Anticipated Scare interval at the end of the video corresponded to the original scare video found in the middle and was measured from 5:40-6:00 minutes. We had to pick 3 random 10 second intervals from the Controls. We had to take the difference between the control and the other intervals because each person had a different physiological baseline for each factor.

The average differences between the Anticipated Scare interval and the Control 2 interval were compared to the average differences between the Control 1 and Control 2 intervals for all three parameters. A significant difference between these two categories would show that during the anticipated fear, the subject had an increased heart rate, brain activity, and/or facial muscle activity compared to the control intervals. The average differences between the Actual Scare interval and the Control 2 interval were also compared to the average differences between both control intervals for all three parameters. A significant difference between these categories would also show that during the Actual Scare interval, at least one of the physiological factors increased.

A paired t-test was conducted using Rstudio, with each physiological parameter being tested for women, men, and both combined. The results were determined to be significant if the p-value was <.05.

Results

Muscle Activity

The average difference between the Anticipated Scare interval and Control 2 interval for all subjects was 0.00000 mV (SD=0.00000), for males only was 0.00000 mV (SD=0.00000), and for females only was 0.00001 mV (SD=0.00079). The average control variance between the first and second control intervals for all subjects was -0.00008 mV (SD=0.00078), for males only was -0.00001 mV (SD=0.00077), and for females only was -0.00012 mV(SD=0.00081)). When comparing these two categories for all subjects, just males, and just females, no significant differences were found (p=0.8885, p=0.8772, p=0.7569).

The average difference between the Actual Scare interval and Control 2 interval for all subjects was 0.00208 mV (SD=0.00569), for males only was 0.00004 mV (SD=0.00108), and for females only was 0.00354 mV (SD=0.00713). When comparing this category to the control variance for all subjects, just males, and just females, no significant differences were found (p=0.09806, p=0.9264, p=0.09658).

Brain Activity

The average difference between the Anticipated Scare interval and Control 2 interval for all subjects was -0.06535 μV (SD=0.34118)), for males only was -0.15756 μV (SD=0.45346), and for females only was 0.00051 μV (SD=0.22885). The average control variance between the

first and second control intervals for all subjects was 0.12166 μV (SD=0.68088), for males only was 0.12166 μV (SD=0.62479), and for females only was 0.13396 μV (SD=0.74154). When comparing these two categories for all subjects, just males, and just females, no significant differences were found (p=0.3246, p=0.407, p=0.3246).

The average difference between the Actual Scare interval and Control 2 interval for all subjects was 0.61323 μV (SD=6.82294), for males only was -0.10275 μV (SD=0.50914), and for females only was 1.12456 μV (SD=9.02810). When comparing this category to the control variance for all subjects, just males, and just females, no significant differences were found (p=0.7500, p=0.5151, p=0.7086).

Heart Rate

The average difference between the Anticipated Scare interval and Control 2 interval for all subjects was 1.26542 BPM (SD=5.38094), for males only was 1.11400 BPM (SD=3.53768), and for females only was 1.37357 BPM (SD=6.52170). The average control variance between the first and second control intervals for all subjects was 0.22417 BPM (SD=3.61976), for males only was -0.56200 BPM (SD=1.14499), and for females only was 0.78571 BPM (SD=4.63239). When comparing these two categories for all subjects, just males, and just females, no significant differences were found (p=0.5314, p=0.227, p=0.8309).

The average difference between the Actual Scare interval and Control 2 interval for all subjects was 2.92042 BPM (SD=5.01740), for males only was 3.29200 BPM (SD=3.96372), and for females only was 2.65500 BPM (SD=5.78621). When comparing this category to the control variance for all subjects, just males, and just females, no significant differences were found for

all subjects and just females (p=0.09575, p=0.4674). A significant difference was found when comparing just the male subjects (p=0.02118).

Discussion

The results of our study do not support our initial hypothesis that an anticipation of a fear inducing stimulus will result in a feed-forward response of increased heart rate, increased brain activity, and increased facial muscle activity. Not only are these results inconsistent with our hypothesis, but they are also inconsistent with evidence and results from other similar studies.

No statistical difference was found between the average anticipated response and average control response for the EMG measurements. The majority of subjects have a difference between anticipated response and control near zero mV, similar to the difference found across individual controls (Figure 6). The corresponding results for males and females has the same insignificant difference as the total subject pool. These unexpected results could imply that increased facial muscle activity is not included in the feed-forward fear response mechanism or could be due to an error in experimental design, which is more likely. A potential error was the electrodes did not stick well to the participant's forehead if they had been sweating, or during the control time, whenever the participant exhibited random facial movements not associated with fear, it was recorded.

The results for the EEG measurements were similar to that of the EMG measurements resulting in the same implications (Figure 7). The error encountered in EEG measurements could be due to many factors, including subject skin conductance and electrode adherence, the subject's hair interfering with electrode adherence, or lack of instrumental sensitivity.

The results of the total subject pool for heart rate is consistent with the results from EMG and EMG measurements once again implying that heart rate does not increase in anticipation of fear (Figure 8). Similar to our other unexpected results, this could be due to a number of factors involved in experimental design because it is unlikely that heart rate would not increase during a fight or flight situation. A possible reason for the insignificance could be the intensity of the stimulus, but further studies would need to be carried out to confirm.

Although our study is testing a similar physiological homeostatic response to that of a previous research group (Dennee et al, 2015), only changes in heart rate were measured across both studies. We kept change in heart rate as a study parameter because the results from Dennee et al (2015) were inconclusive due to technical malfunctions. We did not include measurements of respiration rate and skin conductance because these factors were already determined to be involved in the feed-forward fear response, and instead chose to measure brain activity and facial muscle activity in an attempt to expand upon the previous study.

Another interesting result of this study was the lack of difference between an actual scare response and the control measurements for all three parameters. A significant difference between these two categories was expected and evidence for this expectancy was cited previously in this study. This lack of statistical significance for the difference between and Actual Scare and the control measurements is another factor that contributes to the idea that the experimental design was flawed. The only significant difference between Actual Scare and Control was seen in male heart rate. A possible explanation for this is that males tend to have a lower resting heart rate than females, making a change in overall heart rate more detectable in male subjects. Though it is more likely that our scare stimulus was not scary enough, in fact, in the survey following the

video, when asked “On a scale of 1-10, how scared were you during the experiment?”, our participants responded with an average of 5.542 (standard dev=2.303).

There are many ways in which we could have improved our experimental design. The first would be to determine a probable method for setting baseline measurements for all subjects for each parameter. This would make comparing experimental and control values more straightforward and accurate. Another potential experimental set-up could involve repeated exposure of the calm and scary scene several times to better condition the subjects for a feed-forward response. Also, the EEG showed the least amount of difference between conditions. This could be drastically improved by using fMRI imaging of fear associated brain areas such as the amygdala.

The results of our experiment did not coincide with our hypothesis. Muscle and brain wave activity and heart rate for all subjects did not have a significant difference when comparing the differences of Anticipated Scare from Control 1 and Control 2 from Control 1. The one significant difference we were able to find was in male participants was during the Actual Scare interval compared to the control intervals. A simple explanation for our results could just be that the subjects were not scared or startled during the experiment, implying that they wouldn’t anticipate another scary or startling stimulus at the end of the video.

References

Büchel, C. (2015). The neuronal basis of fear generalization in humans. *Nature Neuroscience, 18,* 1811-1818. doi:10.1038/nn.4166

Castaneda, J. O., and Segerstrom, S.C. (2004). Effect of stimulus type and worry on physiological response to fear. *Journal of Anxiety Disorders, 18(6),* 809-23. doi:10.1016/j.janxdis.2003.10.003

Dennee, A., Gunderson, D., Le Gros, G., Sarazen, M., Stieve, B. (2015). Feed-forward mitigation of physiological fear response to a visual and auditory startle stimulus. *Journal of Advanced Student Science 1.* Retrieved from http://jass.neuro.wisc.edu/2015/01/Lab%20601%20Group%2010.pdf

Fridlund, A. J. and Cacioppo, J.T. (1986). Guidelines for human electromyographic research. *Psychophysiology, 23(5),* 567-589. doi: 10.1111/j.1469-8986.1986.tb00676.x

Geangu, E., Quadrelli, E., Conte, S., Croci, E., Turati, C. (2016). Three-year-olds' rapid facial electromyographic responses to emotional facial expressions and body postures. *Journal of Experimental Child Psychology, 144,* 1-14. doi: 10.1016/j.jecp.2015.11.001

Lindner, K., Neubert, J., Pfannmoller, J., Lotze, M., Hamm, A.O., Wendt, J. (2015). Fear-potentiated startle processing in humans: Parallel fMRI and orbicularis EMG assessment during cue conditioning and extinction. *International Journal of Psychophysiology, 98(3),* 535-545. doi:10.1016/j.ijpsycho.2015.02.025

Ruhd, L., Meagher, M. (2001). Noise stress and human pain thresholds: divergent effects in men and women. *The Journal of Pain, 2(1),* 57-64. doi:10.1054/jpai.2000.19947

Teplan, M. (2002). Fundamentals of EEG Measurements. *Measurement Science Review, 2(2),* 1-11. Retrieved from http://www.measurement.sk/2002/S2/Teplan.pdf

Thunberg, M., Dimberg, U. (2000). Gender difference in facial reaction to fear-relevant stimuli. *Journal of Nonverbal Behavior, 24(1),* 45-51. Retrieved from http://search.proquest.com.ezproxy.library.wisc.edu/docview/229275755?accountid=465

Widmaier, E. P., (2015). *Vander's Human Physiology*. New York, NY: McGraw-Hill Education.

Figures and Legend

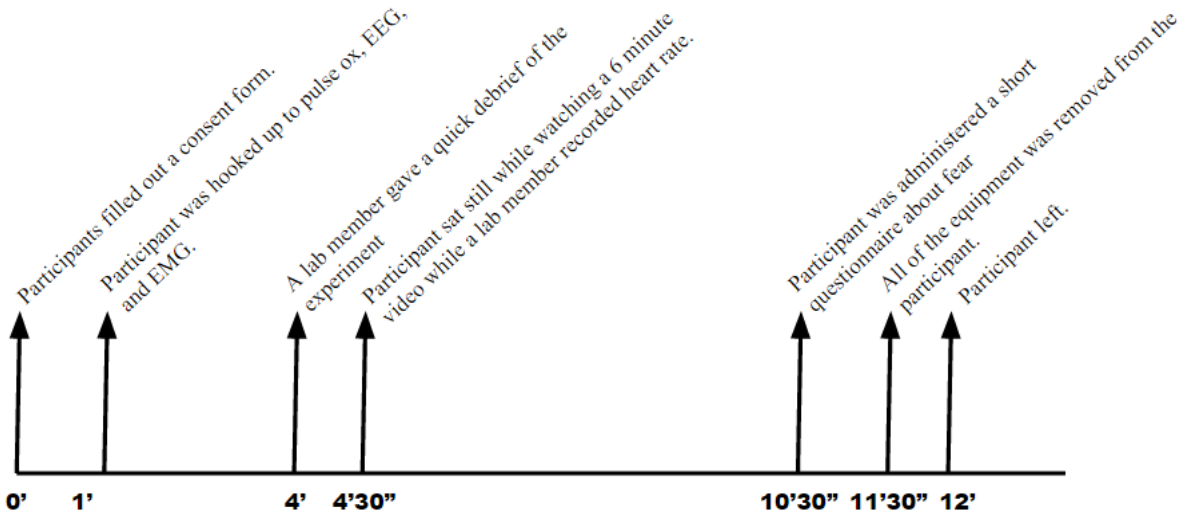

Figure 1: The timeline of experiment used across the study.

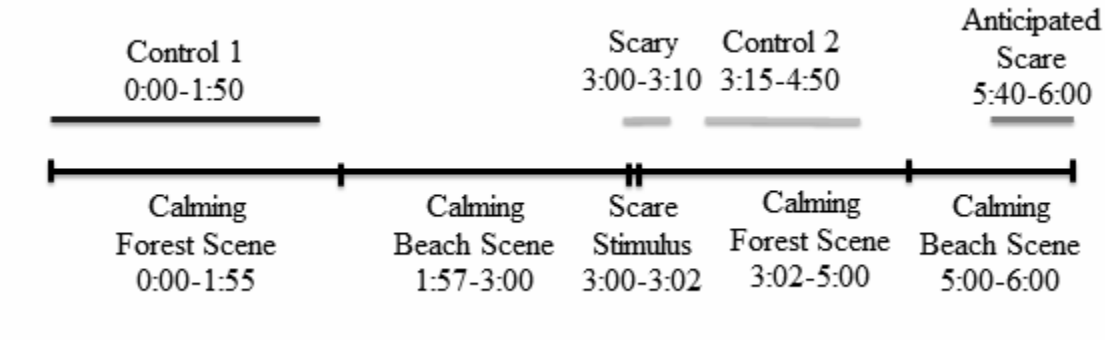

Figure 2: Timing intervals for the presented video. The bottom labels describe the specific video content presented. The top labels represent time intervals that were used in analysis of data.

TIME	INTERVALS
0:00-2:00	10 seconds
2:00-4:00	5 seconds
4:00-5:00	10 seconds
5:00-6:00	5 seconds

Figure 3: The time intervals in which heart rate was recorded during the video duration.

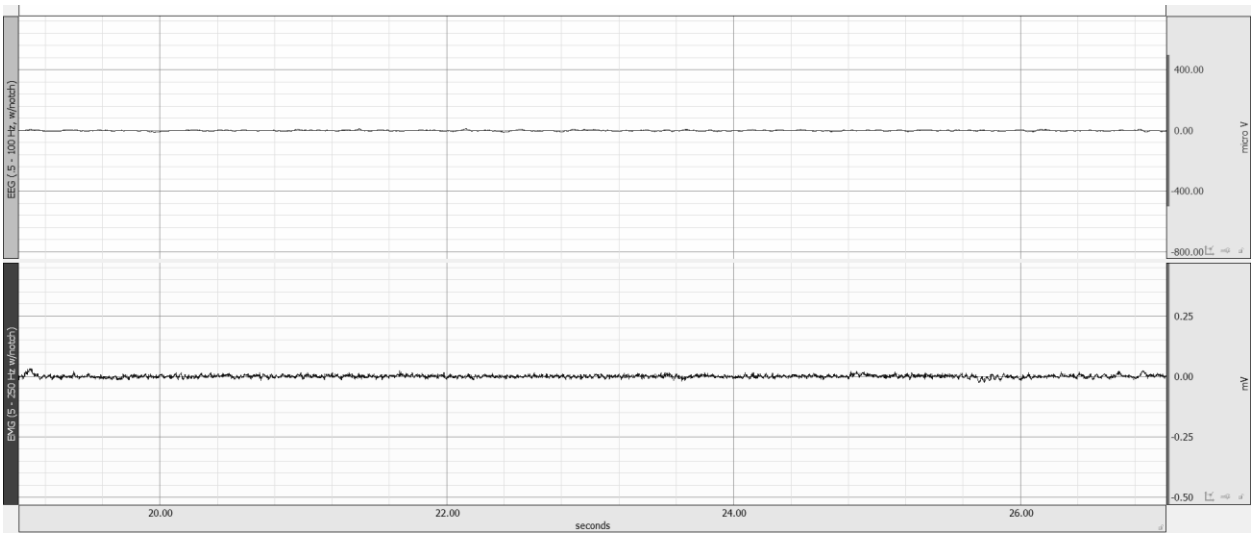

Figure 4: The EEG data is the top fine trace and the EMG is the bottom bold trace while the participant is in a relaxed state using Biopac Systems MP36 Acquisition Unit.

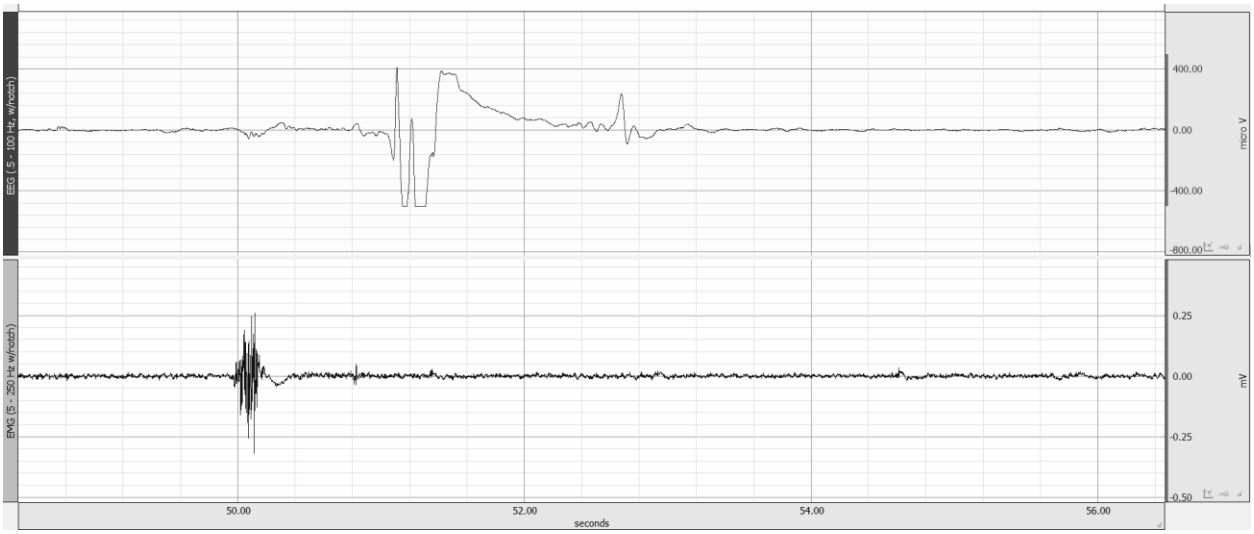

Figure 5: The EEG data is the top fine trace and the EMG data is the bottom bold trace after the participant has been exposed to the random startle stimulus using Biopac Systems MP36 Acquisition Unit.

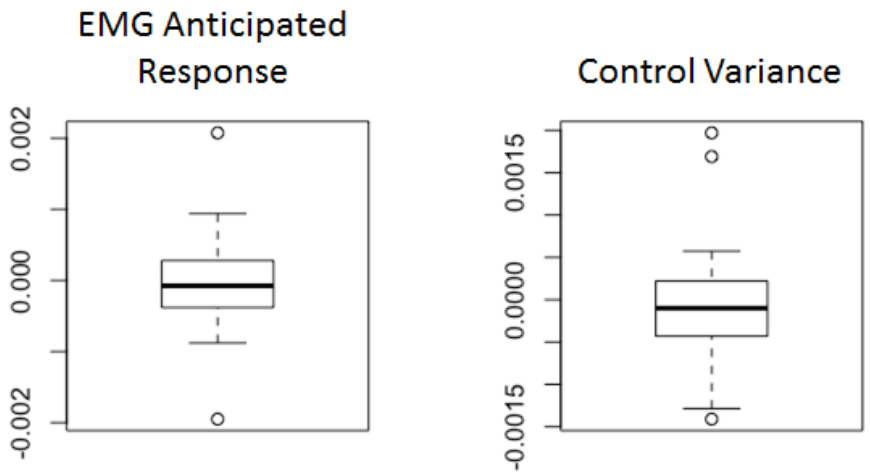

Figure 6: A side by side boxplot comparison of Anticipated response and the variance of control of EMG measurements for all subjects (mV).

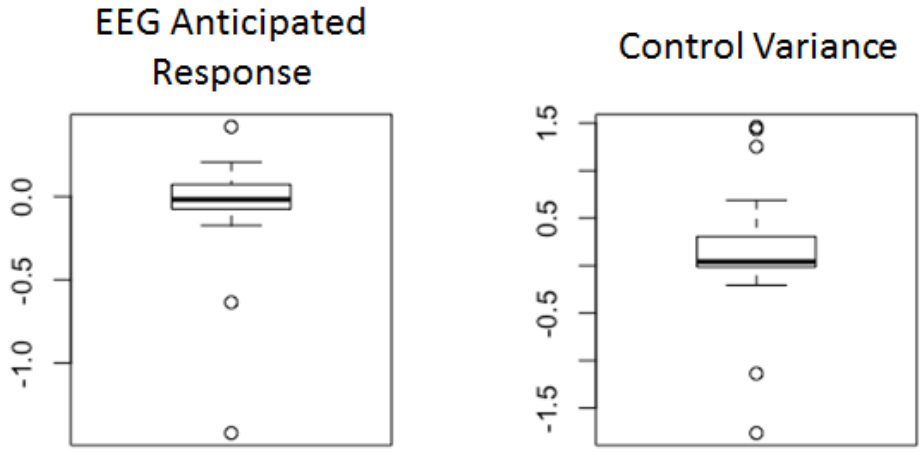

Figure 7: A side by side boxplot comparison of Anticipated response and the variance of control of EEG measurements for all subjects (μV).

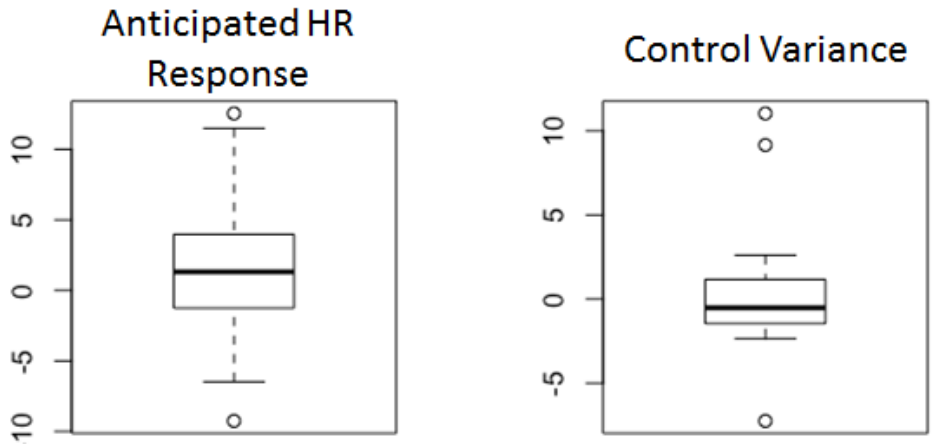

FIgure 8: A side by side boxplot comparison of Anticipated response and the variance of control of Heart rate measurements for all subjects (BPM).