Lab 602, Group 16
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The Effects of Knowledge of an Imminent Threat on the Human Sympathetic Nervous System Response

Abstract

This study aims to explore how knowledge of an imminent threat affects the body's fight or flight response during pre- and post- stimulus stages. More specifically, it examines whether or not an individual's anticipation of a stress stimulus affects the magnitude of the sympathetic nervous system response. Fifteen subjects were tested and three variables were measured: heart rate via a pulse oximeter, blood pressure via a sphygmomanometer, and brain activity via an electroencephalogram. The study consisted of three trials with different parameters for the application of the stimulus: no stimulus, stimulus with warning, and stimulus without warning. The results were then normalized in relation to the baseline measurements and a 21.26% average increase from resting heart rate to maximum rate was seen in stimulus with warning, whereas an average increase of 30.81% was observed in the stimulus without warning trial. For brain activity, both trials with the stimulus present showed increases in alpha and beta activity, however, the "no warning" trial showed a sharper beta spike. Feed-forward regulation was thought to be observed in the "stimulus with warning" trial in which there was an anticipatory up-regulation of heart rate, blood pressure, and brain activity in advance of the application of the stimulus. This in turn suppressed the magnitude of the sympathetic nervous system response that would have been expected otherwise had the subject not been warned of the imminent stimulus.

Introduction

Every college student can attest to the fact that stress is an everyday component of human life. Stress in terms of human experience can seemingly be broken down into two categories. One category involves the chronic stress humans feel in reaction to the persistent challenges they face on a day-to-day basis. The second category involves the sudden onset of a stress stimulus such as a loud noise or electrical shock. The reaction to this second type of stress is widely known as the "fight or flight" response and is the subject of a great deal of research.

Research shows that aside from the extreme mental anxiety one immediately experiences following stress stimuli, the body also sets in motion a variety of physical, chemical, and electrical responses. From a physical standpoint, a human fight or flight response typically involves an increase in heart rate, blood pressure and respiration (Seaward 6). Chemically, the body's endocrine response has been seen to increase blood plasma glucose levels and induce fatty acid mobilization in order to satisfy the potential need for fight or flight energy (Altschafl

Lecture, 21 Mar 2011). Finally, changes in electrical activity in the brain have also been observed in response to stress stimuli (Cheng et al., 2007). However, the body's fight or flight reactions are ultimately coordinated and controlled through a central hub called the sympathetic nervous system (Oertel Lecture, 12 Feb 2011).

While it may seem logical that the body responds in the way it does after encountering a stress stimulus, it is also important to note that the mere anticipation of a stress stimulus may cause a sympathetic nervous system (SNS) response before the stimulus is even experienced. This phenomenon is referred to as a "feed-forward" response. When placed in a situation of imminent threat, one study found a significant shift in brain activity prior to the application of the actual stress stimulus (Mobbs et al., 2007). Given this "anticipatory" change in brain activity, can other typical SNS responses such as elevated heart rate and blood pressure be detected in the presence of the imminent threat of a stress stimulus?

The "predatory imminence continuum" describes the stages– "'pre-encounter,' where there is risk in the absence of immediate danger; 'post-encounter,' where the threat is detected; and 'circa-strike,' defined as distal or proximal interaction with the threat stimulus" – surrounding a distinct threat (Fanselow & Lester 185-211). Our study uses a modified "stress stimulus imminence continuum." The continuum is composed of only two stages, pre- and post-stimulus. We investigated and compared SNS responses during each stage, of patients both aware and unaware of an imminent stress stimulus. This study aims to explore how knowledge of an imminent threat affects the body's fight or flight response during each of these stages. Specifically, it aims to answer whether or not an individual's anticipation of a stress stimulus affects the magnitude of the sympathetic nervous system response.

Materials and Methods

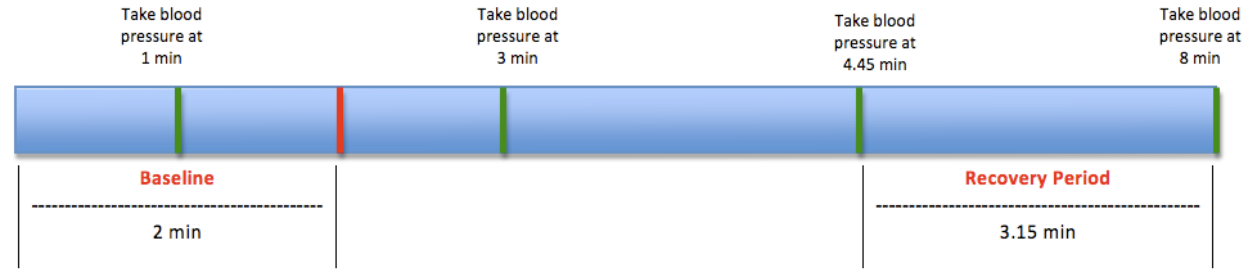
This study measured three variables as proxy measurements of SNS activity: heart rate, blood pressure and brain wave activity. For each variable, it assumed that certain changes in the baseline measurement were indicative of a SNS fight or flight response. A pulse oximeter was used to detect increases in heart rate, a sphygmomanometer for increases in blood pressure, and finally an electroencephalogram (EEG) with a Biopac interface for shifts from beta (or relaxed state) to alpha (awareness state) brain waves. Measurements of each variable were taken at varying intervals during the course of each trial.

Three separate control trials were run with subjects distributed evenly among all three (each subject completes only one of the trials). These trials consisted of:

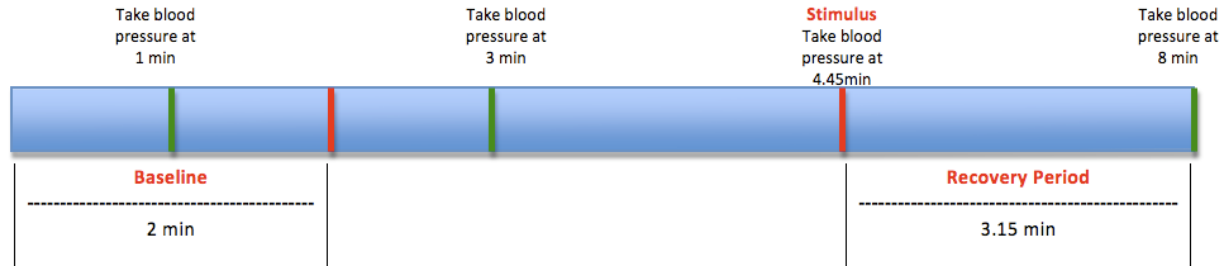
1. **No Stimulus-** Subject views a video clip of a calming beach scene at sunset with calming classical music playing in the background. At no point is a stressful stimulus applied nor is the subject given any reason to suspect one.
2. **Stimulus No Warning-** Subject views the same clip of beach scene with classical music, however at 4 minute 45 seconds of total elapsed time, the clip suddenly switches over to

a disturbing image of a bloody, and demon-possessed teenage girl from the movie *The Exorcist* accompanied by audio of a woman screaming. The horrid image along with the audio lasts two seconds then immediately switches back to the calming beach scene and classical music. The subject is given no warning and is unaware of the imminent stress stimulus

3. **Stimulus With Warning-** Subject views the same clip as in "Stimulus No Warning," however, after recording their baseline measurements, they are warned of the imminent stimulus and approximately when they can expect to experience it (i.e. - "...you'll experience the stressor *around* three minutes")



Timeline 1: No Stimulus Trial



Timeline 2: Stimulus No Warning and Stimulus With Warning

At the beginning of each trial, baseline measurements of all variables were taken for two minutes: blood pressure was taken one minute in and heart rate is taken every 15 seconds. After the baseline measurements was taken, blood pressure was recorded one minute later, immediately following the stimulus (or at the same time in the non-stimulus trials) and again at eight minutes (conclusion of experimental run). Heart rate was recorded at fifteen-second intervals throughout. Due to the nature of the stimulus, and the subjects potentially drastic reactions therein, special precautions were taken to ensure the measurement equipment did not detach from the subject. The pulse oximeter was taped to the subject's finger and immediately after the stimulus, subjects were assured they would not experience the stimulus again and were asked to attempt to relax as they viewed the rest of the video clip.

Due to the fact that subjects typically exhibit different baseline measurements in all three variables, processing and analyzing this study's results will include looking at relative changes (or percentage increases/decreases) in each subject's variables as opposed to absolute changes.

Results

Below we have summarized our data by type (heart rate, blood pressure, and EEG), and explained the statistical manipulations that have yielded our final processed results.

Heart Rate

Due to variation in different subjects' baseline (resting) heart rates, processing the heart rate data in a manner that allowed for comparison between trials was critical. One of the ways this was accomplished was by standardizing the data according to a standard normal distribution. A mean and standard deviation was first calculated from the first two minutes of baseline measurements. Using these values, we assigned normal scores (Z-scores) to each heart rate measurement in the subsequent six minutes of the experimental run. For example, in Figure 1 below, Table 1 represents the raw heart rate measurements of one subject with the baseline highlighted in yellow. Table 2 contains the calculated mean and standard deviation, as well as the resulting Z-scores.

Table 1

	Heart rate
0	71
0:15	76
0:30	73
0:45	71
1:00	74
1:15	77
1:30	77
1:45	79
2:00	75
2:15	82
2:30	84
2:45	85
3:00	78
3:15	77
3:30	79
3:45	78
4:00	82
4:15	81
4:30	83
4:45	85
5:00	82
5:15	84
5:30	80
5:45	78
6:00	77
6:15	73
6:30	68
6:45	67
7:00	71
7:15	68
7:30	69
7:45	71
8:00	68

Table 2

	Baseline Resting Heartrate:
Mean	74.77777778
Std Dev	2.773886163
Time	Standardized Heartrates
2:15	2.603647662
2:30	3.324657784
2:45	3.685162845
3:00	1.161627418
3:15	0.801122358
3:30	1.522132479
3:45	1.161627418
4:00	2.603647662
4:15	2.243142601
4:30	2.964152723
4:45	3.685162845
5:00	2.603647662
5:15	3.324657784
5:30	1.88263754
5:45	1.161627418
6:00	0.801122358
6:15	-0.640897886
6:30	-2.44342319
6:45	-2.803928251
7:00	-1.361908008
7:15	-2.44342319
7:30	-2.08291813
7:45	-1.361908008
8:00	-2.44342319

Figure 1

Plotting the heart rate Z-scores against time for each of the five subjects in each of the three trials ("Stimulus No Warning," "Stimulus with Warning," and "No Stimulus") yields the three scatter-plot graphs shown in Figures 2 through 4 below. Positive Z-scores represent standardized heart rates that were measured at values above those recorded at baseline. Conversely, negative Z-scores represent standardized heart rates that were measured at values below those recorded at baseline. The time on the x-axis begins at two minutes, when the baseline measurements ended and the experimental run measurements began. Each individual subject is represented by their own unique shape and color of data points. Note that in all graphs, the vertical red line represents the point at which the scare stimulus is applied.

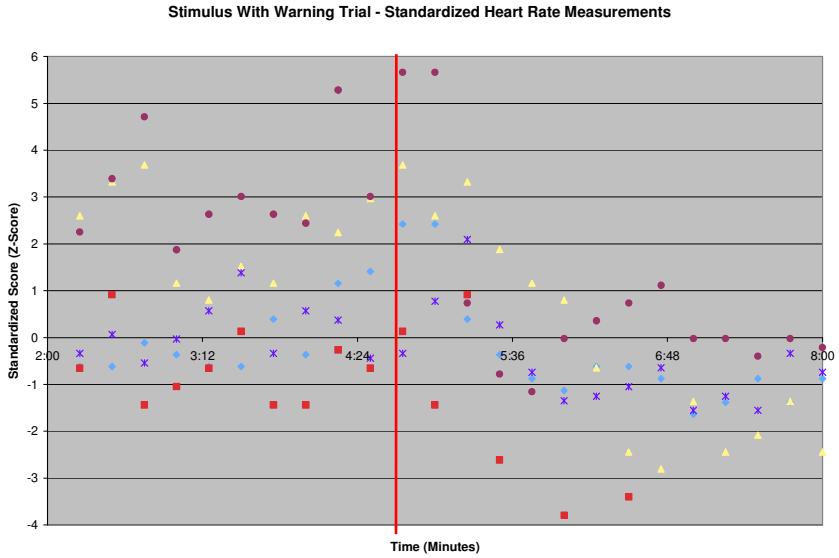


Figure 2

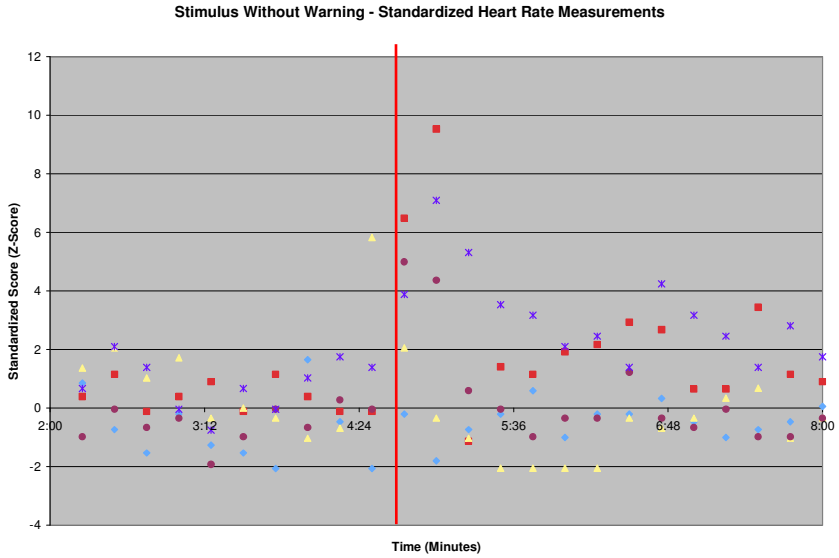


Figure 3

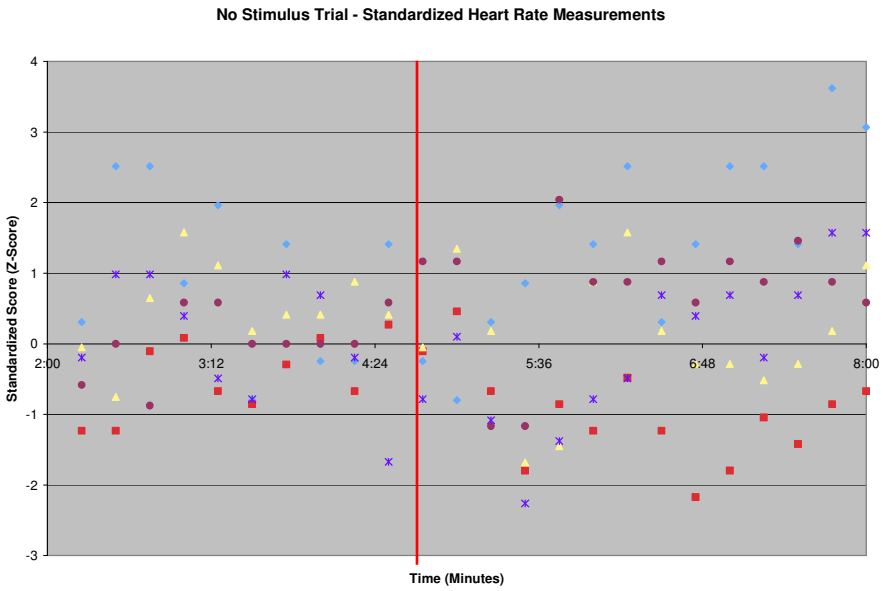


Figure 4

In order to condense the data shown in Figures 2 through 4 above, and extrapolate each trial's general trend, means of the five data points at each time interval were taken and, again, plotted against time. A line of best fit was then applied using a two period moving average approach. The results are shown below in Figures 5 through 7. Again, positive Z-scores represent mean standardized heart rates that fell above baseline recordings and negative Z-scores represent mean standardized heart rates measured at values below baseline recordings.

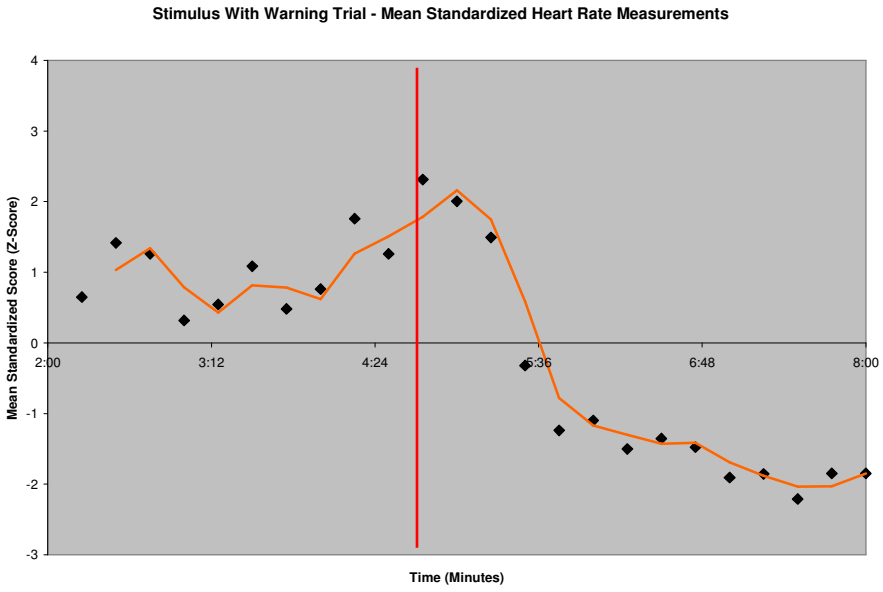


Figure 5

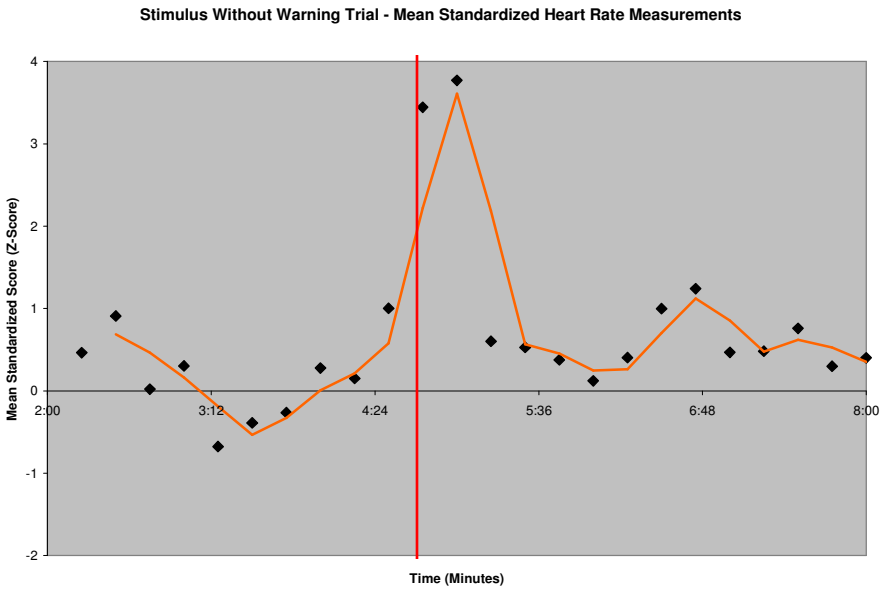


Figure 6

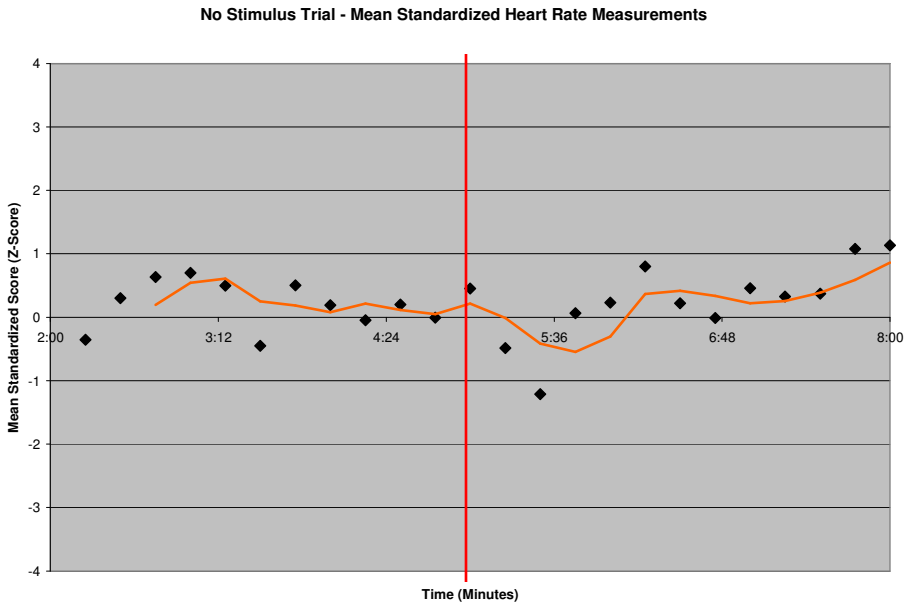


Figure 7

During the trials in which the scare stimulus was applied, the highest mean standardized heart rates were observed within thirty seconds of the subjects experiencing the stimulus (at 2:40). Z-scores of 2.31 and 2.01 during the "stimulus with warning" trial, and 3.44 and 3.77 during the "stimulus without warning" trial were observed at times 4:45 and 5:00, respectively. These Z-scores yield p-values of approximately .01072 and .02275 for the observations during the "stimulus with warning" trial, and .00028 and .00009 during the "stimulus without warning" trial (p-values are approximations taken from standard normal distribution chart). Given such

low p-values (<.05) we can reject the null hypothesis that these spikes in heart rate were simply variations in the observed baseline, and rather, conclude that the spikes were the result of forces not previously present during baseline measurements.

The opposite is true for the "no stimulus" trial, in which the maximum mean standardized heart rate occurred at 8:00, more than 3:00 after the time at which the stimulus *would have* been experienced. In addition, with a Z-score of 1.13, and corresponding p-value of approximately .12507, this jump in heart rate falls within a reasonable level of variation from baseline measurements.

Another way that we were able to standardize data between subjects and hence compare results between trials was by simply looking at the magnitudes of the increases in heart rate that occurred during each of the trials. Each subject's maximum post-stimulus heart rate was compared to their mean baseline heart rate, and a percent increase was calculated. All subjects' percentages were then averaged within each of the three trials (Figures 8 through 10)

Stimulus With Warning

Subject	Measurement	Heart Rate	Percent Increase	Average Percent Increase
1	Mean Baseline	50.44	18.94%	21.26%
	Maximum	60		
2	Mean Baseline	88.67	2.63%	
	Maximum	91		
3	Mean Baseline	74.78	23.03%	
	Maximum	92		
4	Mean Baseline	88.11	35.06%	
	Maximum	119		
5	Mean Baseline	81.33	26.64%	
	Maximum	103		

Figure 8

Stimulus Without Warning

Subject	Measurement	Heart Rate	Percent Increase	Average Percent Increase
1	Mean Baseline	82.78	7.52%	30.81%
	Maximum	89		
2	Mean Baseline	75.44	49.78%	
	Maximum	113		
3	Mean Baseline	79	21.52%	
	Maximum	96		
4	Mean Baseline	75.11	23.82%	
	Maximum	93		
5	Mean Baseline	58.11	51.43%	
	Maximum	88		

Figure 9

No Stimulus				
Subject	Measurement	Heart Rate	Percent Increase	Average Percent Increase
1	Mean Baseline	68.44	9.58%	7.43%
	Maximum	75		
2	Mean Baseline	81.56	3.00%	
	Maximum	84		
3	Mean Baseline	74.22	9.13%	
	Maximum	81		
4	Mean Baseline	80	8.75%	
	Maximum	87		
5	Mean Baseline	79.67	6.69%	
	Maximum	85		

Figure 10

This aggregated data shows that the largest "spike" in heart rate occurred in subjects who were unaware of the impending scare stimulus. Whereas these subjects experienced an average heart rate increase of 30.81%, subjects who were aware of, and anticipated the stimulus experienced an average increase of only 21.26%. Finally, subjects that experienced no stimulus only experienced an increase of 7.43%. It is important to note, however, that in the "no stimulus" trial, subjects' maximum heart rates occurred with no observable pattern, whereas maximum heart rates were reached in the other two trials within 45 seconds of the application of the scare stimulus. We can therefore reason that the 7.43% increase in heart rate observed in the "no stimulus" trials wasn't due to some sort of consistent external factor similar to the stimulus experienced in the two other trials.

Blood Pressure

Since only four blood pressure measurements were taken (at inconsistent intervals) during each trial, plotting blood pressure against time didn't produce any sort of viable correlation. However, when the blood measurements are examined in terms of the experimental context in which they were taken, more viable relationships emerge. Similar to heart rate, we observed appreciable variation in different individuals' blood pressure measurements. We therefore processed the data in a manner similar the heart rate data above, expressing blood pressure changes in terms of percent change from each individual's baseline blood pressure measurement. To provide additional insight into the types of blood pressure changes that were occurring, we also processed systolic and diastolic blood pressure measurements separately. The results are shown in Figures 11 through 13 below.

Stimulus With Warning		
	Average % Increase In Blood Pressure	
	Systolic Blood Pressure	Diastolic Blood Pressure
Baseline	-	-
Minute One - Exp. Run	2.85%	-0.52%
At Stimulus	12.30%	-10.42%
Final	-2.10%	-2.10%

Figure 11

Stimulus Without Warning		
	Average % Increase In Blood Pressure	
	Systolic Blood Pressure	Diastolic Blood Pressure
Baseline	-	-
Minute One - Exp. Run	0.84%	-1.01%
At Stimulus	11.01%	-7.28%
Final	1.23%	-2.27%

Figure 12

No Stimulus		
	Average % Increase In Blood Pressure (Compared to Baseline)	
	Systolic Blood Pressure	Diastolic Blood Pressure
Baseline	-	-
Minute One - Exp. Run	-0.18%	1.90%
At Stimulus	-1.23%	-3.55%
Final	-1.80%	-4.70%

Figure 13

For the two trials in which a stimulus was experienced, maximum systolic blood pressure peaked just after the stimulus was applied, with increases of 12.30% and 11.01% for the "stimulus with warning" and "stimulus without warning" trials respectively. Interestingly, diastolic blood pressure experienced its largest drop upon application of the scare stimulus, with decreases of -10.42% and -7.28% for the "stimulus with warning" and "stimulus without warning" trials respectively. As for the "no stimulus" trial, aside from an initial 1.90% increase in diastolic blood pressure, both systolic and diastolic blood pressure decreased steadily.

Electroencephalogram (EEG)

We plotted each group's standard deviations in alpha and beta wave values across six time intervals (measured in seconds): 1) 0-60, 2) 60-120, 3) 120-225, 4) 225-300, 5) 300-330, 6) 330-480 (Figures 14-16). These timeframes each represent a unique portion of the experiment.

Intervals 1 and 2 are from the baseline portion, 3 and 4 are before the stimulus (if applied), 5 is during and immediately following the stimulus (if applied) and 6 is during recovery.

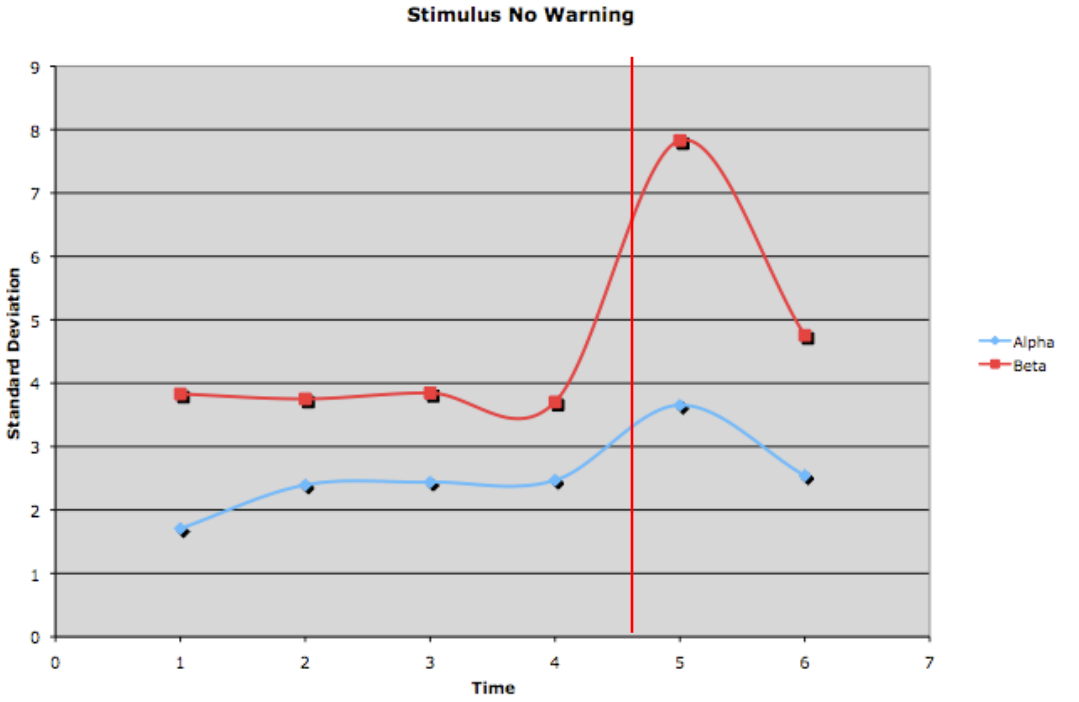


Figure 14

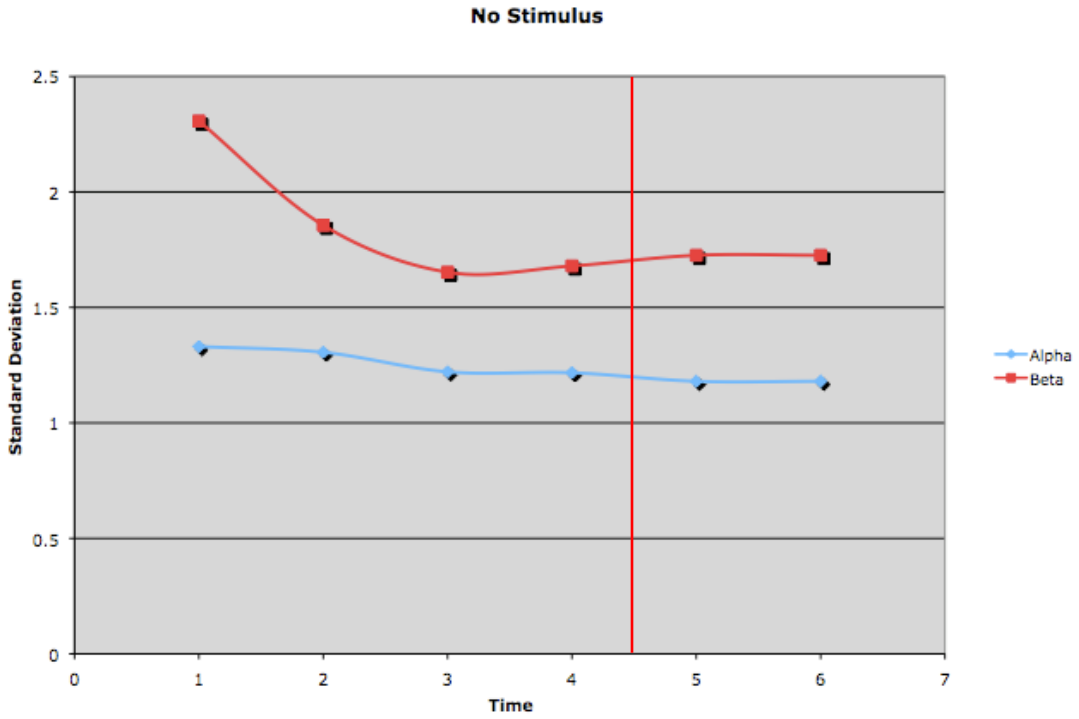


Figure 15

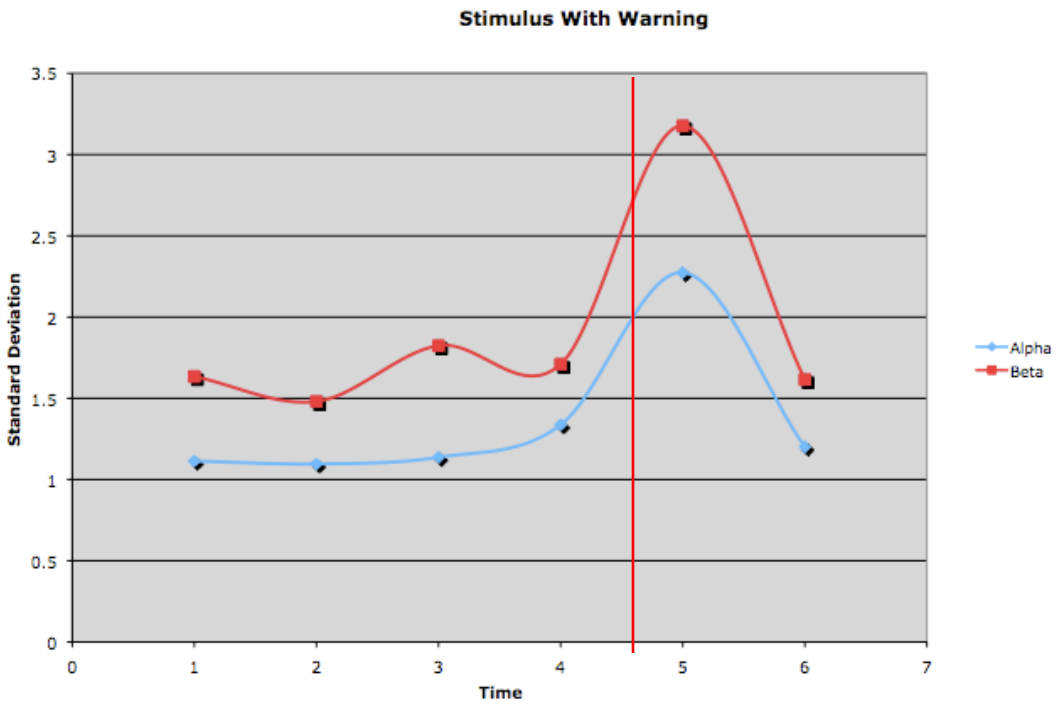


Figure 15

Discussion

The purpose of this study was to determine whether an individual's anticipation of an imminent stress stimulus affects the magnitude of the sympathetic nervous system response. In terms of heart rate, changes were observed that were characteristic of the fight-or-flight response due to the application of a stress stimulus. In the no stimulus trial, when subjects did not receive a scare, the means of the standardized heart rates (normalized in relation to the baseline measurements) showed no significant positive or negative correlation as time progressed (see Figure 7). In other words, the standardized heart rates remained relatively stable over the eight-minute testing period. When looking specifically at the maximum heart rate achieved during the no stimulus trial, there was an average increase of 7.43% from the mean resting heart rate.

In comparison, for the stimulus with warning trial, the means of the standardized heart rates showed a positive correlation, a gradual increase showing a maximum Z-score of 2.31 at the time of the application of the stimulus (see Figure 5). This increase in heart rate is indicative of the expression of a sympathetic nervous system response since this increase was not expressed in the control trial with no stimulus. Furthermore, the gradual increase in heart rate up until the stimulus illustrates feed-forward regulation of the sympathetic response, as the subjects anticipated the impending stimulus in the form of elevated sympathetic nervous system activity. During the recovery period, after the application of the stimulus, the mean of the standardized heart rates decreased to heart rates even lower than baseline measurements. The fact that this

parameter went below baseline measurements during recovery time is most likely due to the fact that subjects of the warning trial were also told that they would not be scared again after the application of the stimulus (unlike the subjects in the no warning trial, who received no warning or description of the procedure beforehand). Furthermore, the maximum heart rate achieved by each subject showed a 21.26% average increase from the mean resting heart rate. This increase from resting heart rate yet again depicts the fight-or-flight response at work in which the mean maximum heart rate achieved was higher than in the no stimulus trial.

Lastly, in the stimulus without warning trial, the means of standardized heart rates also showed a positive correlation up until the application of the stimulus, but unlike in the trial with warning, the increase was not gradual. Instead, the heart rates were relatively stable before the application of the stimulus, and then a rapid increase and subsequent decrease (displayed in the form of a spike) was observed at the time of the stimulus. Thereafter, the heart rates returned to normal resting heart rates. Furthermore, when looking at maximum heart rates achieved by subjects, this trial displayed a 30.81% average increase from the mean resting heart rate. Together, the rapid increase in heart rate and the higher average increase from mean resting rate observed during the stimulus without warning trial give evidence to the existence of an increased sympathetic nervous system reaction in response to an unanticipated scare stimulus.

The blood pressure was another crucial element that we chose to characterize the sympathetic nervous response for our test subjects in each of our three trials. Although the data was not as indicative as that of heart rates, the blood pressure did show a difference in the magnitude of change between the group that was aware of the stimulus and the group that was not aware.

The control group, which received no stimulus throughout the experimental time, showed less than 5% change in magnitude from the baseline at all three time points. The changes are much less significant compared to those of the stimulated groups, which shows that in terms of blood pressure, the stimuli had properly triggered a sympathetic nervous response in the test subjects.

The initial analysis showed that the group that was warned about the stimulus had a greater change in magnitude in both systolic and diastolic blood pressure than the group that was not warned. However, a closer look at the data made us realized that one of the test subjects in the non-warning group had an abnormally calm response to the stimulus, which may have skewed the average change in magnitude of the non-warning group. With the data corrected without the outlier, the non-warning group actually showed a slightly more significant increase in magnitude for both the systolic and diastolic than that of the warning group.

(As an aside, we were curious to know what could have possibly caused the outlying subject to have such an abnormally low sympathetic response to a no-warning stimulus. It turns out that this particular subject is a trombone performance major in one of the best music programs in the Midwest. A major skill that every aspiring musician must learn is that of staying "cool" and "loose" during stressful performance situations. It can therefore be hypothesized that this subject's *abnormally low sympathetic response was due in part to the fact that he has conditioned his mind and body to stay calm in the face of stressful situations.)*

Another overall trend observed in the blood pressure data was tendency for subjects who experienced a stimulus with warning to have final, post-stimulus blood pressures below those recorded in their baselines. In other words, warned post-stimulus subjects tended to overshoot their baseline blood pressures on their way "back down" from the elevated blood pressures they experienced immediately after the stimulus was initially applied. This phenomenon is easily explained by a similar trend in the heart rates of these same subjects (mean standardized heart rate also dipped below baseline in these subjects during the "relaxation period" following the stimulus). Once subjects assessed the stimulus as being of little threat (realizing, "...oh, this is the stimulus the experimenter warned me of…"), their parasympathetic nervous system most likely kicked in at levels above baseline in order to counteract the drastic sympathetic nervous system set into motion by the stimulus. With the sympathetic and parasympathetic responses being dialed down and up respectively, the vasomotor and cardio-acceleratory centers would be depressed, while the cardio-inhibitory center would increase its action. The resulting decrease in heart rate below baseline levels would decrease overall cardiac output and hence also lead to a drop in mean arterial blood pressure.

To analyze the EEG data, we measured the standard deviation of both alpha and beta waves for each group. We noticed that during blood pressure readings, subjects' brain activity spiked as they apparently briefly lost focus on the video. Therefore, we did not include data from these times and split the recording into six time intervals, the first two from the baseline portion and the rest from the experimental portion. In the no stimulus group, the subjects showed high beta activity at the beginning, which gradually decreased, as well as relatively constant alpha activity throughout. This is indicative of the subjects becoming gradually more relaxed as they watched the calming video. In the warning group, subjects showed low beta and alpha activity, followed by a small increase in beta waves and a large spike of both beta and alpha waves. The small increase in beta waves before the stimulus may be attributed to the subjects' anticipation of the scare. Once the stimulus was received, the subjects showed a large increase in both beta and alpha activity, although the beta spike was much sharper. After the stimulus, the subjects eventually returned to a level that was still slightly elevated from baseline. In the no warning group, there was no preemptive rise in beta waves, only a massive, beta-dominated peak in response to the stimulus. At the last reading, the subjects' beta activity was still much higher than in the baseline measurements. These data point to the subjects' lack of anticipation of the

stimulus and much more gradual recovery period. Just as we observed in our heart rate and blood pressure data, a higher overall sympathetic reaction was observed in the no warning trials, whereas a more gradual feed-forward sympathetic reaction was observed in the warning trials.

We attribute the groups' differences in y-axis scale values to be a function of the subjects' physiological variance, as some showed a high baseline level of standard deviation and others were relatively flat. However, this did not affect our ability to observe the peaks in response to the stimulus as they appeared as a consistent spike relative to the baseline value regardless of the initial amplitude of activity.

Given the data and analyses presented above, and using increased heart rate, blood pressure and brain activity as proxies for a sympathetic nervous system response, we can reasonably come to a couple conclusions. One, the overall magnitude of a sympathetic response is greatest when the imminent threat stimulus that catalyzes it is unknown to the victim. Two, while it is smaller in magnitude overall, the sympathetic response in victims aware of the imminent threat stimulus gradually ramps up shortly before the stimulus, displaying feed-forward characteristics due to victims' anticipation of the stimulus. If this study still hasn't convinced readers (particularly those of the student genre), consider these conclusions in the context of a practical example. If a student has been warned of a test or quiz ahead of having to take it, they will no doubt experience a slight, gradually increasing sympathetic response in the minutes leading up until the quiz is passed out (elevated heart rate, sweaty palms, etc). However, when the time comes to actually take the quiz, the overall stress response is dampened because the student expected the quiz and has had time to prepare for it. Contrast this with a "pop-quiz" situation. The student is unaware of imminent quiz, so there is no feed-forward, anticipatory sympathetic build-up. However, when the pop quiz is announced there is a huge overall spike in sympathetic activity (highly elevated heart rate, extreme perspiration, etc.)

So in conclusion, we invite our peers to attempt to replicate our methods and confirm our conclusions. However, we would also hope they realize that, without the help of our complex experimental equipment, they've most likely replicated these experiments hundreds of times already in their daily lives.

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