Physiological Response to An Auditory Startle Stimulus Under Relaxed and Occupied Conditions

University of Wisconsin-Madison Department of Physiology

Physiology 435 Lab 601-5: Maddy Eckelaert, Jai Patel, Michelle Cancel, Zach Bennet, Miki Hirano, Ashley Verhasselt

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Abstract

This study aims to investigate if an individual's physiological response to a auditory startle stimulus, preceded by a auditory warning stimulus, depends on whether or not they are in a relaxed versus occupied condition. Two groups of eight participants were respectively placed into one of these conditions. Three physiological responses were measured throughout the duration of the study: heart rate, respiration rate, and skin conductance. During both the relaxed and occupied conditions, subjects first focused on an object (30s). Then, relaxed condition participants continued this task while occupied condition participants began work on a word search (60s). Next, both conditions received a warning stimulus and 10s after introduction, the startle stimulus was administered. A greater physiological response (increased heart activity, increased respiratory activity and increased perspiration) was expected in response to the startle stimulus in a relaxed condition versus an occupied condition. The relaxed and occupied groups only exhibited a significant difference in max GSR values ($p = 0.04$). Comparing the post-warning and post-stimulus conditions, both relaxed ($p = 0.01$) and occupied groups ($p = 0.05$) exhibited a significant difference in GSR values. Comparing positive and negative controls, the relaxed group exhibited significant values for max heart rate ($p = 3.89x10^{-6}$) and max respiration ($p = 0.03$) and the occupied group exhibited significant values for ($p = 3.89x10^{-6}$) and max respiration ($p = 0.03$). Further research must address a larger sample size and a refinement of the experimental procedure in order to best understand differences between relaxed and occupied conditions.

Introduction

As one of the most primitive defensive responses in the human body, the startle reflex demonstrates the body's quick, involuntary reaction to sudden external stimuli. These stimuli may be visual or tactile, but are most commonly auditory (Dreissen et. al, 2011). In response to the stimuli, a person may exhibit one or more motor responses such as an eye blink, a head movement, an abdominal contraction, or a flexion of limbs (Cook et. al, 1991). In addition to motor responses, the body's cardiovascular and adrenal catecholamine systems will activate, increasing the heart rate and dilating the bronchioles (Jansen et. al, 1995). The body's startle response is also characterized by increased blood pressure, as well as increased skin perspiration. These responses result from the activation of the sympathetic division of the autonomic nervous system (ANS). The other division of the ANS, the parasympathetic division, controls the body at rest and is responsible for resolving sympathetic responses after danger has passed (Seeley et. al, 2004).

Previous studies have shown that the strength of the startle response can be suppressed if a weaker startle stimulus or warning is presented prior to the actual startle stimulus (Braff et. al, 2001). This reduction in amplitude of the startle response reflects the ability of the nervous system to temporarily adapt when given a warning. This has been recognized as pre-pulse inhibition (PPI). Much like the startle reflex, a variety of visual, auditory, or tactile 'prepulses' can be applied to elicit adaptive physiological responses (Braff et. al, 2001). In addition, if a 'prepulse' is emotionally striking, it can elicit a greater reduction in the startle response (Filion et. al, 1998). The duration and intensity of the startle reflex in response to a startle stimulus is a reflection of the individual's personality and their emotional and physical state at the time of the stimulus. Thus, the intensity of the startle reflex has been identified as a reflection of emotion, particularly negative emotions such as fear and anger (Roy et. al, 2009). Since emotion arises unconsciously, like the startle reflex, the individual's autonomic responses offer reliable signs of emotional reaction, such as anticipation, from the warning stimuli.

Previous research suggests that if subjects are introduced to a prepulse prior to the excitatory stimulus, they were more prone to worry than those who did not receive the 'prepulse'. A consequence of worrying about the impeding stimulus resulted in PPI and a reduction in the physiological response to the stimulus. For example, in a study of arachnophobic subjects, those instructed to imagine the spider before exposure to an actual spider exhibited a smaller startle response, as represented by heart rate and skin conductance responses (SCR), than those suddenly exposed to the actual spider (Castandeda & Sergerstrom, 2004). In addition, it has been shown that a subject sitting relaxed in a dark room had a smaller cardiac response to the auditory stimuli when it was preceded by an auditory warning stimulus, known as the prepulse, rather than by itself (Eder et. al, 2008).

In addition, researchers saw a reduction in a subject's startle response when he or she was focused on something other than the stimulus (Silverstein et. al, 1981). This is an example of an adaptive physiological response the body creates depending on its environment. In a study by Lang et. al, subjects engaged in an interesting activity exhibited a significantly smaller startle reflex, as determined by their blink response, than those engaged in a dull activity (1990). This adjustment of the startle response likely reflects the subject's emotional anticipation at the time of the stimuli, as the researchers believed interesting activity was more evocative of emotion. The same research also concluded that subjects with anxiety often showed exaggerated startle responses.

The purpose of this study is to determine whether a participant's physiological response to startle and warning stimuli are dependent on whether they are relaxed or occupied. In both conditions, we expect the occupied group to exhibit a smaller physiological response than the relaxed group. Since the subject is engaged in an activity they will allocate more of their attentional resources to the primary task, leaving little attentional resources for the introduced stimuli. In contrast, relaxed condition subjects will be able to allocate more attentional resources to the stimuli since they are engaged in a dull activity (resting). We expect them to a have a greater physiological response to both the warning and startle stimuli.

Materials & Methods

Sixteen participants (n = 16) were randomly recruited from Physiology 435: Lab 601 for participation in this study. The participants included eight males and eight females. Participants were divided into two groups, one of which involved an occupied condition (word search) and the other of which involved a relaxed condition (no word search). Mean ages of all participants were 22.19 years with a range of 20-45 years. The median age was 21 years. Each group consisted of an 1:1 ratio of males to females.

Before beginning the study, each participant was made aware of possible risks and asked to sign a consent form as well as answer a brief questionnaire about their fearfulness (see Supplemental Materials I-II). Fearfulness was rated on a scale of one to five, with five being the most fearful. Each participant was informed the study would last approximately twenty minutes. The participant was then taken to a quiet room and connected to the following equipment: respiration transducer, electrocardiography (ECG), and galvanic skin response (GSR or SCR) (see Supplemental Materials III). Experimental sessions took place individually for approximately five minutes.

Upon attachment to all equipment, each participant was calibrated for respiration, ECG, and GSR in the BIOPAC student lab program (see Supplemental Materials IV). All participants were calibrated in the same way for each testing session. (Note: Comments were recorded directly into BIOPAC Student Lab Program in order to track experimental progress.) The participants next received verbal instructions as to what the task entailed. Participants were informed of the commands issued in each part of the experiment. When the experimenter said, "begin", the participant would focus on a specific object for thirty seconds in a relaxed position (see Figure 1). The data collected during this time period served as the negative control. Each participant was his or her own baseline. The baseline provided the participant's parasympathetic, as well as sympathetic, activity while he or she was at rest.

Thirty seconds after beginning, the participant was told to either begin the relaxed condition (RC) or occupied condition (OC) activity. He or she would continue this activity until the experiment was finished. Thirty seconds after beginning the activity, each participant received a warning stimulus from five feet away. The warning stimulus was four medium volume, pure tone beeps produced from a smartphone application. Ten seconds after the warning stimulus was given (total time: 70 sec), the participant was exposed to an auditory startle stimulus. The auditory startle stimulus was a short, loud bang produced by a screwdriver hitting a metal lid. It was introduced approximately one and a half feet behind the participant's head. At 100 seconds, participants were told the experiment was completed and that he or she may leave. The participant was disconnected from all equipment and thanked for their participation. Data was analyzed using Microsoft Excel.

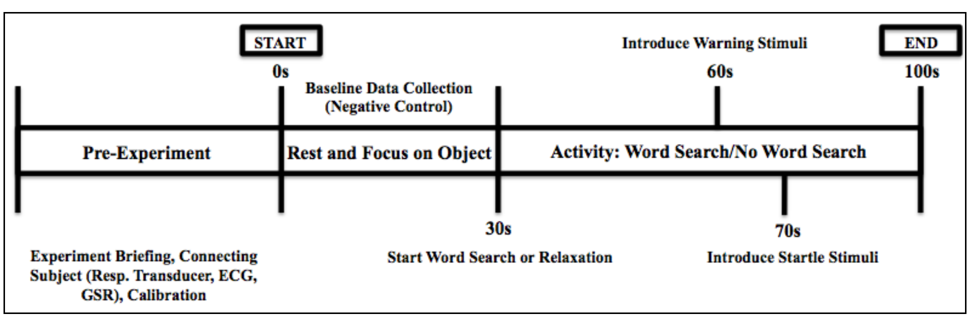

Figure 1. Timeline: Occupied/Relaxed Condition.

On a separate day, each participant was again recruited to obtain his or her positive control data (see Figure 2). The positive control data provided the participant's parasympathetic, as well as sympathetic, activity while he or she was vigorously riding a stationary bicycle. As before, the participant was taken to the quiet room and connected to all measurements. Upon attachment, all measurements were calibrated. Verbal instructions were given to each participant. To start, each participant remained at rest for 30 seconds while on the stationary bicycle. After the 30 seconds, the participant began biking at a moderate speed for an additional 30 seconds. One minute into the experiment, the participants were told to bike for 40 seconds at maximum speed. At the end of the 40 seconds, the positive control experiment was complete. All equipment was disconnected and his or data was analyzed and compared to previously obtained data.

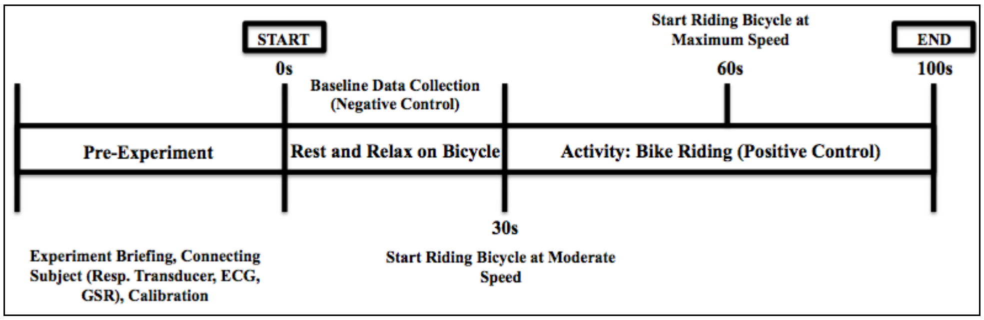

Figure 2. Timeline: Positive Control.

Statistical analysis of the data was done using standard deviations and conducting a t-test in order to determine a p-value. A p-value of less than 0.05 indicated significant differences between the relaxed and occupied condition groups. During data analysis, three participants (RC5, RC7, and OC3) were found to exhibit data points that differed greatly from the other participants. In order to prevent skewing of the overall data, two study participants from the relaxed condition group and one from the occupied condition group were removed. Exclusion of these participants was based on whether or not their data clearly deviated from the calculated averages.

Results

Emotion

Before beginning the study, participants were asked to complete a questionnaire (see Supplemental Materials II) that asked them whether they consider themselves to be a fearful person (fearfulness), whether loud noises tend to startle them (easily startled), and whether they feel anxiety while completing mental puzzles (task anxiety). Group averages of subject responses can be found in Table 1. Subjects in the relaxed condition had a 1.25 times higher fearfulness and startle ratings than subjects in the occupied group. This could explain the higher physiological responses observed in the relaxed condition.

Table 1. Questionnaire Averages by Subject Group

	Fearfulness (1-5)	Easily Startled (1-5)	Task Anxiety (1-5)
Occupied	2.625	3.25	3.125
Relaxed	3.25	4.125	2.875

Heart Rate

As illustrated by Figure 3, average maximum heart rates were similar in the occupied condition and relaxed condition group for the negative controls, post-warning condition, and post-stimulus condition. Maximum heart rates peaked at 150 bpm for both the occupied and relaxed condition groups due to software limitations. In the relaxed condition group, the max heart rates averaged 150 bpm in the positive controls, 103.7 ± 30.3 bpm in the negative controls, 79.2 ±16.3 bpm in the post-warning condition, and 95.5 ± 29.6 bpm in the post-stimulus condition. In the occupied condition group, the max heart rates averaged 150 bpm in the positive controls, 104.9 ± 32.2 bpm in the negative controls, 92.6 ± 28.5 bpm in the post-warning condition, and 93.5 ± 30.2 bpm in the post-stimulus condition.

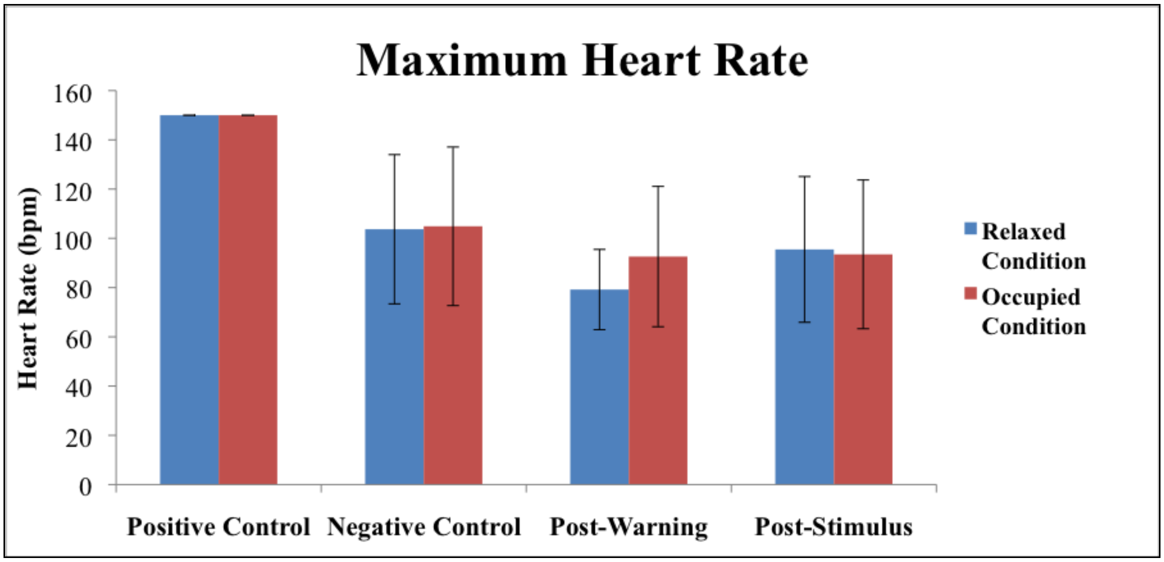

Figure 3. Average Maximum Heart Occupied Versus Relaxed Conditions.

Respiration

As illustrated by Figure 4, average maximum respiration circumferences were similar in the relaxed condition and occupied condition group for the positive controls, negative controls, post-warning condition, and post-stimulus condition. In the relaxed condition group, the max respiration circumference averaged 0.9 ± 0.6 cm in the positive controls, 0.3 ± 0.1 cm in the negative controls, 0.2 ± 0.1 cm in the post-warning condition, and 0.6 ± 0.6 cm in the post-stimulus condition. In the occupied condition group, the max respiration circumference averaged

0.8 ± 0.6 cm in the positive controls, 0.5 ± 0.5 cm in the negative controls, 0.5 ± 0.2 cm in the post-warning condition, and 0.5 ± 0.4 cm in the post-stimulus condition.

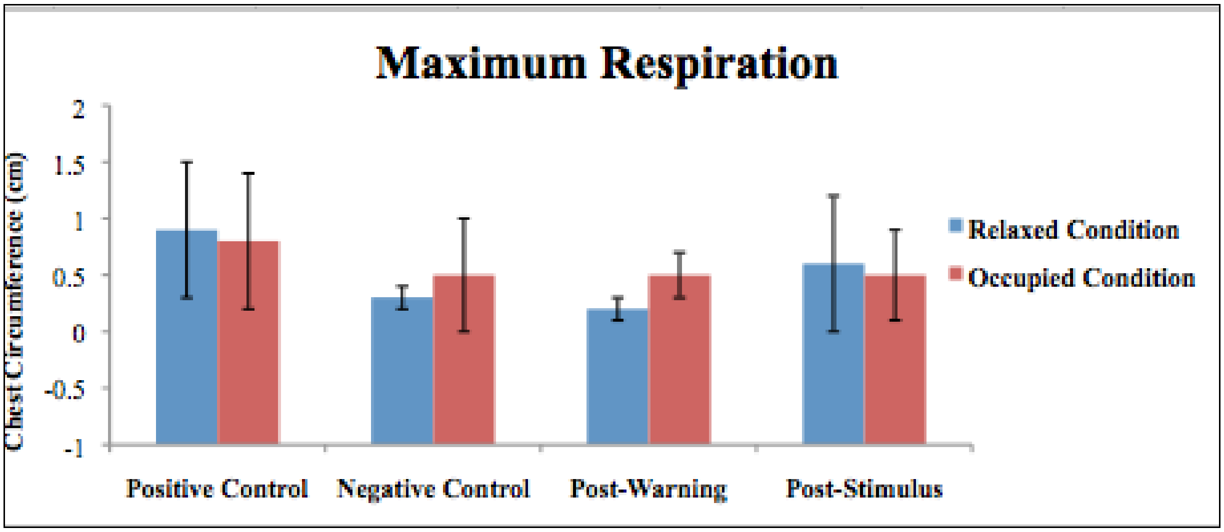

Figure 4. Average Maximum Respiration Occupied Versus Relaxed Conditions.

Galvanic Skin Response

As illustrated by Figure 5, average maximum GSR responses were similar for the relaxed condition and the occupied condition group in the positive controls, negative controls, and post-warning conditions. In the relaxed condition group, the max GSR averaged 0.8 ± 0.5 microsiemens in the positive controls, 0.4 ± 0.4 microsiemens in the negative controls, 0.3 ± 0.2 microsiemens in the post-warning stimulus condition, 1.3 ± 0.7 microsiemens in the post-stimulus condition. In the occupied condition group, the max GSR averaged 0.9 ± 0.5 microsiemens in the positive controls, 0.2 ± 0.1 microsiemens in the negative controls, 0.2 ± 0.4 microsiemens in the post-warning condition, and 0.5 ± 0.3 microsiemens in the post-stimulus condition.

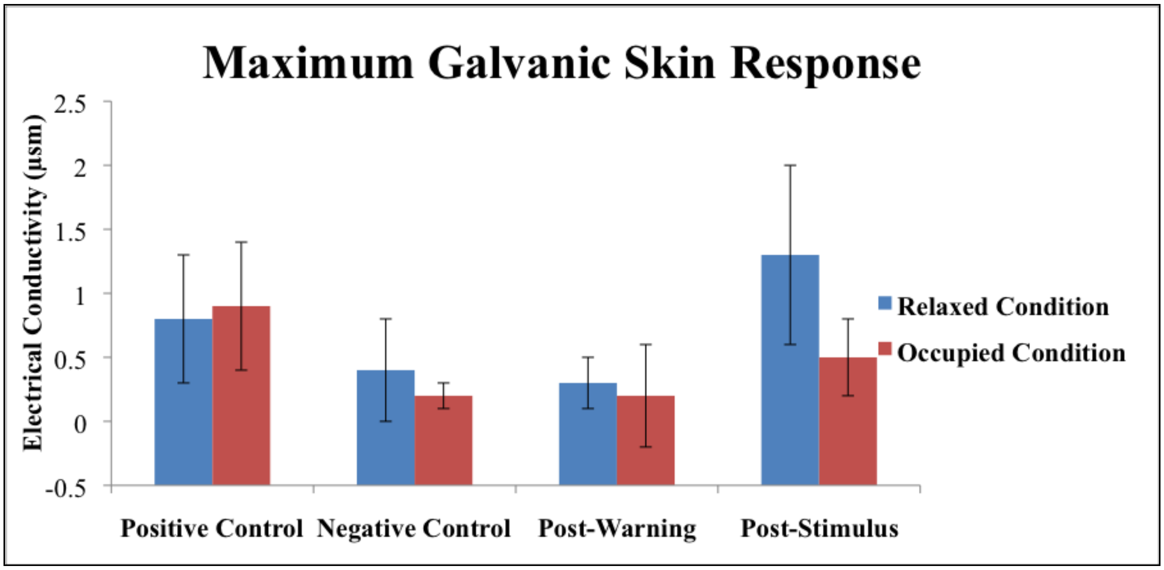

Figure 5. Average Maximum GSR Occupied Versus Relaxed Conditions.

Statistical Analysis

Comparing the relaxed and occupied groups during the post-stimulus condition, the GSR response exhibited a significant difference ($p = 0.04$) (Figure 5). The max heart rate and max respiration values did not differ significantly between the relaxed and occupied condition groups.

Comparing post-warning and post-stimulus conditions in the relaxed group, max GSR values exhibited a significant difference ($p = 0.01$) (Figure 5). Comparing post-warning and post-stimulus conditions in the occupied group also exhibited a significant difference in max GSR values ($p = 0.05$) (Figure 5).

Comparing positive and negative controls in the relaxed group, max heart rate ($p = 3.89 \times 10^{-6}$) (Figure 3) and max respiration ($p = 0.03$) (Figure 4) exhibited a significant difference. Comparing positive and negative controls in the occupied group, max heart rate ($p = 5.60 \times 10^{-5}$) (Figure 3) and max GSR ($p = 0.01$) (Figure 5) exhibited a significant difference.

Discussion

The purpose of this study was to determine whether a participant's physiological response to startle and warning stimuli are dependent on whether they are relaxed or occupied. At this time, data from relaxed and occupied conditions is not significant and thus conclusions supporting or rejecting our original hypothesis cannot be made. However, there are certain limitations presented in the study should be considered.

First, since the study had a limited sample size ($n = 16$) this data cannot accurately represent the population as a whole. It is likely that with the addition of more participants, the results could change and indicate significance. Furthermore a limited sample size increased the chances of human error. It is possible errors arose during collection of measurements for each participant. For example, a misunderstanding by the study participant or a lack of clarity when giving instructions resulted in a number of study participants engaging in the wrong activity (i.e. stare, relax, crossword, bike versus bike fast) at the incorrect time. This may have resulted in unwanted changes in their autonomic responses. Additionally, the ECG leg electrodes fell off of a few study participants during the positive control biking session. This was resolved by starting the biking session over.

Technological errors could have also occurred. In our data analysis from BIOPAC Student Lab Program, limitations in measuring heart rate were found. In the positive control data, the maximum heart rate for all participants leveled out at 150 beats per minute. Further, minimum heart rate for select participants recorded numbers as low as 29 beats per minute. The normal resting heart rate also ranged from 60 to 100 beats per minute. As a result of this likely error in measurement, it is quite possible that the calculated average heart rates were affected but not significantly.

In order to activate the physiological startle response properly, an effective stimulus must be used. However, since the intensity of the startle reflex is a reflection of emotion, each participant's autonomic response to the same stimuli varied, creating mixed results (Roy et. al, 2009). In addition, it was observed that the emotion and reaction of the researchers surrounding the participants may have affected the participants' reactions to the warning or startle stimuli. To limit this effect, we limited the amount of people present in the room during data collection to three: participant, auditory startle stimulus administrator, and data collector. By doing so, the amount of background noise was reduced. This was important to do because as a previous study reported, increased levels in background noise, paired with a stimuli, resulted in a reduced startle response (Davis, 1974).

The physiological response to a startle reflex further depends on the presence of a warning stimuli. Prepulse-to-pulse, or warning-to-stimulus, intervals in most human studies range from 30-240 ms (Braff et. al, 2001). However, in this study, a warning-to-stimulus interval of approximately 10 seconds was used. This may have diminished the effect of PPI. In other words, the time interval used in the study surpassed the period in which the brain had diminished responsiveness to stimuli. Thus, the body's physiological response to each stimuli would already be similar before the occupied or relaxed condition was introduced. In this case, our results would be significantly affected.

In conclusion, we were unable to confirm or refute our original hypothesis and conclude whether relaxation versus occupation determined the degree of physiological response to an auditory stimulus. Our recordings of heart rate, respiratory rate, and skin conductance did reveal a physical response to stimuli, but the effect was not statistically significant within our sample size. The limitations discussed may have contributed to this result. For future research it is suggested that a more controlled environment, higher sensitivity data equipment, a larger sample size, and a variation in stimulus duration be implemented.

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Supplemental Materials

I. Consent Form

University of Wisconsin-Madison
Physiology 435 – Group 5
Research Participant Information and Consent Form

Title of the Study: Physiological Response to an Auditory Startle Stimulus under Relaxed and Preoccupied Conditions.

Principle Investigators: Maddy Eckelaert, Michelle Cancel, Jai Patel, Zach Bennett, Miki Hirano, Ashley Verhasselt

Description of the Research and Participant Involvement:
I understand that I will be asked to either attempt to complete a word search as quickly as possible or relax with my eyes closed during this experiment. While I am completing my given task, I will hear a warning stimuli and I may or may not experience an auditory startle stimulus. I agree to have my skin conductance, respiratory activity and heart rate monitored throughout the duration of the experiment. The experiment should take approximately 20 minutes to complete.

Risks:
There should be no significant health risks associated with this experiment but please do let the experimenters know if you have a heart condition that may be provoked from being startled.

Benefits and Compensation:
I understand that I do not stand to gain any favors by agreeing to take part in this experiment and that I will not be penalized should I decide not to volunteer as a subject, or should I decide to withdraw from participation. I also understand that there are no direct benefits to me by participating in this experiment, but I may ask any of the experimenters of this study to participate in my study.

Confidentiality:
Personal information will be kept confidential. Only group characteristics will be published.

Questions:
I understand that I may ask questions about my rights as a subject, or register any complaint I might have about the experiment with any of the researchers listed above.

Name (printed): ____
Gender: _________________
Age: ________
Signature: ____
Date: __________________

II. Participant Questionnaire

University of Wisconsin-Madison
Physiology 435 – Group 5
Research Participant Questionnaire

Respond to the following questions by circling a number 1-5 to indicate your agreement/disagreement with the following statements

Strongly Disagree		Neither Agree nor Disagree		Strongly Agree
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5

Would you consider yourself a fearful person?
Do loud noises tend to startle you?
Do you consider yourself proficient at mental puzzles?
Do you feel anxiety while completing mental puzzles?
Do you have a history of heart disease?

Name (printed): ____
Gender: ________________
Age: _______
Signature: ____
Date: _________________

III. Equipment and Justification for Use

Respiration Transducer (Attached to Channel 1)

Measurements indicate inhalation, expiration and breathing strength. Using this device, it is possible to observe the physiological effects of an auditory startle stimulus on the breathing rate and breathing pattern of RC and OC experimental groups. For best results, straps are snugly wrapped around the subject's chest under their arms. If possible, the bagginess of the subject's clothing is limited.

Electrocardiogram (ECG) (Attached to Channel 2)

An ECG assesses the electrical and muscular functions of the heart. An ECG is used specifically to monitor and detect heart activity changes in the participants. A disposable snap electrode is fixed to the inside of the right wrist and the inside of the left and right ankle. The snap electrode on the right wrist is clamped with a white electrode clip, the right ankle with a black electrode clip, and the left ankle with a red electrode clip.

Finger Electrode (Attached to Channel 3)

Resistance of the skin is be measured by analyzing the activity of sweat glands in the fingers. This indicates the amount of physiological stress the participant experiences during the course of the experiment.

Auditory Warning Stimulus

Once baseline data is collected, the warning stimulus is presented to the participant. The stimulus is a ringing noise produced from a smart phone application.

Auditory Startle Stimulus

Approximately 10 seconds after presentation of the Auditory Warning Stimulus, an Auditory Startle Stimulus is be presented to the participant by another investigator. A loud banging of a screwdriver against metal serves as the stimulus.

Occupied Task

A word search is presented to OC group.

IV. BIOPAC Student Lab Program Overview

1. To begin testing, click 'Correlate'
2. To add comments, type in the comment bar at the top and click F9 on the keyboard to save the comment in time with the data being collected
3. Integrate data with Microsoft Excel
    a. In BIOPAC Student Lab Program, press 'I' and highlight the area of interest.
    b. Go to Edit → data windows → copy wave data
    c. Open Microsoft Excel → Paste data