The Effect of a Mismatched Audiovisual Stimulus on Heart Rate, Respiration Rate, Skin Conductance, and Beta Waves

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Key Terms: sensory, auditory, visual, electromyography, electrodermal activity, stress, mismatch

Word Count: 5119

Abstract

Previous research has shown that sensory stimuli can induce a stress response. This response occurs as a result of a sympathetic response involving the hormones epinephrine, norepinephrine, and cortisol. Sensory stimuli initiate this response through immediate preconscious processing and longer-term conscious processing in the central nervous system. The stress response results in increased heart rate, respiration rate, skin conductance, and beta wave frequency among other physiological measures. Overactivation of the stress response has been found to cause stress-related diseases. The purpose of this study was to assess if a novel audiovisual (AV) stimulus synthesizing previous research provoked a greater stress response than traditional audiovisual stimuli. This study utilizes a novel mismatched AV stimulus that paired emotionally connotated images with oppositely connotated sounds.The traditional matched AV stimulus paired emotionally connotated images with correspondingly connotated sounds. 25 subjects between the ages of 20 and 22 were treated with a three minute video from one of the two conditions. Their heart rate, respiration rate, skin conductance, and beta wave frequency were recorded. The mismatched AV stimulus caused a significantly greater increase in heart rate, skin conductance, and beta wave frequency compared to the matched AV stimulus. The increase in respiration rate was not found to be significant. Overall, the research findings offer support that a mismatched AV stimulus elicits a greater stress response than a traditional, matched AV stimulus. These findings have future implications in neurological disorders and the optimization of stress reduction therapies.

Introduction

Higher organisms use sensory processing to understand and navigate their environments, and when a threat is sensed, some have evolved a stress response utilizing epinephrine, norepinephrine, and cortisol that help them to fight the stressor or to flee the situation. This stress response is useful in life-threatening situations; however, it is often invoked in non-life-threatening situations. An overexposure to cortisol has been used to explain cases of cardiovascular disease, anxiety disorder, respiratory disease (Schneiderman 2005), and osteoporosis (Wang 2016). Unfortunately, reported levels of stress in American adults have been on the rise since 2007 (APA 2015). Because of its association with disease, it becomes important to understand what non-life-threatening situations the human body perceives as threats and the underlying physiological mechanisms of this response.

Traditional research on stressors such as treadmill tests (Goldschlager 1976) has expanded to include a vast array of stressors such as timed mathematics tests (Ramirez 2013) and high altitude rock climbing (Schöffl 2007). Despite this expansion, the field of research directly measuring physiological responses to stressful *sensory* stimuli is relatively small despite the sensory system's role in preconscious cognitive processing (Fox 2010), which plays an active role in the stress response (Dendovic 2005). With regards to sensory stress tests, Thayar *et al* reported in 1983 that an audiovisual (AV) stimuli could induce a stress response in participants as measured by skin conductance. Another method of investigating sensory stimuli is mismatch negativity, which measures participants' physiological responses to speech-related images paired with sounds shown at a fast rate. Mismatch negativity research has gained insight into several subconscious processes related to stress (Näätänen 2007). Research in this field has supported the notion that mismatch negativity AV stimuli have predictable physiological responses--on the scale of hundreds of milliseconds--because these responses precede higher conscious processing (Surraka 1998). Similar research has shown longer term predictable physiological responses--on the scale of seconds--to emotionally connotated AV clips (Brouwer 2013). This research begets a question that has not been asked: How does the body react to emotionally connotated images paired with oppositely connotated sounds with time to process the "mismatched" stimuli?

At a physiological level, stressful stimuli invoke the sympathetic nervous system, which is responsible for what is colloquially known as the fight-or-flight response. During this response, the hormones epinephrine, norepinephrine, and cortisol are released into the bloodstream. These hormones temporarily increase metabolic rate, mobilize energy, and prepare for healing a potential wound while inhibiting processes not pertinent to the situation, such as those involved in resting and digestion. For instance, heart rate and respiratory rate increase to increase oxygen availability to skeletal muscle and the brain (Masaoka 1997). Similarly, sweat glands temporarily fill and then empty in case physical exertion becomes necessary, which is best measured by electrodermal activity (EDA) (Storm 2002). At a neurological level, brain waves move into higher frequency beta waves, which are indicative of alertness (Lee 2014). These are among many other physiological changes that occur during a sympathetic stress response, and they are the components that this study will examine. Moreover, stress can be positive or negative depending on the situation. A positive stress, or eustress, helps someone complete a task or challenge, such as completing an examination in a timely manner (Ramirez 2013). A negative stress, or distress, is in response to increased anxiety or fear, such as during an injury (Krivokapich 1993). The stimuli in this study were designed to induce negative stress on the subject because they were not performing a task while watching the video. Subsequently, we expect to see an increase in the aforementioned physiological measures if the AV stimulus is successful in inducing negative stress.

The design of our two AV stimuli as well as the physiological responses we consider are built on previous research. In 2013, Brouwer *et al* measured skin conductance and heart rate in response to emotional pictures paired with emotional sounds. In this study, positively connotated images were paired with positively connotated sounds and negatively connotated images were paired with negatively connotated sounds. We define this pairing of similar stimuli as “matched” stimuli. For example,Brouwer *et al*’s study used an image of a water slide paired with an audio clip of a boy laughing as a positively connotated matched stimuli, and a photo of a dead body paired with an audio clip of a siren as a negatively connotated matched stimuli. When shown negatively matched stimuli, the researchers found lower heart rates when compared to pleasant stimuli. In addition, skin conductance increased in both pleasant and unpleasant stimuli. Our study investigates this physiological relationship further with a “mismatch” of emotionally connotated AV stimuli, which pairs positively connotated images with negatively connotated sounds. In 2014, Lee *et al* studied how brain waves were affected by emotionally connotated visual stimuli using an electroencephalogram (EEG) to measure the ranges of alpha (8-12Hz), beta (13-30Hz), gamma (31-50Hz), and theta waves (4-7Hz). These stimuli were meant to elicit positive emotions including amusement and surprise, neutral emotions, or negative emotions including fear or disgust. Pertinent to our study, they found that negatively connotated clips showed a significantly higher frequency of beta waves, which is a another variable we will consider.

To further the field investigating stress responses to sensory stimuli, we are creating a novel “mismatched” AV stimuli and comparing subjects’ physiological response to a “matched” AV stimuli. This research could find applications in inducing stress, such as in horror movies or videogames, or reducing stress, such as in listening to music during locally anesthetized surgical procedures. We hypothesize that the mismatched AV group will see a statistically significant increase in average respiration rate, heart rate, skin conductance measured by EDA, and beta wave activity measured by EEG compared to the matched audio-visual group in accordance to a greater negative stress response in the mismatched AV group. We believe this difference will exist because higher order processing will initiate more of a stress response to the unexpected “mismatched” stimuli than to the “matched” stimuli.

Methods

Participants

Twenty-four students participated in this study. These participants were pulled from the University of Wisconsin-Madison’s Physiology 435 class on a voluntary basis. Of these participants, 13 were female and 12 were male. Their ages ranged from 20 to 22. We asked each participant how many long they had slept the previous evening and the amount of caffeine they had consumed the morning of the experiment. The amount of sleep ranged from 4 to 10 hours , and on average the participants got 6.8 hours of sleep. 10 participants had consumed coffee the morning of the experiment, consuming an average of 9.6 ounces. These demographic characteristics were not considered to be confounding and are discussed further in the “statistical methods” section. All participants were given a consent form before starting the experiment warning them of possible disturbing material in the video. Participants were ensured their identities would be kept confidential.

Materials

Heart rate, respiration rate, beta brain waves, and electrodermal skin response were recorded while participants sat in a chair, wore headphones, and watched one of two recorded videos with either matched or mismatched AV stimuli. Both videos contained 6 positively connotated images and 6 negatively connotated images, each 15 seconds in duration. The images were retrieved from various websites, and the songs were obtained from Youtube (see Table 1 for references). Participants used Bose Quietcomfort 25 Acoustic Noise Canceling headphones connected to a Mac Laptop computer (Assembled in China, Model #A1466) for the stimulus. Nonin pulse oximeter (Nonin Medical Inc. Plymouth, MN. Model #9843) was used to measure the participants pulse in beats per minute (BPM). A respiratory transducer (Biopac Systems Inc. Goleta, CA, Model #SS5LB) was used to measure participant respiration rate in the units of respirations per minute. To measure the presence of beta brain waves, EEG data was collected using the Biopac EEG system assembled in Goleta, California with the following parts: EEG lead set (Model #SS2L), disposable electrodes (Model# EL503), Electrode Gel (Model #GEL1), and an abrasive pad (Model #ELPAD). Lastly, electrodermal response was measured using the Biopac disposable electrodermal (EDA) set up with the following parts also assembled in Goleta, California: EDA lead (Model #SS3LA) and isotonic electrode gel (Model #GEL101).

Experimental Stimuli

Two stimuli were used in this experiment. The research team created both of the videos, each three minutes in length. The positively connotated images and audio clips were chosen to induce positive emotions such as happiness or joy. The negatively connotated images and audio clips were chosen to induce negative emotions such as fear or disgust. Both image types were generated with Brouwer *et al* as a guide. The pattern was a positive image followed by a negative image repeated six times in total. The "matched" video had positive images with positive music and negative images with negative music. The "mismatched" video had the same image order, except the audio clips were reversed for each pattern and so had positive images with negative music and negative images with positive music. Figure 1 includes the images and corresponding audio clips used in each video.

Experimental procedure

Participants were assigned either matched or mismatched stimuli randomly. A fair coin--1965 D quarter--was flipped 35 times to assign participants a treatment; heads for matched treatment and tails for mismatched treatment. The participants were not told which group they were assigned prior to the experiment. Participants were seated in a stationary chair in front of the laptop. Researchers gave out the consent form, which was read by the participant. If the participant was unwilling to sign the consent form, they would have been released from the study; however, this was not encountered. They were then given a short survey that asked their age, biological sex, caffeine intake that day, and hours slept the night before in case these became a confounding variable or a source of biasedness. Analysis of this survey was performed and is reported on in the following "statistical methods" section.

Upon consent, researchers attached the respiratory belt around the upper chest (Figure 2), attached the three EEG electrodes with conducting gels (Figure 3), wrapped the 2 EDA electrodes with conduction gel around the participant's left middle and index fingers, and placed the pulse oximeter on the participant's right index finger (Figure 4). These attachments were made in no particular order. The pulse oximeter was manually read every 10 seconds for heart rate (BPM) and recorded into a Google Sheet. The respiration belt produced a waveform that the researchers used to count breaths. Breaths were considered to be any peak with a corresponding trough that had a range of at least half the amplitude (mV) between them (Figure 5). Researchers then took a baseline measurement for 2 minutes while the participant looked at the laptop with a blank screen. The baseline was compared to the treatment to measure physiological change. After baseline measurements, researcher started the proper AV stimulus: matched or mismatched. Once the video ended, researchers stopped recording, removed the equipment, and debriefed participants to the full nature of the experiment, strongly advising the participant not to alert their peers to the nature of the study. The total time of the experiment was approximately 17 minutes. Figure 6 denotes an overall timeline for this procedure.

Statistical Methods

For each measured variable, a right-sided paired sample t-test for mean difference in change in physiological variable due to mismatched AV stimuli versus matched AV stimuli was performed. Results will be considered significant if the t-value with n-1 degrees of freedom has a corresponding α-value $< .05$.

$$t_{mm-m,df=n-1} = \frac{\frac{dy}{dx}(mm) - \frac{dy}{dx}(m)}{\sqrt{\frac{s_{mm}^2}{n_{mm}} + \frac{s_m^2}{n_m}}}$$, where m=matched audiovisual stimuli and mm=mismatched audiovisual stimuli.

Considered significant when $P(t_{mm-m,df=n-1} < \infty) < .05$.

A t-test was performed because the sample size was less than 30 subjects and the variance was unknown for the physiological response variables. The last 30 seconds of the 2 minutes of baseline data were used for baseline physiological measurements to control for possible physiological arousal at the beginning of data collection. The first 30 seconds of the 3 minutes of treatment data were considered in accordance to the length of time that Brouwer *et al* considered AV stimuli to have a predictable physiological response. The derivative of beta waves frequency (12-40 Hz) was calculated in Biopac (Figure 7) because this method controls for large variation in ranges due the amount of conducting gel applied and human counting error.

Demographic analysis of the sample was conducted to identify possible biases. Biological sex was approximately uniform with 13 females and 12 males. Age approximately followed a normal distribution centered around 21.2 years. Time slept the previous night approximately followed a normal distribution centered at 6.8 hours slept. 10 subjects consumed caffeine; however, most reported their consumption as at least 4 hours before the experiment with the average amount being 9.8 ounces. Moreover, a preliminary t-test for mean differences in heart rate was conducted between caffeine consumers and non-caffeine caffeine consumers in the control group, and the difference was not found to be significant. In summation, analysis of the demographic does not appear to indicate bias towards a particular college demographic in the sample. Yet, it is worth noting that the sample is only representative of scientifically oriented college-aged persons in a laboratory setting.

Controls

Before the methods were finalized, controls were conducted to determine if the equipment was operational and if the AV stimuli elicited a physiological response. A negative control was performed to ensure that physiological responses did not occur when stimuli were absent. The subject was placed in front of a blank screen with monitoring devices. No remarkable physiological responses occurred with the negative control, and so the experimenters moved onto the positive control. The positive control was performed on two group members. Participant 1 was presented with the matched AV stimuli and participant 2 was presented with the mismatched AV stimuli. Experimenters noticed that participant 2 had a wider range of heart rate compared to participant 1. Participant 2 also experienced a decreased respiration rate while participant 1 had increased respiration rate. While their baseline EDA measurements varied greatly, the change in skin conductance was similar. Participant 2 experienced an increased rate in frequency of beta waves measured by the EEG compared to participant 1.The positive control experiments proved the methods to be executable and that the stimuli did induce a physiological response.

Results

Heart Rate

The mismatched AV group had a significantly larger increase in heart rate over the matched AV group (p*=.0458). As an example, the experimental data for participant 25 and participant 10 are shown in Figure 8. Participant 25--mismatched AV treatment--saw a rise in heart rate of 22

BPM, and participant 10--matched AV treatment--saw a fall in heart rate of 7.5 BPM. The average increase in the mismatched treatment was 4.27 BPM (n=14, s=7.26 BPM), and the average increase in the matched treatment was .09 BPM (n=11, s=3.36 BPM). Figure 9 shows this data graphically.

Respiration Rate

The difference in change in respiration rate was not significant (p=.3611). The average increase in the mismatch treatment was 1.85 breaths per minute (n=14, s=2.36 breaths per minute), and the average increase in the match treatment was 1.52 breaths per minute (n=11, s=2.09 breaths per minute). Several participants held their breaths during the videos, which we discuss further in the discussion section. Figure 10 shows this data graphically.

Electrodermal Activity

The mismatched AV group had a significantly larger increase in EDA over the matched AV group (p*=.0312). As an example, participant 25 of the mismatched treatment showed an increase of 4.3 microsiemens (Figure 11), while participant 20 of the matched treatment showed a decrease of 1.8 microsiemens. The average increase in the mismatch treatment was 1.1926 microsiemens (n=12, s=1.32 microsiemens), and the average increase in the match treatment was .1678 microsiemens (n=9, s=.94 microsiemens). The decreased n-values were due to data recording errors: the skin conductance electrodes did not measure any conductance for participants 8, 9, 16, and 18. Figure 12 shows this data graphically.

Electroencephalogram

The mismatched AV group had a significantly larger increase in their rate of beta wave frequency over the matched AV group (p*=.0337). As an example, participant 14 of the mismatched treatment showed an increase of 6.99 Hz, while participant 20 of the matched treatment showed a decrease of 10.93 Hz. The average increase in the mismatch treatment was 1.1238 Hz (n=14, s=1.77 Hz), and the average decrease in the match treatment was 1.0854 Hz (n=11, s=3.33 Hz). Figure 13 shows this data graphically.

Discussion

This research provides evidence that a novel mismatched audiovisual stimulus evokes more of a stress response in individuals than a traditional matched audiovisual stimulus. Although it has limitations, it offers valuable insight into sensory stressors and finds applications in future neurological research and stress reduction therapies.

Heart rate, skin conductance, and beta wave brain wave activity increased significantly in the mismatched treatment group compared to the matched treatment group. While these measurements are useful in evaluating a stress response and many researchers have used them, a more direct method would be to directly measure the catecholamines involved in the stress response--norepinephrine and epinephrine--using saliva samples and biochemical techniques. A baseline measurement could be compared to the treatment response of an individual, and the average percentage change could be used to test the hypothesis. This method is only useful in a longer conscious stress response because it takes time to collect the sample, but it would bring researchers closer to the physiological source of the stress response. Future researchers looking at sensory stressors may consider utilizing this method.

The respiration rate was expected to increase for the mismatch treatment group; however, no difference was observed. Some participants actually held their breaths during their treatment. This was not expected with a stress response in which increased oxygen is advantageous.

Perhaps an increased heart rate mobilized oxygen and because there was no physical activity occurring, the lungs compensated by slowing respiration and decreasing pulmonary oxygen perfusion. Counting breaths also proved problematic. Although a rigorous methodology was defined in the experimental procedure, this was difficult to apply in practice. Some participants would have the occasional shallow breath or long exhalation that interfered with the data as only thirty seconds of baseline and treatment data were being considered. Statistically speaking, on average these events should not interfere with the results, but as the sample size was relatively small (mismatched n=14; matched n=11), four or five of these events may not affect the groups equally. Moreover, a larger sample size would have produced more robust results in all measurements as variances decrease and the likelihood of a false positive significant result decreases. That said, we believe our results to indicate that a stress response was indeed occurring, and it was occurring more so in the mismatched AV stimulus treatment group.

The results of this experiment are believed to be sound and repeatable, but there were some potential sources of error. Although participants were instructed to keep their hands on their laps and not move, some hand movement occurred and some participants were spinning and rolling in their chairs. These movements could have affected skin conductance or EEG monitoring, caused a non-stimulus related change in heart rate, or altered the pulse oximeter position. Nail polish is also known to affect pulse oximeter sensors (Cote 1988), and some of the participants were wearing nail polish. In general, the environment of the experiment could have been more controlled. The subjects were surrounded by peers that might have affected their interpretation of the stimuli due to social expectations. The amount of light in the room and glare on the screen varied from day to day of data collection. The subjects were also anticipating a stimulus that would elicit a physiological response, as was the nature of the experiments the class was performing on one another. These potential sources of error could be controlled for by having a participant not enrolled in Physiology 435 view the video in a dim lit room by themselves in a stationary chair.

Device calibration would have also contributed to more robust results. The EEG data did not involve a calibration step, and this step is crucial in insuring proper signal strength from electrodes. Without this step, the signal to noise ratio of the readout can be large (Teplan, 2002). Moreover, EEG and skin conductance measurements relied in part on the amount of conducting gel used. The more gel that was applied, the higher the initial reading of skin conductance. Although this research investigated the *change* in skin conductance and and should not necessarily be affected by the amount of gel used, a more controlled application of gel or some form of calibration is desirable for future research. Calibration would have also helped to detect when the equipment was not correctly connected to the individual. As noted in the results section, a few participants had data that was not recorded correctly, and calibration could have alerted the experimenter to a null reading before the treatment was applied. While calibration should produce more accurate results, we still believe our data to be precise, and this precision is what counts when comparing mean differences.

Participants were asked to verbally give feedback on the stimuli they watched, and this provided valuable information about their processing of the stimuli. The stimuli were physiologically stimulating as shown with our data, but they were also mentally stimulating. A few participants responded after the video, saying “that was interesting.” This provides preliminary evidence that some higher conscious processing was involved in their response. A few of the participants stated that they were able to figure out the pattern between the images and sounds after a few AV pairings into the mismatched stimuli, implying that they were “puzzled” in some sense by the pairings. Perhaps this processing of the mismatched stimulus evoked more of a stress response than the matched stimulus group, but perplexion is not a quantifiable experiences and serves only as conjecture in explaining our results. Other comments reaffirmed that the music and images in the stimuli successfully elicited the emotions of happy and scared. More than one participant stated when “Walking on Sunshine” by Katrina and the Waves was playing, they were trying not to dance or sing. Another stated that she had a personal fear of “Slender Man,” an image used in the stimuli. The participants’ comments were informative, and they gave us insight to the schemas participants and how it could have impacted the results.

Cultural background should be considered in future research of sensory stimuli. The emotionally connotated stimuli were created from our knowledge based on Western culture, so participants in this study understood the different images’ and music’s connotations. For example, they perceived the image of the beach as positive, and they perceived the horror music as negative. While this particular set of mismatched AV stimuli elicits a greater stress response than matched AV stimuli, this may not hold true across cultures. However, we believe that this general greater stress response to mismatched AV stimuli over matched AV stimuli should hold across cultures with appropriately connotated cultural symbols and sounds.

This research has applications in neurology. Mismatch negativity research, which focuses on preconscious physiological responses to AV stimuli, has investigated neurological differences between persons with Alzheimer’s disease, Parkinson’s disease (Pekkonen 2000), and Autism Spectrum disorders (Dunn, 2007). These individual’s preconscious processing in response to mismatch negativity has been found to differ from the general population, and this has provided researchers with valuable information on the nature of these diseases and disorders. We believe that a longer term, conscious approach to this research could also provide insight into these diseases and disorders. Research could investigate if those with neurological diseases and disorders have different structures in the brain involved in consciously processing the novel mismatched AV stimuli than the general population, and this information could be valuable in clinical diagnosis.

In environments where someone is subjected to potentially frightening visual stimuli and auditory stress reduction therapies are used, this research suggests that playing positively connotated music may not reduce their stress levels. For example, when a child is in the dentist's office for a filling, or when someone is watching their own locally anaesthetized procedure, playing positively connotated music may actually make the situation more stressful. Future research could investigate physiological measurements or salivary hormone measurements during the procedure under different auditory conditions, and their results could prove useful in creating optimal auditory stress reduction therapies.

In summary, our results indicate that a novel mismatched audiovisual stimulus induces more of a stress response than a traditional matched audiovisual stimulus potentially because of higher order processes involved in releasing greater amounts of catecholamines. Further research could confirm these processes and find applications in neurological disorders and stress reduction therapy.

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Figures and Tables:

	Associated Music		
Image	Matched	Mismatched	Duration (s)
duckling	The Benny Hill Theme Song	Toccata and Fugue by Bach	15
slenderman	Toccata and Fugue by Bach	The Benny Hill Theme Song	15
Nemo from Finding Nemo	party noises and laughing	a chainsaw	15
an outline of a face in the dark	a chainsaw	party noises and laughing	15
a happy family	Octopus' Garden by The Beatles	The Murder by Bernard Herman	15
a person with their head on backwards	The Murder by Bernard Herman	Octopus' Garden by The Beatles	15
a beach	Walking on Sunshine by Katrina and the Waves	The Jaws Theme Song	15
the girl from The Ring	The Jaws Theme Song	Walking on Sunshine by Katrina and the Waves	15
a hot air balloon	Blue Danube Waltz by Strauss	chains rubbing together	15
aliens	chains rubbing together	Blue Danube Waltz by Strauss	15
people graduating from college	springtime birds	The Funeral March by Chopin	15
realistic ghosts	The Funeral March by Chopin	springtime birds	15

Figure 1. The pairing of images and music for both matched and mismatched AV stimuli in order of appearance. See "Table 1" for references.

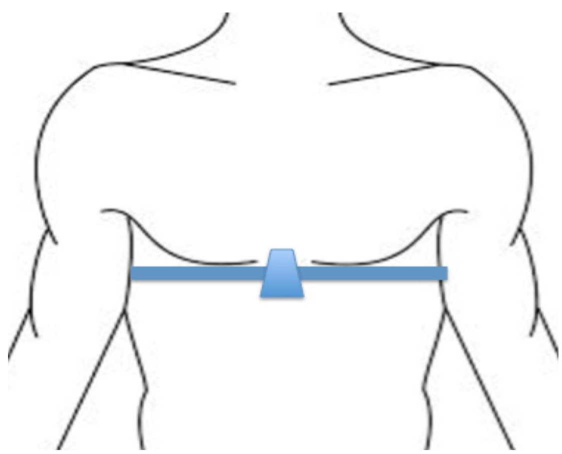

Figure 2. Example placement of the respiratory belt used on each participant. The sensor labeled with the "Biopac Systems" logo was placed on the sternum, below the breast line.

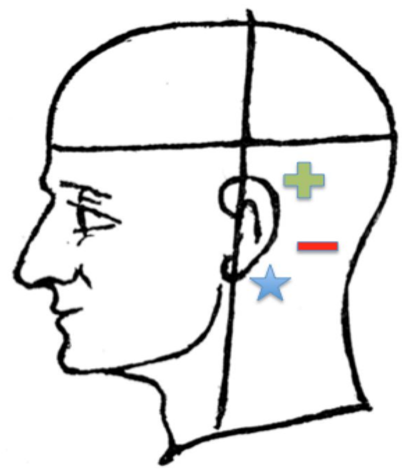

Figure 3. The biopotential amplifier modules for the Biopac system use a three-electrode arrangement: VIN+ (+), VIN− (-), and GND (Star). This diagram was used as a guide to place the EEG electrodes on participants.

Figure 4. Electrodermal activity (EDA) sensing skin conductance finger electrodes were placed on the participant’s left index and middle fingers. The pulse oximeter was clipped on participant's right index finger.

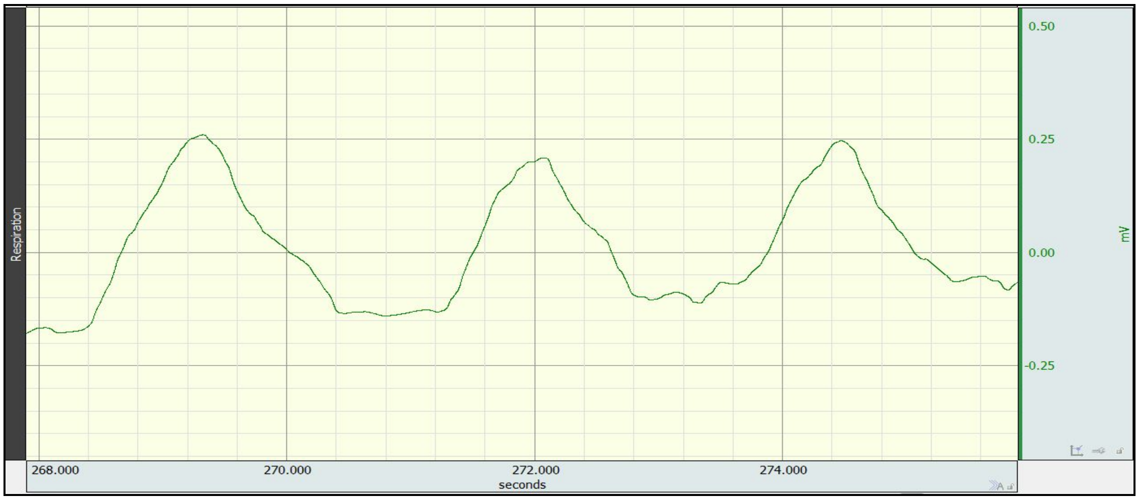

Figure 5. Screenshot of a portion of a participant's respiration graph measured in millivolts (mV) over time (seconds). Each peak denotes one breath.

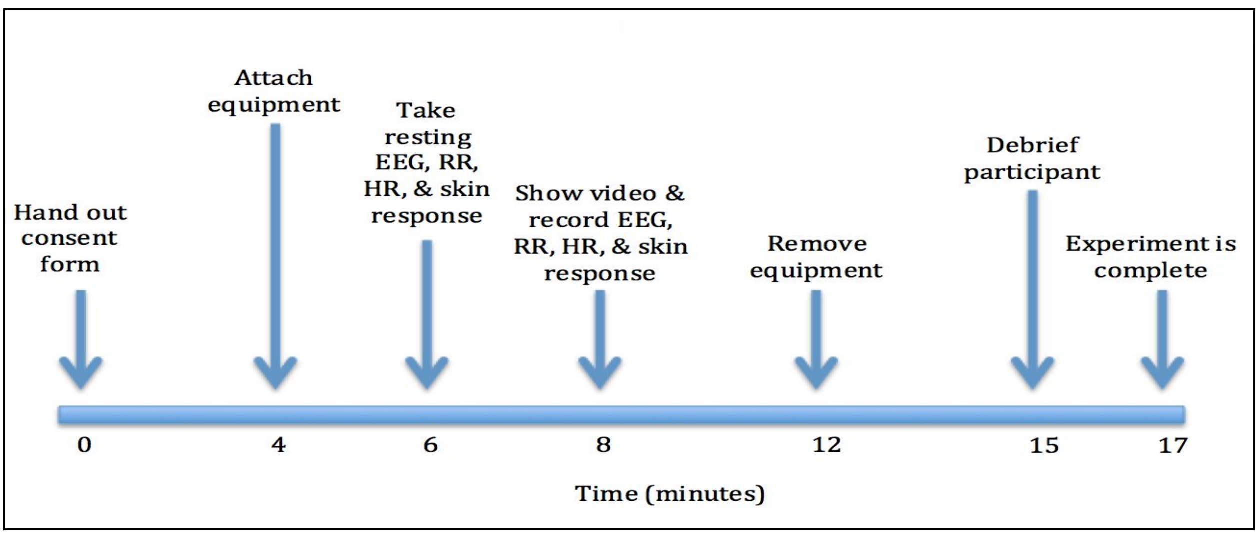

Figure 6. General timeline of events participants experience in a single trial over time (minutes). Following the participant's signed consent, experimenters attached equipment to the participant and a baseline reading consisting of a continuous measurement of skin conductance response, EEG, respiratory rate, and recorded the heart rate every 10 seconds. After the baseline, the video was played and the same factors were recorded. Equipment was then removed, the participant was debriefed about the study, and the experiment was complete.

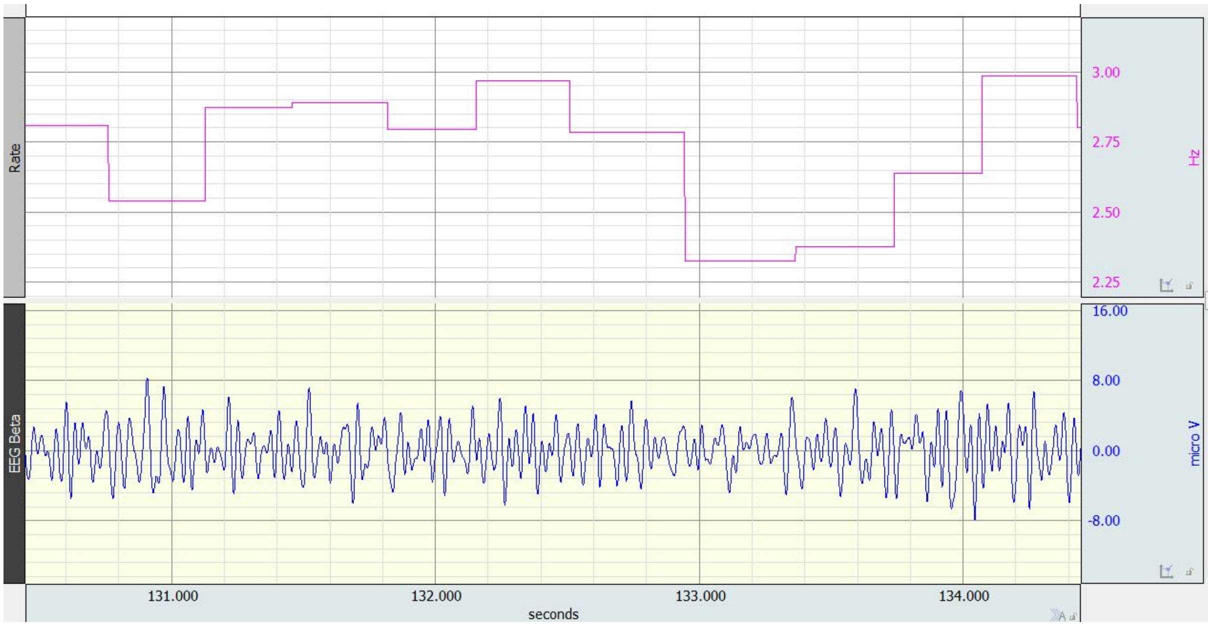

Figure 7. Screen shot of EEG beta wave collection data during the first four seconds of the mismatched video. The derivative of beta wave frequency (Hz) is graphed above beta wave frequency (mV) and was calculated using the Biopac program.

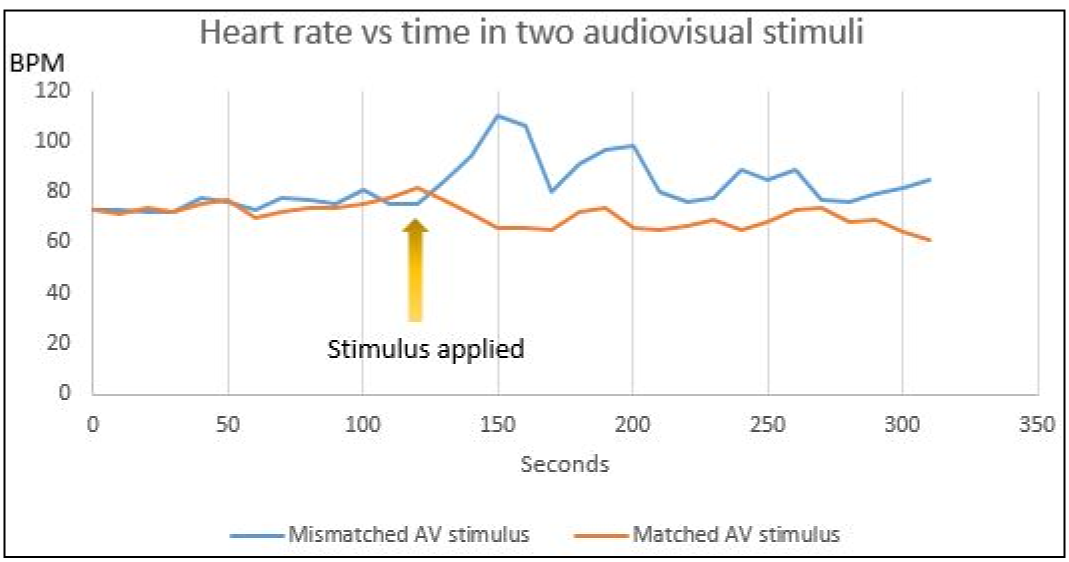

Figure 8. Comparison of two participants' heart rate measured in beats per minute over time (seconds). The arrow on the graph indicates when the video was started, and to the left of the arrow is the baseline measurement.

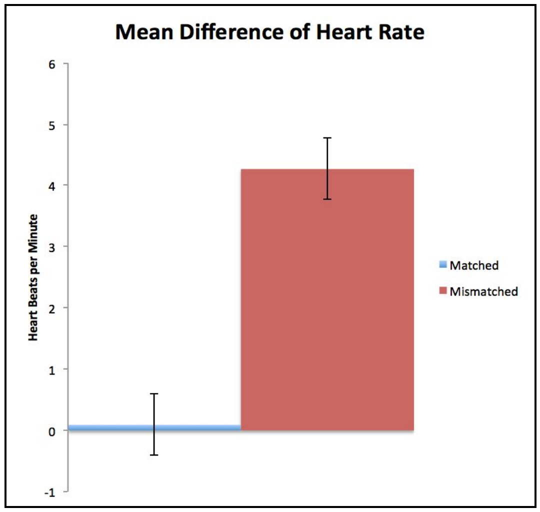

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Figure 9. Comparison of matched and mismatched average heart rate difference during assigned audio-visual stimuli. The data was found by finding the difference between the heart rate during the last 30 seconds of baseline and heart rate during the first 30 seconds of the experiment (treatment-baseline). The average increase in the mismatch treatment was 4.27 BPM (n=14, s=7.26 BPM), and the average increase in the match treatment was .09 BPM (n=11, s=3.36 BPM). p*=.0458

Figure 10. Comparison of matched and mismatched average respiration rate difference during assigned audio-visual stimuli. The data was found by finding the difference between the respiration date during the last 30 seconds of baseline and respiration rate during the first 30 seconds of the experiment (treatment-baseline). The average increase in the mismatch treatment was 1.85 breaths per minute (n=14, s=2.36 breaths per minute), and the average increase in the match treatment was 1.52 breaths per minute (n=11, s=2.09 breaths per minute). p=.3611

Figure 11. Screenshot of a participant's skin conductance data measured in microsiemen over time (seconds). The data to the left of the black line is the baseline measurement. Data to the right of the black line is the measurement took while the participant watched the mismatched video.

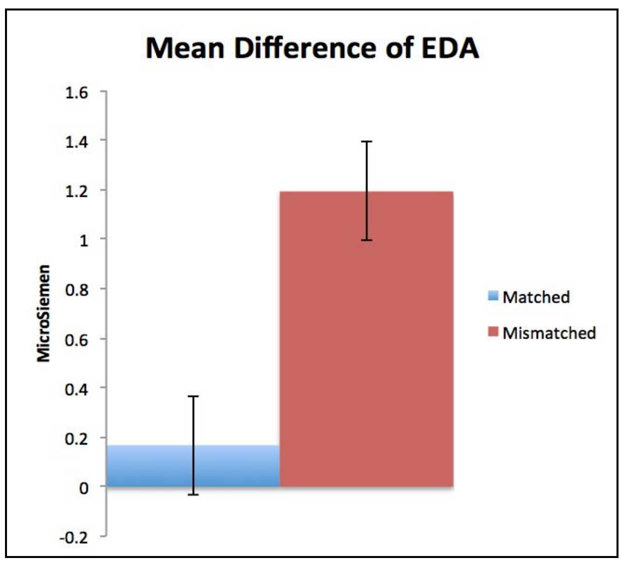

Figure 12. Comparison of matched and mismatched average EDA difference during assigned AV stimuli. The data was found by finding the difference between the EDA reading during the last 30 seconds of baseline and EDA reading during the first 30 seconds of the experiment (treatment-baseline). The average increase in the mismatch treatment was 1.1926 microsiemens (n=12, s=1.32 microsiemens), and the average increase in the match treatment was .1678 microsiemens (n=9, s=.94 microsiemens). p*=.0312

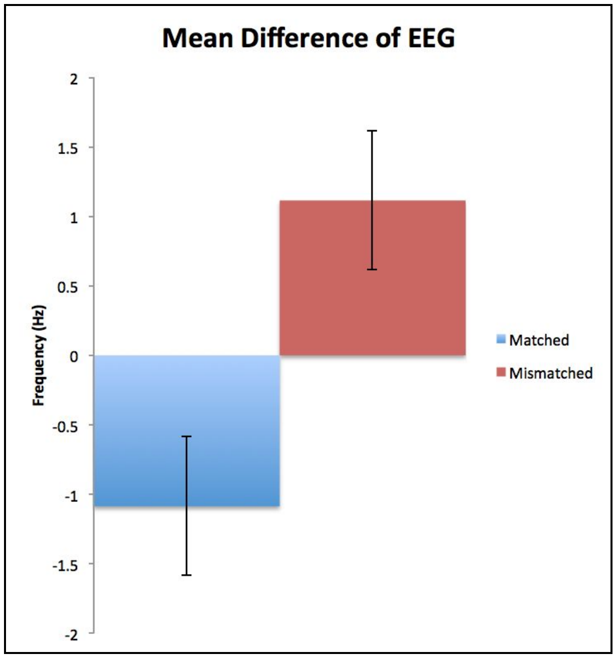

Figure 13. Comparison of matched and mismatched average EEG difference during assigned audio-visual stimuli. The data was found by finding the difference between the EEG reading during the last 30 seconds of baseline and EEG reading during the first 30 seconds of the experiment (treatment-baseline). The average increase in the mismatch treatment was 1.1238 Hz ($n=14$, $s=1.77$ Hz), and the average decrease in the match treatment was 1.0854 Hz ($n=11$, $s=3.33$ Hz). $p^{*}=.0337$

Images	**Audio clips**
Baby duckling (https://s-media-cache-ak0.pinimg.com/originals/20/ca/71/20ca71a79f57fcffa5c7f76a7b235251.jpg)	Sound of laughter https://www.youtube.com/watch?v=iiYGN05vfgM
Slenderman http://media2.s-nbcnews.com/j/msnbc/components/video/__new/tdy_snow_slender2_140604.nbcnews-ux-1080-600.jpg	Benny Hill theme song https://www.youtube.com/watch?v=MK6TXMsvgQg
Baby dory (https://i.ytimg.com/vi/rKjxJqIQTsE/maxresdefault.jpg	Octopus's Garden https://www.youtube.com/watch?v=c0vFUxE3SrM
Scary face in the dark http://www.wallpaperup.com/56223/The_House_Creepy_Black_movies_demon_blood_face_dark_horror_evil_monster.html	Walking on sunshine https://www.youtube.com/watch?v=CKh0dLIuIu8

Smiling family (http://scarffl.com/wp-content/uploads/2013/09/FamilyCover14.jpg)	Tchaikovsky - Waltz of the flowers https://www.youtube.com/watch?v=QxHkLdQy5f0
Head on backwards https://s-media-cache-ak0.pinimg.com/564x/4a/ac/8a/4aac8a96110196b8590a0ca32445bcaa.jpg	Sound of spring/birds https://www.youtube.com/watch?v=NU9RO_v52e4
Beach (http://weknowyourdreams.com/image.php?pic=/images/beach/beach-05.jpg)	Toccata and Fugue in D Minor https://www.youtube.com/watch?v=ho9rZjlsyYY
Scary face in the dark http://www.wallpaperup.com/56223/The_House_Creepy_Black_movies_demon_blood_face_dark_horror_evil_monster.html	Chain saw sound effect https://www.youtube.com/watch?v=I4bnWAI3Oww
Hot Air balloon http://media.thecelebrityauction.co/picture/c/05/CwMdBE5KXRoIBEBIRk0DABkRDBwQSw0XEVsTEgYEGABdGkpRQlRCTBkMAwdKQ0VVVEBQWwsYHVkVDABeBwIeCRsMGURFRFdGXQ8T/FQ==.jpg	Psycho murder music https://www.youtube.com/watch?v=cyIxdOctioo
Scary alien https://i.ytimg.com/vi/Keb5XyXF7Xc/maxresdefault.jpg	Jaws theme song https://www.youtube.com/watch?v=BX3bN5YeiQs
Graduation http://www.kiplinger.com/slideshow/college/T014-S003-best-colleges-with-lowest-debt-at-graduation-2015/images/intro.jpg	Chain sound effect https://www.youtube.com/watch?v=jAZHtD0y6oo
Real ghost http://inyminy.com/wp-content/uploads/2014/12/5.-ghost-of-oak-grove-kentucky.jpg	Funeral march https://www.youtube.com/watch?v=47qUk8B1A3s

Table 1. Description of the images and audio used in both matched and mismatched videos including the link to the website from which they were retrieved from. Image references are in sequential order of appearance in both videos, audio references are in no particular order.