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Classical Conditioning of Cognitive States

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Abstract

Classical conditioning has been a fundamental concept and practice throughout the history of psychology. While classical conditioning traditionally seeks to elicit target behaviors in correlation to specific stimuli, we sought to do the same with cognitive states in place of behaviors. Specifically, we wanted to determine the effectiveness of conditioning states of cognitive arousal in human participants in conjunction with cues presented in a designed learning task. We designed a cognitive task specifically for this research, referred to as "the Tone Pitching Task", which utilized a combination of working memory and mental processing in order to elicit cognitive arousal and focus in participants. By presenting participants with cognitive tasks designed to elicit arousal, we aimed to create associations with the induced cognitive state and the neutral cues presented throughout the conditioning. By recording pupil dilation via eye tracking technology as well as EEG data, which served as measures of sympathetic and parasympathetic nervous system activity, we were able to determine the intensity of the induced cognitive state. In this initial study we sought to test the effectiveness of the "Tone Pitching Task," which was designed specifically for this study, as an unconditioned stimulus for cognitive arousal. While this study suggests that the tone pitching task requires adjustments to serve as a robust unconditioned stimulus, we did observe within trial effects on pupil size as well as a negative correlation between pupil size and delta brain wave activity in the frontal cortex, as well as trends that suggest changes could be made to the tone pitching task in an attempt to improve future research.

Keywords: Classical Conditioning, Cognitive Arousal, Attention, Focus, Learning, Pupil Dilation, EEG.

Introduction

Classical conditioning has been a corner stone of the school of behaviorism, and psychology in general, ever since it was first observed in Ivan Pavlov's dogs. Since then, countless associations have been shown to be possible using a wide verity of conditioned and unconditioned stimuli to elicit numerous varying responses. Classical conditioning works primarily by creating associations between stimuli, unconditioned stimuli (US) that naturally elicit a response, unconditioned response (RS), and originally neutral stimuli that are paired with the unconditioned stimulus, conditioned stimulus (CS). If conditioning is successful during this acquisition phase then the originally unconditioned stimulus can know be elicited in the absence of the US and now in response to the CS, while this is the same response it is now referred to as a conditioned response (CR). The acquisition of this conditioning takes time and multiple pairings of the US with the CS in order to create these associations in subjects or participants. Over time, after the US is removed and the response is now being elicited only by the CS, the association between the US and CS can diminish to the extent where weaker and weaker CR is seen to the point where the CR is no longer observed in a process known as extinction. If extinction is observed then reacquisition can be applied where the US is reintroduced to create the association with the CS again to the point where the CR can be observed once again. The process of reacquisition often takes less time than the initial acquisition of the CR as the association between the US and CS has already been made before and are easier to reinstate. A phenomenon in classical conditioning called spontaneous recovery can also be observed when extinction starts to occur and the CR begins to appear weaker, after waiting a period of time before presenting the CS again the strength of the CR can jump back up to levels near where they were after the association was initially acquired. Another phenomenon, known has habituation, might also play a role in classical conditioning where in repeated exposure to the same stimulus eventually causes the lack of any initial response to this stimulus. Habituation might pose a threat during acquisition if the stimulus is not strong enough to elicit the same level of response over time. While extinction and habituation are similar, they are two distinct phenomena as extinction occurs when the response to a condition stimulus diminishes, and habituation occurs when a naturally elected response to an unconditioned stimulus diminishes. Classical conditioning has been successfully demonstrated on a wide variety of animals, such as dogs and mice, as well as on humans and been used to elicit a wide range of responses, such as simple behaviors such as button pressing as well as physiological responses such as increased salivation. Behaviorist experiments with classical conditioning such as the “little Albert experiments” done by John B. Watson have illustrated those emotional states could be conditioned in human participants. The goal of this research was to design an experiment in which it could be tested whether more complex cognitive states, as opposed to behaviors and emotional states, could be conditioned thorough traditional learning paradigms. Arousal and relaxation were chosen as the cognitive states of interest in this research because of the relative ease in eliciting these states in participants as well as their potential utility given successful conditioning.

While most classical conditioning is approached though a behavioral lens, we sought to focus primarily on the cognitive aspect of the conditioned state of the subjects and its effect on performance of cognitive tasks. Behaviorists argue that classical conditioning targets behaviors including conditioning of emotions, such as fear, cognitive states often have similar neurological origins as these types of emotions. For instance, fear response arises primarily through sympathetic nervous system activation while arousal and relaxation are also related to changes in autonomic nervous system functioning. Specifically, arousal is associated with the activation of the sympathetic nervous system and inhibition of the parasympathetic nervous system. Conversely, relaxation is associated activation of the parasympathetic nervous system and inhibition of the sympathetic nervous system. The sympathetic nervous system and parasympathetic nervous system are the two main components of the autonomic nervous system. The sympathetic nervous system is commonly known to be responsible for the “fight or flight response” and does this by antagonizing the prismatic nervous system. The para sympathetic nervous system is commonly known to promote a “rest and digest” response and does so by antagonizing the sympathetic nervous system. These two opposing aspects of the autonomic nervous system help maintain homeostasis in the body by regulating each other. Since emotional conditioning related to the emotional states that are regulated and influenced by autonomic nervous system functioning has already been demonstrated, we hypothesized that conditioning of cognitive states would be plausible as well considering cognition and emotion are both influenced by autonomic nervous system function. Since classical conditioning elicits behaviors through similar neurological mechanisms as the ones that determine cognitive states, it is reasonable to predict that classical condition could be utilized to create associations between stimuli that naturally elicits these cognitive states and stimuli that could be later represented in the absence of uncontained stimuli in order to produce those same cognitive states on command.

In deciding what unconditioned stimulus to use to naturally elicit cognitive arousal and focus we designed a cognitive task specifically for this experiment we named the Tone Pitching Task. We wanted to assure that no changes in pupil size, one of the metrics we used to measure cognitive arousal, was due to visual stimulation and changes in the intensity of light exposure to the participants pupils. To do this we designed a task that was auditory only, instead of visually oriented as most cognitive tasks traditionally are. We wanted to utilize a combination of working memory and cognitive processing through comparison and the design of the tone pitching task reflects both of these components. The tone pitching task presents participants with a sequence of tone, all varying in pitch, and instructs participants to listen to each tone and indicate whether the newly presented tone is higher, lower, or equal in pitch to the previously presented tone. Participants were first presented with a fixation circle to draw their attention to the display the task was presented on, followed the flash of a bright red circle that served as the conditioned stimulus. After this, one of four audio clips comprised of a four-tone sequence of three distinct tones, a high, medium, and low tone. This task is not unlike an auditory n-back task in that it requires participants to remember previous tones in relation to the newly presented tone. It also requires participants to utilize cognitive processing by instructing them to make a comparison between tones in a limited period of time before the next tone is presented. Out goal in designing this task was to increase mental processing and require heightened focus and attention from participants in hopes that this focus and attention could be associated with a neutral unconditioned stimulus. The enviable goal of creating this association is to later remove the task in hopes to observe the same cognitive arousal and focus in participants in response to the conditioned stimulus alone as is seen in response to the tone pitching task.

While emotion and cognition both have subjective aspects, cognitive states in particular could be observed and measured though performance on cognitive tasks. We decided to measure cognitive state through a combination of pupil dilation, EEG data, and performance on cognitive tasks. Pupil dilation has already been demonstrated to be elicited by arousing stimuli through means of sympathetic nervous system activation (Bradley et al., 2008). Conversely, pupil constriction has been shown to be elicited through the activation of specific parasympathetic nervous system pathways. These opposing pupillometric responses provide for relatively easy to obtain measures of the activity of these opposing branches of the autonomic nervous system, which are indicative of an individual's relative levels of relaxation and arousal. Studies on pupillometry have shown that pupil responses are both reflexive and voluntary, meaning while visual stimuli, particularly increases in bright light, can influence pupil dilation and constriction without any control of the individual exposed to the stimuli, choosing to focus on or ignore the visual stimuli, as well as focusing on cognitive tasks, can increase the degree to which an individual's pupils dilate or constrict (Mathôt, 2018). It has been shown that an individual's pupils dilate during working memory tasks due to increase mental effort as a result of the task (Kahneman and Beatty, 1966). Pupil dilation has also been shown to increase during tasks involving mental processing (Hess and Polt, 1964). By utilizing cognitive tasks that implement both working memory and mental processing, we can effectively elicit cognitive arousal and mental attention in subjects and use pupil dilation as a measure of this increase in attentiveness and determine if this attentiveness is maintained even in the absence of these cognitive tasks, indicating that an association has been made between the conditioned stimulus and the cognitive arousal elicited by the task. By measuring variation in pupil size, we can determine if participants are relaxed and or aroused throughout conditioning and if these cognitive states are maintained in the absence of the unconditioned stimulus. It is also important to note the more well-known response of the pupils to light. An individual's pupils will reflexively dilate in response to exposure to bright light. In order to control for this reflexive pupil light response, the conditions in which subjects are tested must remain consistent between trials to assure consistency both within and between subjects. By having all subjects tested in conditions with consistent light exposure, we can reduce any confounding changes in pupil size as a result of the pupil light response.

Analysis of EEG data can also be utilized to determine the cognitive state of participants. EEG has been utilized to measure electrical activity in the brain for more than 100 years, and the first human EEG recordings were taken by German physician Hans Berger in 1924. Since then, scientists have been utilizing EEG technology to analyses brain activity for a number of applications ranging from exploratory research on working memory and attention to clinical applications and is used as a diagnostic tool for a number of neurological conditions such as seizure disorders. The brain waves measured by EEG have been classified into distinct ranges of frequencies that are associated with various cognitive states and activities. Gamma brain waves (38+ Hz) are associated with the highest levels of concentration, although the EEG technology available to us does not measure gamma brain waves which would be particularly useful to know in a study that is examining concentration and attentiveness. Beta brain waves (13-38 Hz) are associated with mental activity and a general state of alertness. Alpha brain waves (8-12 Hz) are associated with a more relaxed state of mind and indicative of inattentiveness. Theta brain waves (4-8 Hz) are associated with even deeper relaxation than alpha brain waves as well as inwardly focused cognition. Delta brain waves (0.5-4 Hz) are most associated with brain functioning during sleep. As a functional neuroimaging technique, EEG has strong temporal resolution, meaning it takes readings of precise time points, but quite poor spatial resolution, and it is difficult to pinpoint which regions of the brain the readings are coming from. The EEG technology utilized in this study focuses on brain waves arising in the frontal cortex, which is appropriate considering that the frontal cortex is where all higher-level thinking and processing takes place, and therefore, brain activity in this region is indicative of the cognitive states we are focusing on. Increases in beta brain wave levels have long been associated with increased visual attention (Wróbel, 2000). Unfortunately, the utilization of visual tests of attention would prove problematic in a study focusing on pupil dilation, as changes in visual stimulus could confound cognitive effects on pupil size due to the reflexive responses of pupils to light and visual cues. In place of visual stimuli to elicit attention and alertness we opted for auditory tasks that do so by requiring subjects to utilize their working memory as well as their cognitive processing skills. Changes in the levels of brain waves in the frontal cortex throughout condition can serve as a measure of attentiveness and relaxation in response to unconditioned stimuli as well as whether or not these cognitive states are maintained after the removal of the stimuli that naturally elicit them. Beta brain wave activity is the primary type of brain wave activity associated with attention and focus and therefore, would be the primary type of brain wave activity we would be looking to see in association with the unconditioned stimulus of the tone pitching task. By collecting EEG data from participants, we have another measure to examine when determining the cognitive state of participants during testing. In pairing this data with performance on cognitive tasks, we are able to determine the cognitive benefit of the induced cognitive state.

More aroused and attentive participants should perform better on more difficult cognitive tasks while more relaxed participants should perform better on relatively easy cognitive tasks.

Before testing if the elicited cognitive states remain in the absence of the unconditioned stimulus, the tone pitching task, we first wanted to determine if this task serves as a robust unconditioned stimulus for these cognitive states. Over the course of the summer and the course of the fall semester, seventeen students were recruited to participate in this study and underwent the acquisition portion of the learning paradigm.

If conditioning of these cognitive states is successful, it could prove beneficial for a multitude of applications for both clinical and personal use. Success in conditioning cognitive states through traditional learning paradigms could open up a world of applications particularly in the field of cognitive behavioral therapy. By being able to consistently condition cognitive states such as arousal and relaxation, clinicians might be able to assist patients with anxiety and panic disorders as well as patients with disorders effecting their ability to pay attention such as ADHD. The implementation of this type of conditioning into treatment could be utilized as alternatives to drug treatments that are currently the standard for these types of treatments and often come with unpleasant sorter side effects as well as problematic side effects from long term use. This type of conditioning could also be used in combination with the current standard drug treatments.

Methods

Participants

Participants were recruited both from Dr. Bish's concussion research lab as well as though the Ursinus SONA software and received SONA credit for participation in this research.

Seventeen participants came in for three testing sessions over the summer for preliminary testing with the conditioning task. Participants each completed a demographic questionnaire which included questions about age, gender, race, and conditions that might affect their ability to relax or pay attention, whether or not they wore glasses, and whether they were right or left-handed. Participants ages ranged primarily from 18 to 21, with one outlier age 27. The mean participant age was 19.8 years old with a standard deviation of 2.1 years. Participants were predominantly White/Caucasian with 82.35% of participants identifying as White. 11.76% of participants identified as mixed race with one participant identifying as both White and Black and one participant identifying as White and Asian. One participant identified as Asian. Participants where asked if they identified as Hispanic or Latino, but no participants indicated coming from this ethnic background backgrounds. 58.82% of participants identified as male and 41.18 identified as female. 29.41% of participants reported having conditions that impaired their ability to pay attention with seven citing having ADHD, one of whom reported also having two concussions. 11.76% of participants reported having conditions that impaired their ability to relax, both reporting having generalized anxiety. 47.06% of participants reported that they needed either glasses or contacts. All participants reported that they were right-handed.

Eye Tracking

Participants' pupil sizes were measured throughout the duration of the test using the Tobii eye tracking software and Tobii T60 eye tracking computer which includes a computer display as well as specialized eye tracking cameras mounted at the base of the display, which can record eye movements across the screen as well as pupil size.

EEG

Participants' brain waves were recorded throughout the duration of the test using the Biopac MP36 and two channel EEG electrodes. Participants first had their forehead and area behind their left ear sterilized using alcohol wipes before having recording electrodes attached on the right and left side of their forehead and a ground electrode placed behind their ear.

Tone Pitching Task

In designing a conditioning task that would allow for multiple interchangeable unconditioned stimulus, a task was created involving an open circle (FC) which served as a fixation point, closed red circle (RC) that served as the conditioned stimulus, and an audio clip with instructions, the tone pitching task (TP), that served as the arousing US. In the Tobii eye tracking software, the subjects were presented with instructions followed by four pretest segments consisting of the open fixation circle followed by the red closed circle and then, again, the open fixation circle, this time along with a sequence of four tones of varying low, medium, and high pitches. Four septate preset tone sequences were created, comprised of four tones each, and recycled throughout the test, and subjects were presented with a tone pitching task a total of 18 times, including two trial sequences, one before the pretest portion of the test and one before the portion of the test including the letter number sequencing task, in order to assure the subjects fully understood the task. The subjects were instructed to press the arrow keys on the keyboard to indicate whether the current tone is higher, lower, or the same pitch as the previous tone. Subjects were instructed not to respond to the initial tone, as there was no previous tone to compare its pitch too, and were only required to indicate the relative pitch of the three subsequent tones in the task. This tone pitching task served as the arousing US as it required the subjects to maintain attention on the tones in hopes to elicit a state of arousal or excitation as opposed to passive listening, although the passive listening task was not utilized in these preliminary trials. This tone pitching task served as an US for cognitive arousal by requiring a combination of working memory function and mental processing in participants who had to both remember the relative pitch of the previous tone while comparing the newly presented tone and determining its pitch relative to the previous one. A version of this task was also created that instructs participants to listen to the tones with no feedback required to serve as a relaxing US, but this version was not used during the preliminary testing to determine whether or not this task was robust enough for further testing.

LNS

After the pretest portion, subjects were presented with the same sequences again, but this time they were instructed to complete a letter number sequencing task following the tone pitching task, wherein they are presented with a set of numbers and letters and were asked to repeat them in numerical order and then alphabetical order. Three letter number sequences of each four, five, six, and seven digits were presented during the second portion of the learning task. The same sequence of digits was utilized across all trials, although upon being asked no subjects had recognized that the same digit sequence was recycled. Subjects' performance was reordered for each of the letter number sequencing tasks and scored as a pass or fail.

Controls of Variance

In order to keep testing conditions consistent across all trials, all testing was conducted in a windowless room to reduce confounding of pupil size data due to variances in ambient light from outside. Participants who arrived to their first testing session wearing either glasses or contacts were instructed to wear glasses or contacts during all subsequent testing sessions. The brightness of the computer display was kept consistent across all testing sessions and between all participants in order to mitigate variances in pupil size due to light exposure.

Data Processing

Integration brain wave data from the red circle and the tone pitching portion of the task was taken from the Biopac analysis software and exported into a compiled data file. Pupil size data was exported from the Tobii eye tracking software, and averages of the pupil size consisting of the first ten and last ten consecutive readings from each portion of interest were taken and compiled in a separate spread sheet. If consecutive recordings were not available, the first ten and last ten readings were used. Because differences between pupil size in the right and left eye were not observed to be significantly different, only left eye pupil size was exported into the compiled date file, although only data was collected from readings where both the right and left eye were visible enough to be recorded. LNS performance was recorded and added to the compiled data file. Data was taken from all four sequences in the pretest and the second of the three sequences for each digit length in the letter number sequencing portion of the learning task. All subsequent statistical analysis was done in IBM SPSS 24.

Other Versions of the Test

A version of the learning task was created that utilized a rapid visual information processing task where a sequence of numbers is presented, and subjects are instructed to press the space bar every time a sequence of three odd or three even numbers are presented in a row, in place of the pitching task in order to see if a visual US yields differing results than an auditory US, as well as whether or not it would confound pupil dilation data, although this learning task was not utilized in the preliminary testing to determine the robustness of the cognitive response elicited by the unconditioned stimulus.

Results

Pupil Dilation

Statistical analysis illustrated there was no significant difference in pupil size during the fixation circle, red circle, or tone pitching task. There was also no significant difference in pupil size between each of the three trials of the learning task. These results do not support the hypothesis that the tone pitching task serves as an effective unconditioned stimulus for cognitive arousal. There were however a number of significant findings. Firstly, there was a significant difference in pupil sizes between each test segment during the fixation circle as revealed by repeated measures ANOVA ($F(7. 301) = 2.495$, $p=.017$). Specifically, pupil size in segment 3 ($F(1,43) = 6.2$, $p=.017$) and 4 ($F(1,43) = 5.123$, $p=.029$) were significantly smaller than all previous segments, and pupil size in segment 8 ($F(1,43) = 4.602$, $p=.038$) was significantly larger than all previous segments. The directionality of differences in pupil size during the fixation circle is illustrated in figure 1. The data from the statical analysis on differences in pupil size during the fixation circle are outlined in table 1 and table 2. There was also a significant difference in pupil sizes between each test segment during the red circle as revealed by repeated measures ANOVA ($F(7, 315) = 7.391$, $p<.0005$). Specifically, pupil size in segment 2 ($F(1,45) = 14.479$, $p<.0005$) and 3 ($F(1,45) = 24.096$, $p<.0005$) and 4 ($F(1,45) = 21.233$, $p<.0005$) were significantly smaller than all previous segments. The directionality of differences in pupil size during the red circle is illustrated in figure 2. The data from the statical analysis on differences in pupil size during the red circle are outlined in table 3 and table 4. There was also a significant difference in pupil sizes between each test segment during the tone pitching task as revealed by repeated measures ANOVA ($F(7, 329) = 3.951$, $p<.0005$). Specifically, pupil size in segment 2 ($F(1,47) = 25.860$, $p<.0005$) was significantly smaller than all previous segments. The directionality of differences in pupil size during the tone pitching task is illustrated in figure 3. The data from the statical analysis on differences in pupil size during the tone pitching task are outlined in table 5 and table 6. All together this data suggests that there is some effect on pupil size between segments within each of the three main components of the test. Another significant finding that was observed when splitting the test into two portions, a pretest portion comprised of the first four segments which did not include letter number sequencing, and a test portion comprised of the last four segments which included letter number sequencing. Statistical analysis revealed that pupil sizes were significantly different between the pretest and test portions within all three components of the learning task across all three trials. Firstly, there was a significant difference between pupil sizes in the pretest and test during the fixation circle as revealed by a paired samples t-test ($t(49) = -3.313$, $p=.002$). Specific mean pupil sizes across the three trials for both the pretest and test are plotted on figure 4. There was also a significant difference between pupil sizes in the pretest and test during the red circle as revealed by a paired samples t-test ($t(50) = -4.451$, $p<.0005$). Specific mean pupil sizes across the three trials for both the pretest and test are plotted on figure 5. There was also a significant difference between pupil sizes in the pretest and test during the tone pitching task as revealed by a paired samples t-test ($t(50) = 2.126$, $p=.038$). Specific mean pupil sizes across the three trials for both the pretest and test are plotted on figure 6.

EEG Data

There were no significant differences in brain wave activity between either the fixation circle, the red circle, or the tone pitching task. There was also no significant difference in brain wave activity between each of the three trials of the learning task. These results do not support the hypothesis that the tone pitching task serves as an effective unconditioned stimulus for cognitive arousal. There was also no significant correlation between alpha, beta, or theta brain wave activity and pupil size throughout the learning task. This does not support previous research that both pupil dilation and EEG data are indicative of autonomic nervous system activity. However, there was a statically significant negative correlation between pupil size and delta brain wave activity in the frontal cortex (r (39) = -.346, p = .019). While this is a relatively weak correlation, it does suggest that 13.3% of delta brain wave activity in the frontal cortex can be predicted by pupil size and vice versa. The data points used in this correlation are plotted in figure 7.

LNS

There was no significant difference on the performance on the letter number sequencing between trials. This finding suggests that there was no behavioral effect of the learning task in its current form.

Figure 1

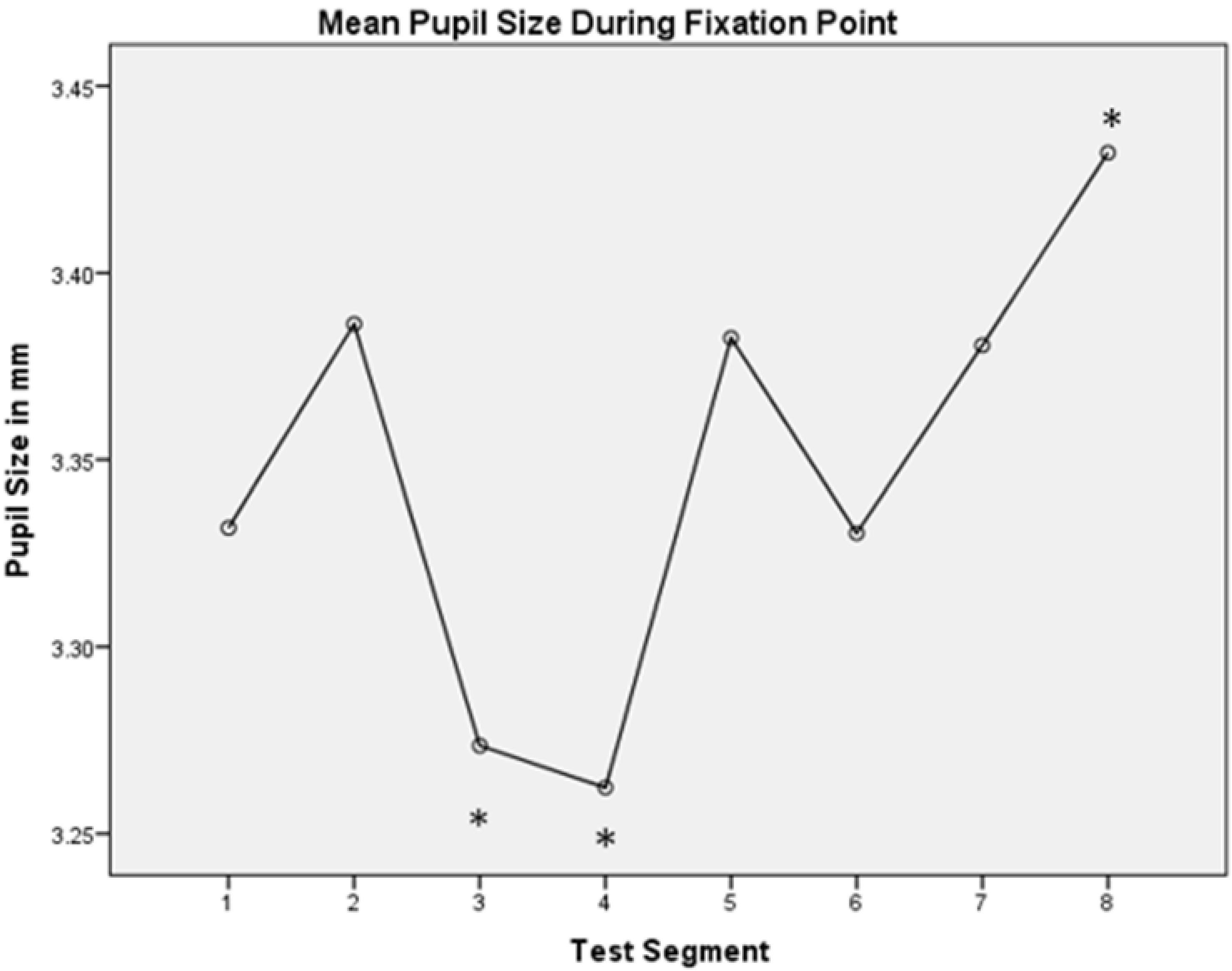

Figure 1. Mean Pupil Size During Fixation Point. Pupil sizes during the Fixation Circle were recorded in mm and plotted. Segments 3 (F (1,43) = 6.2, p=.017), 4 (F (1,43) = 5.123, p=.029), and 8 (F (1,43) = 4.602, p=.038) were found to be significantly different than pupil sizes in all previous segments.

Table 1

ANOVA Data Illustrating a Significant Difference in Pupil Sizes Between Segments Within the Fixation Circle Portion of the Test.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
fc	Sphericity Assumed	1.115	7	.159	2.495	.017
	Greenhouse-Geisser	1.115	3.822	.292	2.495	.047
	Huynh-Feldt	1.115	4.437	.251	2.495	.039
	Lower-bound	1.115	1.000	1.115	2.495	.122
fc * TestSession	Sphericity Assumed	.937	14	.067	1.048	.405
	Greenhouse-Geisser	.937	7.644	.123	1.048	.401
	Huynh-Feldt	.937	8.873	.106	1.048	.403
	Lower-bound	.937	2.000	.468	1.048	.359
Error(fc)	Sphericity Assumed	19.218	301	.064		
	Greenhouse-Geisser	19.218	164.353	.117		
	Huynh-Feldt	19.218	190.777	.101		
	Lower-bound	19.218	43.000	.447		

Table 2

ANOVA Showing Specific Data for the Comparisons Between Each Segment Within the Fixation Circle Portion of the Test.

Source	fc	Type III Sum of Squares	df	Mean Square	F	Sig.
fc	Level 2 vs. Level 1	.136	1	.136	1.444	.236
	Level 3 vs. Previous	.336	1	.336	6.200	.017
	Level 4 vs. Previous	.214	1	.214	5.123	.029
	Level 5 vs. Previous	.219	1	.219	3.004	.090
	Level 6 vs. Previous	.000	1	.000	.004	.952
	Level 7 vs. Previous	.128	1	.128	1.223	.275
	Level 8 vs. Previous	.430	1	.430	4.602	.038
fc * TestSession	Level 2 vs. Level 1	.370	2	.185	1.960	.153
	Level 3 vs. Previous	.169	2	.084	1.555	.223
	Level 4 vs. Previous	.125	2	.062	1.494	.236
	Level 5 vs. Previous	.218	2	.109	1.490	.237
	Level 6 vs. Previous	.043	2	.021	.173	.842
	Level 7 vs. Previous	.286	2	.143	1.366	.266
	Level 8 vs. Previous	.104	2	.052	.557	.577
Error(fc)	Level 2 vs. Level 1	4.064	43	.095		
	Level 3 vs. Previous	2.332	43	.054		
	Level 4 vs. Previous	1.797	43	.042		
	Level 5 vs. Previous	3.141	43	.073		
	Level 6 vs. Previous	5.278	43	.123		
	Level 7 vs. Previous	4.496	43	.105		
	Level 8 vs. Previous	4.022	43	.094		

Figure 2

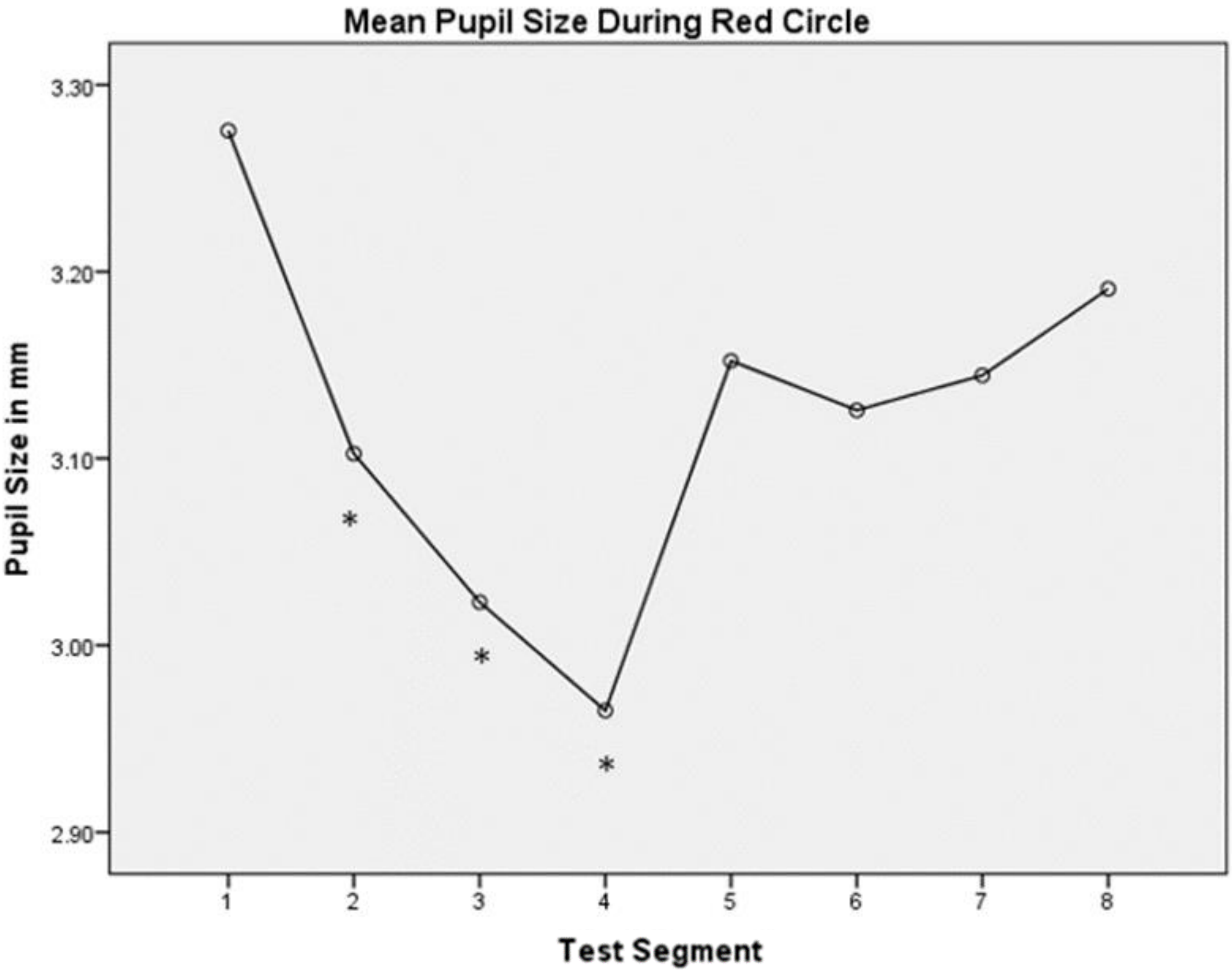

Figure 2. Mean Pupil Size During Red Circle. Pupil sizes during the Red Circle were recorded in mm and plotted. Segments 2 ($F(1,45) = 14.479$, $p<.0005$), 3 ($F(1,45) = 24.096$, $p<.0005$), and 4 ($F(1,45) = 21.233$, $p<.0005$) were found to be significantly different than pupil sizes in all previous segments.

Table 3

ANOVA Data Illustrating a Significant Difference in Pupil Sizes Between Segments Within the Red Circle Portion of the Test.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
rc	Sphericity Assumed	3.086	7	.441	7.391	.000
	Greenhouse-Geisser	3.086	4.636	.666	7.391	.000
	Huynh-Feldt	3.086	5.464	.565	7.391	.000
	Lower-bound	3.086	1.000	3.086	7.391	.009
rc * TestSession	Sphericity Assumed	.355	14	.025	.425	.966
	Greenhouse-Geisser	.355	9.273	.038	.425	.924
	Huynh-Feldt	.355	10.928	.032	.425	.943
	Lower-bound	.355	2.000	.177	.425	.656
Error(rc)	Sphericity Assumed	18.787	315	.060		
	Greenhouse-Geisser	18.787	208.635	.090		
	Huynh-Feldt	18.787	245.877	.076		
	Lower-bound	18.787	45.000	.417		

Table 4

ANOVA Showing Specific Data for the Comparisons Between Each Segment Within the Red Circle Portion of the Test.

Source	rc	Type III Sum of Squares	df	Mean Square	F	Sig.
rc	Level 2 vs. Level 1	1.431	1	1.431	14.479	.000
	Level 3 vs. Previous	1.316	1	1.316	24.096	.000
	Level 4 vs. Previous	1.361	1	1.361	21.233	.000
	Level 5 vs. Previous	.177	1	.177	2.479	.122
	Level 6 vs. Previous	.023	1	.023	.265	.609
	Level 7 vs. Previous	.066	1	.066	.879	.353
	Level 8 vs. Previous	.292	1	.292	2.874	.097
rc * TestSession	Level 2 vs. Level 1	.069	2	.035	.350	.707
	Level 3 vs. Previous	.022	2	.011	.200	.820
	Level 4 vs. Previous	.169	2	.085	1.319	.277
	Level 5 vs. Previous	.060	2	.030	.423	.658
	Level 6 vs. Previous	.036	2	.018	.206	.814
	Level 7 vs. Previous	.106	2	.053	.708	.498
	Level 8 vs. Previous	.011	2	.005	.054	.948
Error(rc)	Level 2 vs. Level 1	4.447	45	.099		
	Level 3 vs. Previous	2.457	45	.055		
	Level 4 vs. Previous	2.884	45	.064		
	Level 5 vs. Previous	3.204	45	.071		
	Level 6 vs. Previous	3.977	45	.088		
	Level 7 vs. Previous	3.369	45	.075		
	Level 8 vs. Previous	4.567	45	.101		

Figure 3

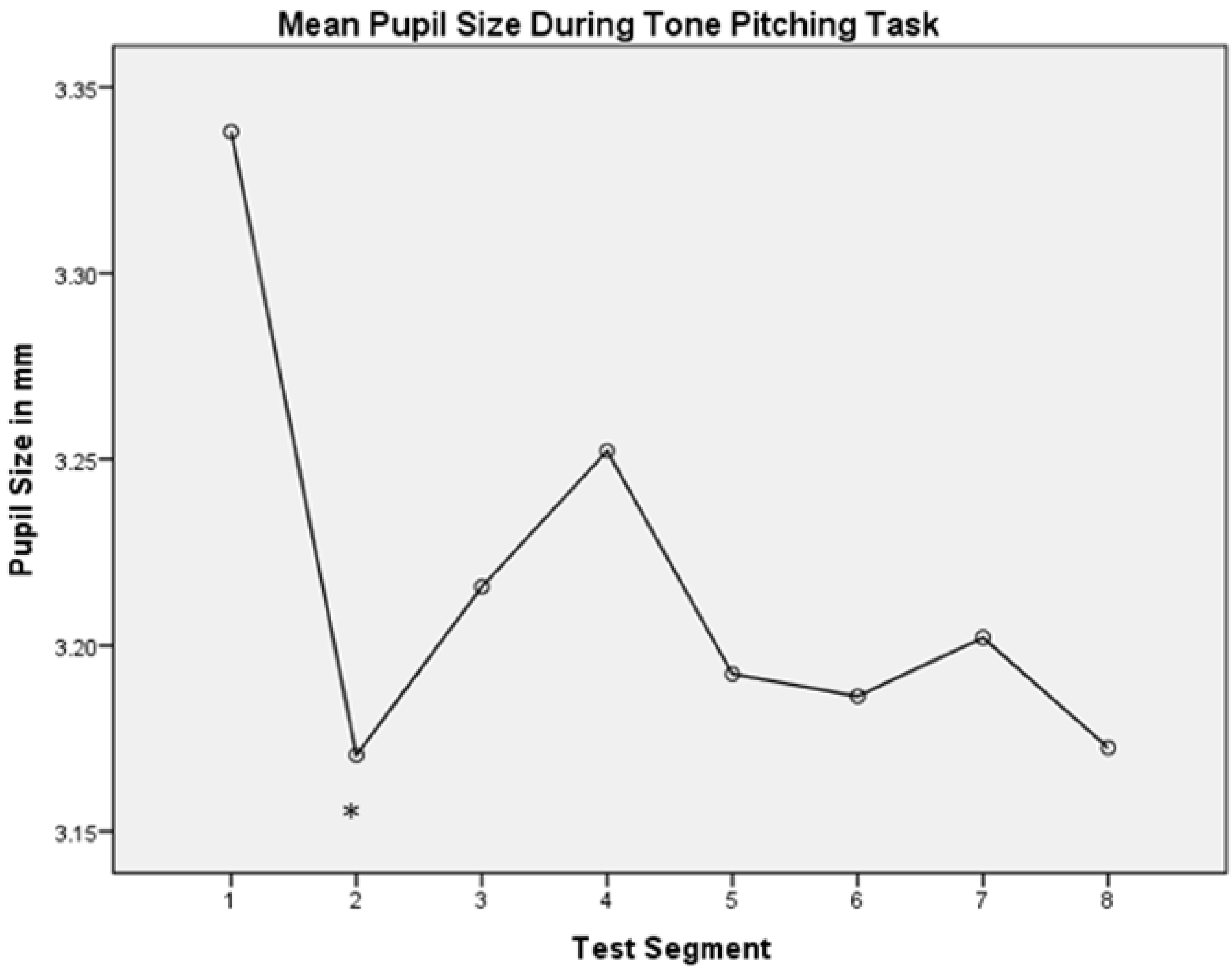

Figure 3. Mean Pupil Size Tone Pitching Task. Pupil sizes during the Tone Pitching Task were recorded in mm and plotted. Only Segment 2 ($F(1,47) = 25.860$, $p<.0005$) was found to be significantly different than pupil sizes in all previous segments.

ANOVA Data Illustrating a Significant Difference in Pupil Sizes Between Segments Within the Tone Pitching Task Portion of the Test.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
tp	Sphericity Assumed	1.090	7	.156	3.951	.000
	Greenhouse-Geisser	1.090	3.593	.303	3.951	.006
	Huynh-Feldt	1.090	4.092	.266	3.951	.004
	Lower-bound	1.090	1.000	1.090	3.951	.053
tp * TestSession	Sphericity Assumed	.563	14	.040	1.020	.432
	Greenhouse-Geisser	.563	7.186	.078	1.020	.420
	Huynh-Feldt	.563	8.185	.069	1.020	.423
	Lower-bound	.563	2.000	.281	1.020	.368
Error(tp)	Sphericity Assumed	12.963	329	.039		
	Greenhouse-Geisser	12.963	168.862	.077		
	Huynh-Feldt	12.963	192.343	.067		
	Lower-bound	12.963	47.000	.276		

Table 4

ANOVA Showing Specific Data for the Comparisons Between Each Segment Within the Red Circle Portion of the Test.

Source	ac	Type III Sum of Squares	df	Mean Square	F	Sig.
tp	Level 2 vs. Level 1	1.402	1	1.402	25.860	.000
	Level 3 vs. Previous	.074	1	.074	2.859	.097
	Level 4 vs. Previous	.006	1	.006	.224	.638
	Level 5 vs. Previous	.134	1	.134	3.594	.064
	Level 6 vs. Previous	.112	1	.112	1.512	.225
	Level 7 vs. Previous	.028	1	.028	.310	.581
	Level 8 vs. Previous	.125	1	.125	2.622	.112
tp * TestSession	Level 2 vs. Level 1	.125	2	.063	1.154	.324
	Level 3 vs. Previous	.115	2	.057	2.208	.121
	Level 4 vs. Previous	.054	2	.027	1.037	.362
	Level 5 vs. Previous	.033	2	.017	.447	.642
	Level 6 vs. Previous	.086	2	.043	.575	.567
	Level 7 vs. Previous	.123	2	.062	.675	.514
	Level 8 vs. Previous	.205	2	.103	2.153	.127
Error(tp)	Level 2 vs. Level 1	2.548	47	.054		
	Level 3 vs. Previous	1.222	47	.026		
	Level 4 vs. Previous	1.226	47	.026		
	Level 5 vs. Previous	1.757	47	.037		
	Level 6 vs. Previous	3.497	47	.074		
	Level 7 vs. Previous	4.288	47	.091		
	Level 8 vs. Previous	2.239	47	.048		

Figure 4

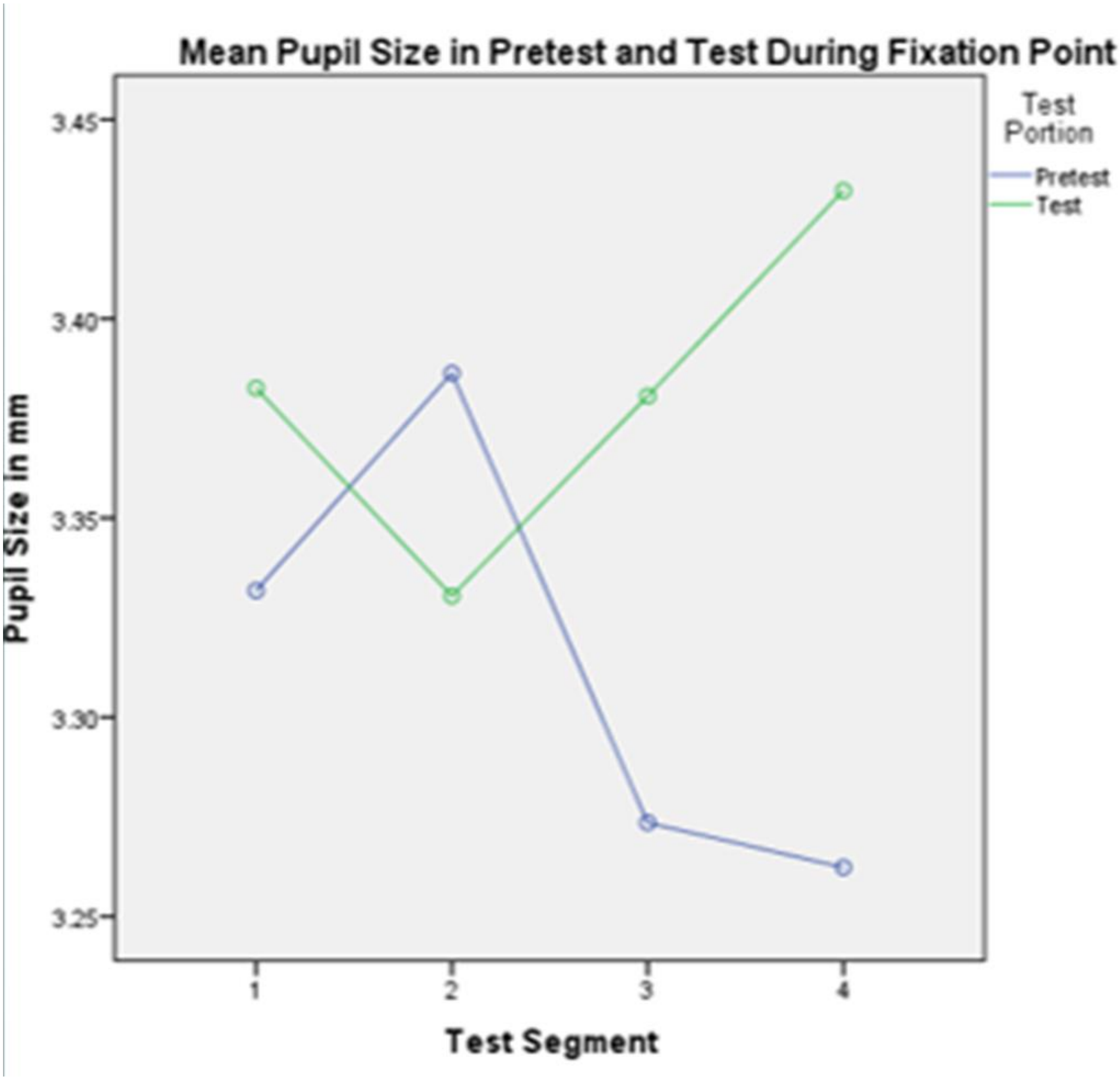

Figure 4. Mean Pupil Size in Pretest and Test During Fixation Point. Pupil sizes during the Fixation Circle were recorded in mm and comparisons were made between the Pretest and Test portions of the learning task. Pupil sizes were found to be significantly different between the Pretest and Test (t (49) = -3.313, p=.002).

Figure 5

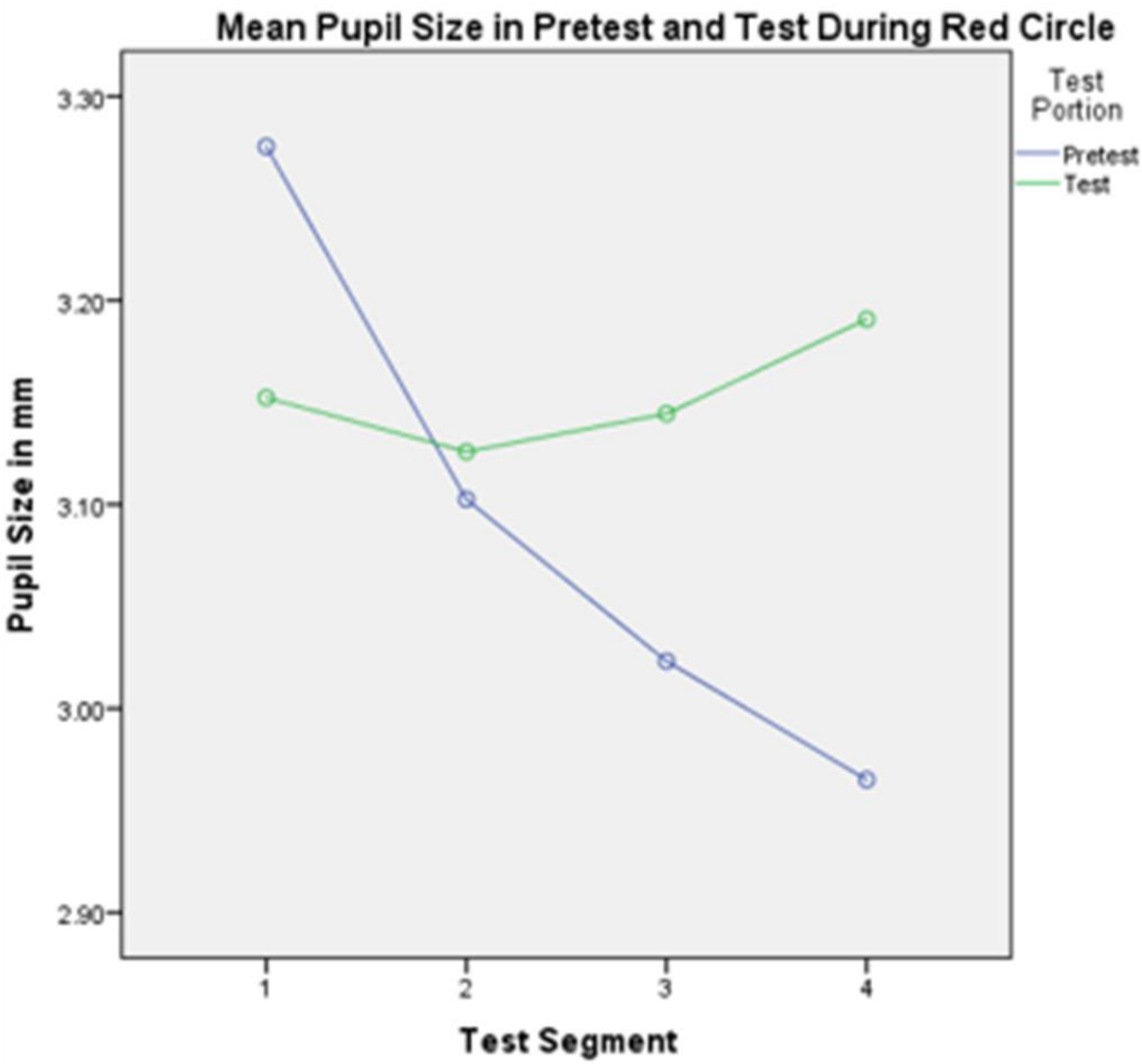

Figure 5. Mean Pupil Size in Pretest and Test During Red Circle. Pupil sizes during the Red Circle were recorded in mm and comparisons were made between the Pretest and Test portions of the learning task. Pupil sizes were found to be significantly different between the Pretest and Test ($t(50) = -4.451$, $p<.0005$).

Figure 6

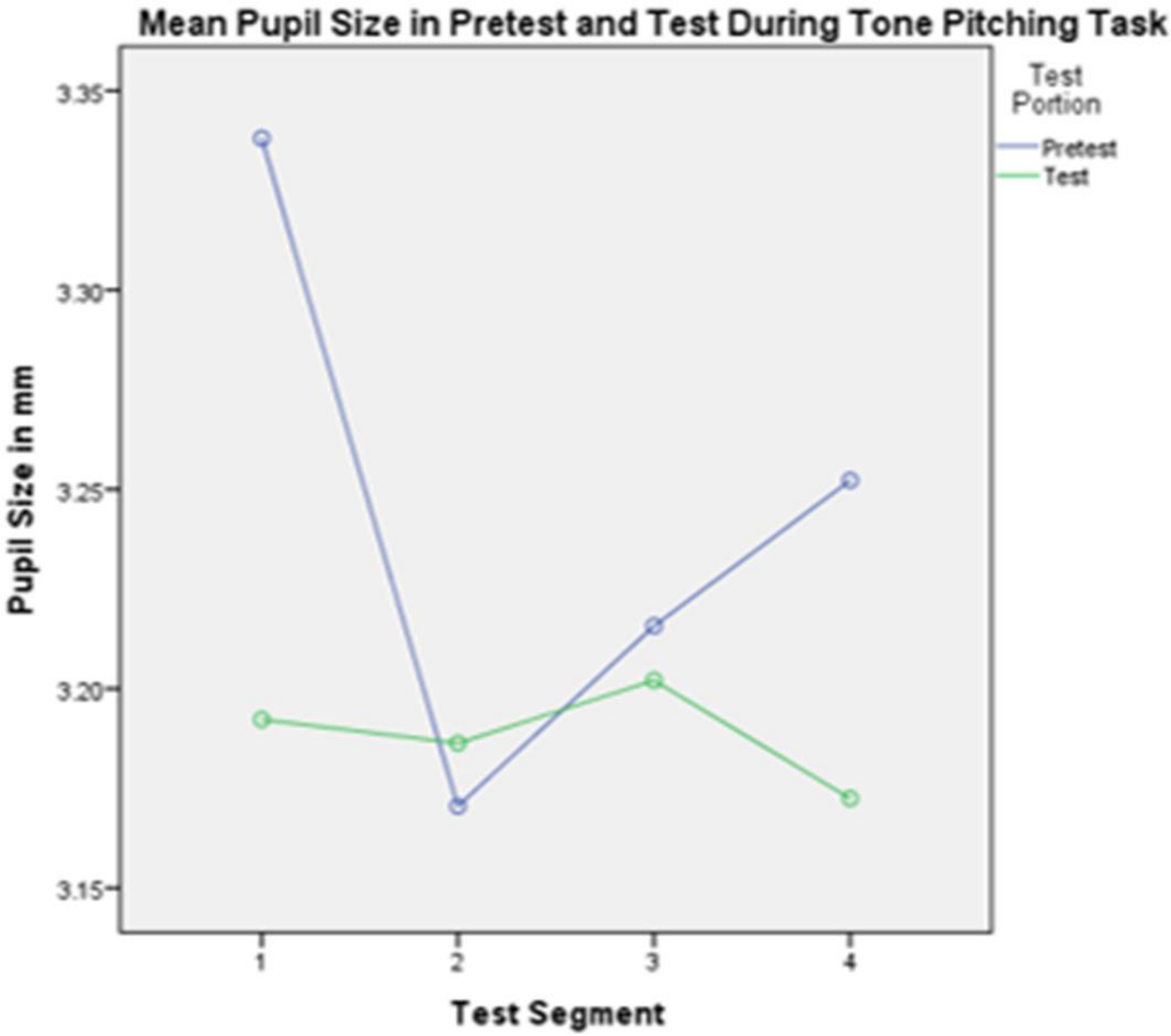

Figure 6. Mean Pupil Size in Pretest and Test During Tone Pitching Task. Pupil sizes during the Tone Pitching Task were recorded in mm and comparisons were made between the Pretest and Test portions of the learning task. Pupil sizes were found to be significantly different between the Pretest and Test ($t(50) = 2.126$, $p=.038$).

Figure 7

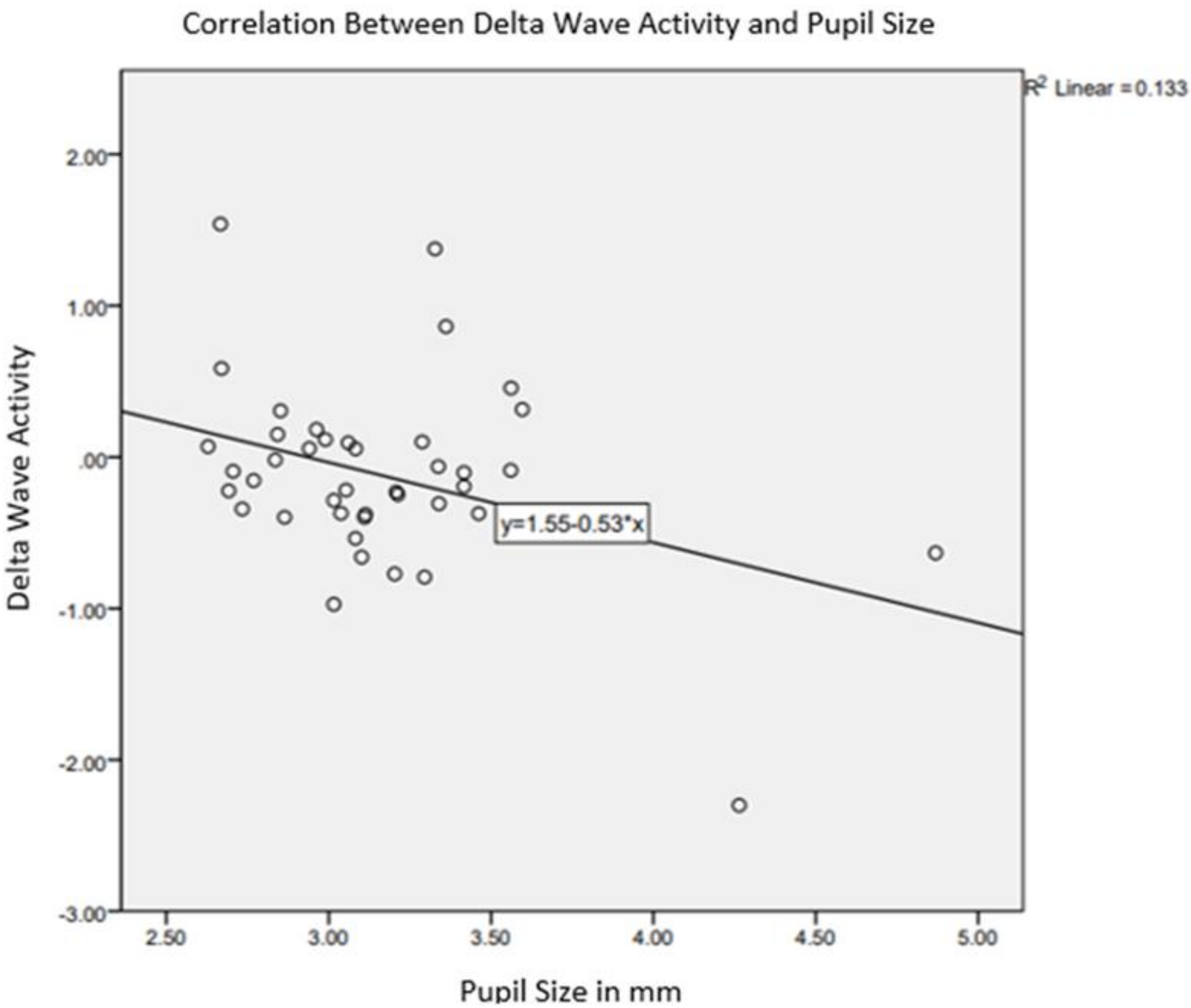

Figure 6. Correlation Between Delta Wave Activity and Pupil Size. Pupil sizes were recorded in mm and plotted against Integral Delta wave data. There was found to be a significant, weak, negative correlation between pupil size and Delta brain wave activity in the frontal cortex (*r* (39) = -.346, p = .019).

Discussion

The fact that no significant difference in pupil size was seen between the three main components of the learning task, and specifically the fact that pupil size was not significantly larger during the tone pitching task, suggests that the tone pitching task, in its current form, does not serve as a sufficient unconditioned stimulus for cognitive arousal. This is potentially due to the tone pitching task not requiring a high enough level of working memory and cognitive process which would be necessary for eliciting cognitive arousal at a level sufficient for conditioning. One potential way to solve this issue is to increase the cognitive load of the tone pitching task. By increasing the number of pitches used in the task and using tones that are less easily distinguished from each other, we would increase the amount of mental processing necessary to be able to determine the relative pitch of the tones. Perhaps by also instructing participants to compare newly presented tones with the tone before the previous tone, as opposed to the previous tone, this would place a large strain on subjects working memory, similar to the difference between a 2-back and a 1-back task, and therefore require the level of cognitive arousal and attention we sought to elicit with this task. Increasing the difficulty of the tone pitching task might also address any issues that could be due to habituation of the unconditioned stimulus. When looking at the patterns changes in pupil sizes in figure 5 and figure 6, it can be noted that while there appears to be a wider variance in pupil size during the pretest, pupil sizes during the test are much more similar which might suggest that participants are becoming familiar with the tone pitching task, and are therefore becoming habituated to the task, and their pupil sizes begin to stagnate and level off. By using distinct tone sequences, instead of recycling the same four sequences throughout the test, as well as increasing the cognitive load exerted by the task, we might be able to utilize the tone pitching task in a way that combats this potential habituation by creating a wider verity of stimulus and causing participants to remain alert throughout the task. Shortening the duration of the test form around 20 minutes to closer to 10 minutes might also reduce any effect of habituation or reduced the number of participants who became bored with the task and put forth less mental effort. The difference in pupil sizes between the pretest and the test that was observed does suggest that there was some effect on pupil size between these two portions of the learning task. If this was not due to habituation alone, this difference could be due in part to the inclusion of the letter number sequencing task in the test, as this was essentially the only difference between the pretest and the test. Perhaps the additional cognitive load of the LNS, and perhaps the fact that LNS task might have been more difficult than the tone pitching task, took away from any effects on cognitive arousal and attention that the tone pitching task might have otherwise had. Participants might have been anticipating the letter LNS task during the letter number sequencing task, as the LNS task directly followed the tone pitching task in the test, and therefore put less effort into the tone pitching task during the test portion of the test. Another explanation for the lack of cognitive arousal observed in the study might also be in the nature of the tone pitching task itself. While studies involving visually oriented cognitive tasks have been illustrated to elicit changes in pupil size due to cognitive functioning and not as a result of visual stimulation (Kang et al., 2014), research has not been done on the use of auditory cognitive tasks and perhaps these types of cognitive tasks do not elicit the same level of pupil dilation as visual tasks. Perhaps the use of isoluminescent visually oriented cognitive tasks might provide for a better learning task and these types of tasks could serve as robust unconditioned stimuli for eliciting cognitive arousal. There was also no real incentive to preform well on either the tone pitching task or the letter number sequencing task. It is possible that by the end of the learning test, participants became bored with the test all together and decided to put forth minimal cognitive effort, since they would receive their SONA credit either way. By incentivizing participants to preform well on these tasks, perhaps by rewarding the top performers with gift cards or some other form of monetary incentive, participants would feel more compelled to put forth their best effort on theses tasks and this might increase their level of cognitive arousal and focus. Statistical analysis on the performance on the tone pitching task was not done in the study and perhaps examining this data might reveal a relationship between pupil size, brain wave activity, and performance on this task. There was also no significant difference in performance on the LNS task. The same digit sequences of this task were used across all three trials of the test, and this could have affected participants' performance, although upon being asked at the end of trial three no participants reported noticing that the sequences were the same each time. There were no significant correlations between pupil size and either alpha, beta, or theta brain wave activity throughout the testing. While this is inconsistent with research that suggests that both pupil size and brain wave activity are indicative of autonomic nervous system activity, this might suggest that other factors influence both pupil size and brain wave activity to an extent at which a correlation would not be observed between the two. It is also important to note that the EEG data collected in this study was collected via a two-electrode recording and the use of a greater number of electrodes would result in more accurate readings and might lead to more significant data in future studies. This research was also my first experience with recording EEG data and perhaps my beginner skill level played a role in sub recordings that could have affected the results. However, a significant negative correlation between pupil size and delta brain wave activity was observed. These findings prove particularly interesting as delta brain waves are primarily associated with sleep and the most inactive cognitive states. These findings might support the suspicion that participants grew tired of the learning task and became exceptionally bored during testing. This might also indicate a relationship between parasympathetic nervous symptom activity and the increase in delta wave activity in the frontal lobe. Further research on this topic may reveal that the relationship between sleep and autonomic nervous system functioning, the “rest” component of “rest and digest” is also somehow related to waking states of consciousness as is suggested by the correlation between decreased pupil size, and indication of sympathetic nervous system functioning, and increased levels of theta brain wave activity in the frontal lobe, brain wave activity that is traditionally only associated with sleep. While this correlation was relatively weak, with only 13.3% of variance in delta brain wave activity being explained by pupil size, this is certainly an interesting finding that deserves further exploration.

If changes are made to the unconditioned stimulus that result in the sufficient levels of cognitive arousal elicited in participants, the next logical step for this research would be to move out of the acquisition phase by removing the unconditioned stimulus and observing the level of cognitive arousal that remains in the presence of the conditioned stimulus alone. It would also be interesting to investigate the effectiveness of conditioning other cognitive states such as relaxation. I believe that adjustments have to be made to the timing in which the study is conducted, maintaining consistency in the time between trials as well as grouping the trials closer together such as once per day in consecutive days, in order to maintain any possible effect of conditioning. Expanding this study to other cognitive states, such as relaxation, could also lead to insights on the effectiveness of using different cognitive states as conditioned responses. Relaxation is more subjective than arousal and focus and therefore it would be more difficult to design an unconditioned stimulus that elicited relaxation for a wide range of participants. Research into other cognitive states might also reveal a difference in the rate at which these conditioned responses are extinguished after the removal of their respective unconditioned stimulus. An individual's ability to relax is influenced by external factors in their day to day lives much more then their ability to focus on a specific task and this may be reflected in the success of conditioning in future research. We also did not factor participants response to demographic questions about whether or not they have conditions that effect their ability to focus or relax into our statistical analysis and future analysis of this data could reveal an effect between participants with these conditions and those without. Because of the extensive amount of data collected on a relatively small number of participants, we were unable to conduct exhaustive statistical analysis of all data collected in the time allotted for this study. By digging into the data further we may discover more interesting significant findings that may shape the ways in which we would chose to adjust the our experimental design were we to repeat and readminister this study as well as results that may inspire future studies regarding the subject matter we examined.

While this study has yet to yield results that are indicative of the successful conditioning of cognitive states, I have confidence that there are a number of adjustments that can be made before we can rule out this type of conditioning as a possibility. If this research proves successful in demonstrating the classical conditioning of cognitive states, I believe this would have huge implications in both the clinical and academic realm. Many individuals who suffer from the impairments in attention caused by ADHD could benefit greatly from the success of this research. Clinicians might be able to implement this type of condition into their patients lives either as alternatives to traditional stimulant medications or in combination with these types of medications. There are a plethora of short-term and long-term effects of these types of medications, and by providing noninvasive alternatives to these treatments, the health and wellbeing of patients could be greatly increased. If conditioning of other cognitive states, such as relaxation, were demonstrated to be effective, similar types of clinical application of this research could be applied to patients with a variety of conditions such as generalized anxiety disorder and panic disorder. Again, the medications used to treat these disorders come with a number of both short-term and long-term side effects and reducing the need for these types of medications could result in the improved health of patients. This type of conditioning might also prove useful in the treatment of medication resistant disorders such as PTSD. Individuals who experience PTSD often have few effective treatment options, and by providing them with some form of relief from their symptoms through the conditioning of relaxation, their quality of life might be greatly improved. This research could also have implications in the academic realm as well as for individuals who experience issues with attention and relaxation at levels that do not meet the clinical threshold for diagnosis. Utilizing this type of conditioning for studying could increase the level of attention and focus for students and improve their efficiency and performance on academic assignments. It could also potentially be utilized in the classroom to increase attention and information retention during lectures. Busy individuals with stressful lives could also benefit from quick, on demand relaxation via the use of conditioned stimuli after they have already developed these associations through undergoing the acquisition phase of this type of conditioning. There is also reason to believe that if cognitive tasks, such as the ones outlined in this study, serve as effective unconditioned stimuli, that the same type of psychiatric medications this conditioning could replace might serve as effective unconditioned stimuli as well. If patients could undergo condition with traditional psychiatric drugs serving as the unconditioned stimuli, they could then be able to stop taking these drugs and instead use conditioned stimuli to elicit the same or similar desired effects as these medications. I believe that the wide range of implications that this research could have on society, if proven successful, warrants further efforts to refine and readminister this study in the hopes of finding effective and realistic ways of classically conditioning cognitive states.

References

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*(4), 602–607. https://doi.org/10.1111/j.1469-8986.2008.00654.x

Hess, E. H., & Polt, J. M. (1964). Pupil size in relation to mental activity during simple problem-solving. *Science, 143*(3611), 1190–1192. https://doi.org/10.1126/science.143.3611.1190

Kahneman, D., & Beatty, J. (1966). Pupil diameter and load on memory. *Science*, *154*(3756), 1583–1585. https://doi.org/10.1126/science.154.3756.1583

Kang, O. E., Huffer, K. E., & Wheatley, T. P. (2014). Pupil dilation dynamics track attention to high-level information. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0102463

Mathôt, S. (2018). Pupillometry: Psychology, physiology, and function. *Journal of Cognition, 1*(1). https://doi.org/10.5334/joc.18

Wróbel A. Beta activity: a carrier for visual attention. Acta Neurobiol Exp (Wars). 2000;60(2):247-60. PMID: 10909182.