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Effects of Auditory Stress on Long-Term Memory Formation in Humans

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Table of Contents:

- Key Point Summary – p.1
- Abstract – p. 2
- Introduction – p. 2
- Materials and Methods – p. 3
- Results – p. 5
- Discussion – p. 6
- References – p. 8
- Acknowledgements – p. 9
- Tables – p. 10
- Figures and Legends – p. 11

Key Points

- The sympathetic nervous system impacts memory formation; however, sources disagree on whether it improves or reduces memory formation.
- This study aimed to broaden the study of stress on memory from purely physical definitions to mental or auditory stress on memory.
- The data collected here on memory formation is inconclusive, suggesting the need for experimental design modifications and an increased sample size.
- Future research should be done to further scientific knowledge on the effects of stress on memory, as it may have detrimental effects on human health and livelihood.

Word count: 92

Abstract

It has been shown that mental stress has a significant effect on the physiological condition of humans including long-term health concerns such as cardiovascular disease. While the influence of physical stressors on memory formation has been the subject of several studies, the effect of mental stress on formation and maintenance of long-term memory is still not clear. In this experiment, the effect of an acute exposure to environmental sounds established as being stressful to most individuals on long-term memory formation is evaluated. The acute stress is an auditory stimulus which accompanies the memorization process in the experimental group. Memorization and commitment of words to long-term memory was assessed by recalling words immediately after testing and one hour post-memorization, respectively. Results show there is no significant difference in memory formation between test and control groups. Interestingly, further analysis reveals that both experimental and control groups showed elevated stress levels according to the physiological parameters measured. Possible flaws in the experimental design and how they might be addressed in future studies are discussed.

Introduction

From the physiological standpoint, stress is defined as the stimulation of the sympathetic nervous system, and this response can be initiated in a laboratory setting by providing a specific stimulus to the test subject (van Stegeren, 2009). This response is extremely significant from an evolutionary standpoint, as it is the source of the "fight or flight" response needed to escape a potential predator or other extremely dangerous situations. In modern culture, however, frequent or prolonged activation of the sympathetic response due to less than life-or death situations can lead to diverse, frequently deleterious, changes in many biological processes leading to a variety of health and quality of life issues.

As part of their daily lives, people are frequently required to memorize and process large quantities of information either in an academic environment or as a portion of their career duties, which can, in itself, be a stressful situation. In recent years the topic of stress affecting memory formation has come to the forefront, mostly due to the fact that many people are suffering from stress related illnesses. It has been shown that stressful events can stimulate physical manifestations such as impaired memory formation (van Stegeren, 2009).

Assessing the effects of stress on long-term memory formation is a complicated process as a result of the numerous variables and hormones that are released during the stress response. There is not always agreement about whether stress inhibits or actually improves the formation of long-term memories. Research has shown that certain types of memory, particularly spatiotemporal memory, can be enhanced by stress. The part of the physiological stress response that is thought to have the most detrimental effect on memory formation is the spike in cortisol levels within the body (van Stegeren, 2009). Studies by Smeets *et al.* have showed that memory is only impaired by stress when the valence of the words were neutral and had no emotional basis (2006). Another study by Smeets *et al.* displayed that acute stress had a positive impact of long-term memory formation when the words were context-congruent personality words (2007). This context-congruency explains the situation when the "memory acquisition phase and stressor share the same spatiotemporal context" (Smeets *et al.* 2007). In laboratory studies on humans, a stronger correlation between cortisol levels and memory formation was observed in a [laboratory] setting than between chronic stressors and memory formation in everyday life (Sauro *et al.*, 2003). Chronic stress in the body is something that can have a negative impact on not only memory formation but also overall health and wellness.

Two variables in previous research have been the type of memory test used and the effect of an emotional attachment to the words or information tested. Researchers have also examined the effect of stress at different times during the memory process. This has lead to examination of the role of noradrenaline, which is released immediately following exposure to the stressor, and glucocorticoids, the primary one being cortisol in humans and is released later in the stress response (Schwabe *et al.*, 2011) .

The numerous types of memory elicit completely different physiological responses within the brain and body. The hippocampus, which is known to be involved in various types of memory formation, is particularly rich in glucocorticoid receptors and so stress will have a direct effect on this part of the brain (Conrad, 2010).

In this study, the effect of stress on explicit memory, and more specifically semantic memory is assessed. From the website "The Brain from top to bottom" it is stated that this includes memory of facts and knowledge, and is accumulated over the subject's lifetime and is independent of the situation under which the knowledge was acquired (Dubuc). This specific type of memory formation is tested using a word-memorization test. Together with previous knowledge, this study will evaluate the effect of stress on long-term memory formation, as noted by the recall of the words one hour post-test. It is hypothesized that in the case of a written, word-memorization memory test, a subject who is under the influence of a stressor during the learning process will convert less information to long-term memory than someone who is not under the influence of the stressor.

Materials and Methods

Ethical Approval:

This experiment complied with all protocols and regulations set by Dr. Andrew J. Lokuta in conjunction with the faculty advisors associated with the Journal of Advanced Student Science (JASS). Written consent was obtained by all twenty participants in both the control and experimental groups. These individuals assured that this study conformed to the standards set by the latest revision of the Declaration of Helsinki. The procedures administered were approved by Dr. Lokuta as well as documented by assistants in the lab.

Procedure:

At the beginning of the experiment, participants will be given a short pre-test questionnaire to assess their current physiological baseline and mental stress levels with questions such as: how much sleep did they get last night; when did they last eat and, on a scale of 0 to 5, how stressed do they feel? The questionnaire will be used in data analysis to control for individual variability. The investigator administering the test will assign a subject number to the participant. There will be two groups, to which each individual is assigned at random by an investigator separate from the test administrator. This is in order to maintain the double-blind quality of this experiment and help maintain a lack of bias during data analysis.

Group Assignment:

The first group will serve as a negative control and will consist of participants who do not receive any stressors while attempting to memorize sixteen words. The second group will contain individuals exposed to a mentally stressful stimulus during memorization. The stressor used in this study consists of an auditory recording made by placing sounds, specifically: a cicada, an alarm clock, a wasp, a baby crying, and finally a CD skipping into a playlist then each sound was copied several times and the playlist shuffled so that the same sound was not heard back-to-back. These sounds, which were determined to elicit a stress response, were played from the generated playlist through headphones to the experimental group and labeled the stressor while no sounds were heard in the headphones of control group participants. Each subject will be connected to a lie detector setup using the BioPac System model MP30, which evaluates respiration, sweat production, and heart rate.

Experiment:

At the beginning participants are informed that they will be performing a memory task and will be given five minutes to perform said task. Participants will be asked to sit up straight and think of non-stressful things at the beginning of the experiment. Calibration of the machine will occur according to a student operator's manual accompanying the device. Data collection will begin first with recording of 30 seconds of baseline activity, and then the subject will either be exposed to the stressor or not, depending on the previously independently established group assignments. The subject will be exposed to the auditory stimulus for one minute, at which point the physiological values will indicate, based on a pilot study performed by the authors (data not shown), a sympathetic response. The stressor remains active in the experimental group during the memorization process.

Memory Tests:

Participants will get five minutes to study the list of sixteen randomly generated words which are set up in a 4X4 grid. In the stress group at this time the stress stimulus will be discontinued, and two minutes later they will be asked to recall as many words from the list as possible. This is performed in order to establish a baseline for short term memory formation and determine how many words each subject was able to commit to memory which is expected to show individual variance independent of the presence of the stressor or not. One hour later, they will again be asked to recall as many words as possible in order to see how their performance compares to the initial short term results. The second memory test is performed to assess how many words were committed to long-term memory, and the results for each individual will be normalized again based on their initial performance in order to compare results between the experimental and control groups.

Data Collection and Analysis:

Heart rate, respiration, and the percentage of the words that were remembered with both short term and long term memory will be analyzed for individual variance and then compared between the two groups. In Excel, various t-tests were performed to show if there are significant differences physiological measures and memory scores between the experimental and control groups. T-tests were also performed to determine if significant stress responses were observed for all individuals.

Results

Physiological measures and memory test scores did not support our hypothesis that control and the experimental groups would show differences in long term memory formation. To analyze differences in groups, paired and unpaired t-tests were used. Low p-values (<0.05) show that the two groups are significantly different, while high p-values show that the two groups are not significantly different. To further define terms used here: the baseline period is defined as the first 30 seconds of data collection, when no memorization or auditory stressors are being applied. The Memorization period is the five minutes each subject was given to memorize 16 words. The experimental group received an acute auditory stressor during the memorization period, while the control group received no auditory stressor. Statistical Tests were only performed on Heart Rate, Breathing Rate, and Memory Scores.

Overall Stress Responses:

Heart rate and breathing rate data show that a stress response occurred on average in both the control group and the experimental group. According to Heart Rate paired t-tests, both the control group and the experimental groups had significant differences between the baseline mean and the mean heart rate during the memorization period (control, experimental; p-value=0.03, 0.03; mean increase in beats per minute = 9.35, 6.96). The average breathing rate in breaths per minute (BPM) was not significantly different in the control group between baseline and memorization period (p-value=0.24, mean increase = 2.32 BPM); while the average breathing rate was significantly elevated in the experimental group (p-value=0.01, mean increase = 3.78 BPM).

Unfortunately, the overall stress response by the control group was not significantly different from that of the experimental group. Performing an unpaired t-test showed no significant difference between the two groups' average breathing rate and heart rate (average breathing rate, heart rate; p-value = 0.74, 0.89).

Memory Scores:

The memory tests also show no significant difference between long term memory formation in the control group versus the experimental group. Two unpaired t-tests were performed to determine if there was a significant difference in long-term-memory formation between the two groups. The first unpaired t-test compared the number of words added to the long term memory in each group; the control and experimental groups did not differ significantly according to this test (p-value = 0.6). Similarly, the second unpaired t-test compared the percentage of words added to the long term memory from the short term memory and showed no significant difference between the control group and the experimental group (p-value = 0.94). These percentages were initially found by dividing the long term memory score by the short term memory score.

In conclusion, the data is not statistically significant and we are therefore unable to support or refute the initial hypothesis that an auditory stressor will affect the formation of long term memory.

Discussion

In this study, the effect of applied stress on the ability of people to form long-term semantic memories was investigated. The effect of physical stressors on both spatiotemporal and objective memory has been the subject of significant amounts of research. In contrast, the effects on memory of stressors that are of an environmental or mental nature, such as ambient noise, are far less well characterized; however, their ability to induce stimulation of the sympathetic nervous system, as measured by production of elevated cortisol levels and possible links to long-term health concerns, has been established (Seidman and Standring, 2010). Given this fact, this study attempted to examine the effect of randomized noises that have been determined by psychologists to be highly annoying (Chang *et. al.*, 2011) on long-term memory formation. On the basis of the existing literature, it was hypothesized that application of noise stressors would impair memory formation. On the contrary, the data obtained in this study indicates that under the conditions used, there is no effect on long-term memory formation in response to stimulation of the sympathetic nervous system. There are several aspects of the experimental design that the researchers believe may need to be addressed in future studies to more conclusively test the hypothesis. As there is the possibility that these factors may obscure an underlying effect, it is believed that both further refinement of the experimental protocol and an increase in the sample size used in future studies.

The testing procedure itself appears to have induced activation of the sympathetic nervous system:

As mentioned in the Results section, both heart and breathing rates showed extensive variation among individuals in both the experimental and control groups. This variability was observed in both baseline readings taken before the onset of the memorization test and during memorization (see Figures 1 and 2). This factor in itself makes analysis of statistical differences between groups problematic, particularly with a small sample size. A statistically relevant increase in heart rate following initiation of the test was observed in both experimental and control groups (see Tables 1 and 2), indicating that both groups experienced some level of stress once word memorization had begun. This might be attributable to a stress that is inherent in test-taking itself or, as subjects did not initially know if they were experimental or control subjects, an anticipatory stress in control subjects waiting to see if they would be exposed to the stressful stimulus. These factors could be controlled for in future studies by including a parallel study in which subjects knew *a priori* which group they belonged to. If there is an anticipatory stress involved, it would be expected that individuals belonging to the control group who knew they would not be receiving the stressor should show no elevation in the physiological parameters used to evaluate stress induction. In contrast, individuals who knew they would be receiving the stressor might be expected to show an anticipatory elevation in heart and breathing rates both during baseline value collection and following initiation of the memory test.

In the case of breathing rates, when average baseline values were compared to those during the memory test, the experimental group showed a statistically relevant elevation in breathing rate while the control group did not. As the experimental group showed significant increases in both heart and breathing rates, while the control group only showed significant elevation in heart rate, it could be surmised that the experimental group did show a higher level of sympathetic activation than members in the control group in response to the auditory stressor. However, when average values of both breathing and heart rates of experimental and control subjects taken during memorization are compared, no significant difference was observed. This indicates that any conclusion that a higher level of sympathetic response was induced in the experimental group can only be regarded as tentative.

It has been reported by others that, of the hormones released during the stress response (noradrenaline and cortisol), it is the elevated level of cortisol that has the strongest effect on memory (van Stegeren, 2009). Given this fact, determining the baseline and post stimulus blood cortisol levels in test subjects would be a valuable quantitative measure of the relative levels of stress response.

Under the test conditions used, application of the auditory stressor does not affect short or long- term objective memory formation:

In both experimental and control groups, no significant difference in the number of words committed to memory or retained in long-term memory was observed. Both groups, within experimental variance, were able to memorize a relatively large raw number of words and retain greater than 90% of the memorized words for an hour post-test. Several factors might account for this. First, it is possible that the test subjects did not find the selected noises, though considered to generally be annoying to most people, to be overly stressful when exposed to them for short periods. It seems that this is likely based on informal post-test interviews with some experimental subjects, where descriptions of their responses to the selected noises ranged from amusement to extreme aggravation. In future studies, having test subjects give a subjective rating of their stress level as was done prior to testing after completion of the test would be valuable. Increasing the length of time that experimental subjects are exposed to the stressor prior to initiation of the memory test and decreasing the amount of time that subjects are given to memorize the words would perhaps be valuable adjustments to the experimental protocol for future studies.

As has been discussed previously in this article, the effect of both the type of applied stress and the type of memory tested show complex relationships where stress may assist the formation of certain memories and impair others. For example: spatiotemporal memory in rats can be enhanced by activation of the sympathetic nervous system (Conrad, 2010) though the duration (McLaughlin *et. al.*, 2007) and time of stress application (Ghiglieri *et. al.*, 1997) have been shown to be significant in terms of the effect of stress. Sympathetic activation has also been tied to both the formation and loss of neuronal contacts in the hippocampus and other structures of the limbic system that are known to be involved in memory formation (Conrad, 2010). In contrast to spatiotemporal memory, the effects of stress on objective memory formation appear to be dependent on the emotional linkages of the material being memorized. In studies using human subjects using word memorization as a test variable analogous to that used in this study, memory has been shown to only be deleteriously affected by the application of acute physical stressors in situations where the words have no emotional ties (Smeets *et. al.* 2006). In contast, if the words lack neutrality, stress appears to enhance memory formation (Smeets *et. al.* 2007).

As mentioned previously, there are several possible factors in the experimental design that might obscure an actual effect on semantic formation. In the experimental protocol used for these experiments, subjects were given a grid containing words that were selected at random and judged to be free of emotional content to avoid the effect of emotional ties noted by Smeets *et. al.*(2007). Furthermore, care was taken to not have groups of related words together on the grid to avoid chunking of memory. However, when the sheets on which experimental subjects wrote the words they had memorized were examined during data analysis, it was noted that most subjects wrote the words they had memorized in the same spatial order that they appeared on the test sheet (data not shown). This suggests that not only semantic, objective memory is being utilized but also spatial memory as to where words occurred in the array. This fact might allow experimental subjects greater ease in memorizing the words presented. While this problem is hard to avoid completely, placing the words randomly on the page and far removed from each other might reduce the contribution of any spatiotemporal component contributing to the subject's ability to memorize the words.

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We would like to acknowledge the Physiology 435 TAs, PLVs and Professors for their guidance and assistance throughout these experiments.

Tables

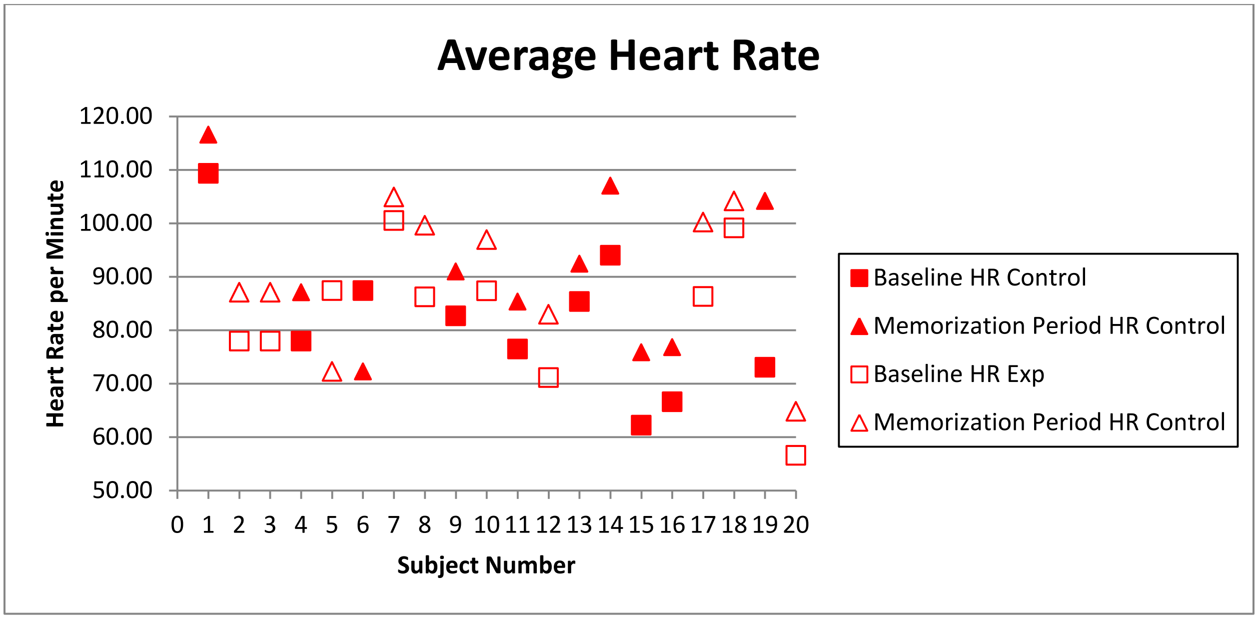
Period	Variable	Mean	Standard Deviation
Baseline	Average BPM	18.08	4.44
	Average Heart Rate	81.50	13.68
	Short Term Memory Score	11.30	2.31
Memorization Period	Average BPM	20.40	3.63
	Average Heart Rate	90.86	14.59
	Long Term Memory Score	10.40	3.03
Comparison	Memory % (LTMS/STMS)	0.91	0.15

Table 1: Control Group Scores
This table lists the means and standard deviations for each variable collected for the control group.

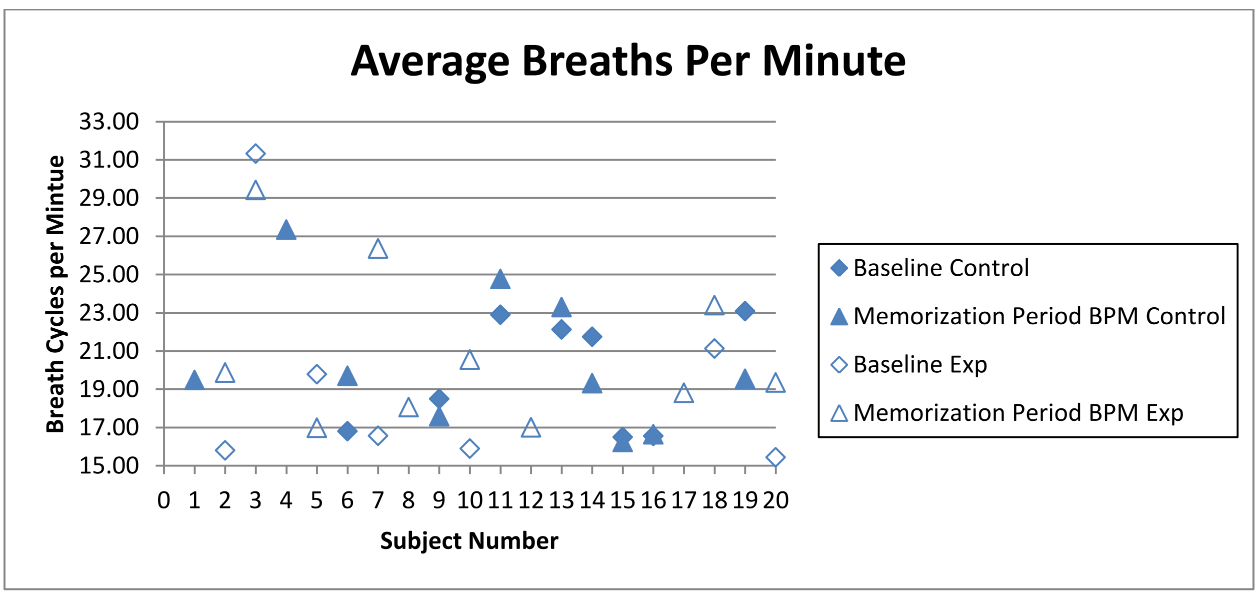
Period	Variable	Mean	Standard Deviation
Baseline	Average BPM	17.12	6.01
	Average Heart Rate	83.05	12.98
	Short Term Memory Score	10.20	3.85
Memorization Period	Average BPM	20.99	4.15
	Average Heart Rate	90.01	13.68
	Long Term Memory Score	9.50	4.35
Comparison	Memory % (LTMS/STMS)	0.92	0.18

Table 2: Experimental Group Scores
This table lists the means and standard deviations for each variable collected for the experimental group.

Figures and Legends



<u>Figure 1: Scatter plot of Average Heart Rate.</u>
This figure shows the heart rate data collected for each subject. This plot shows both the control group and the experimental group. There appear to be no obvious trends in either group.



<u>Figure 2: Scatter plot of Average Breaths per Minute</u>
This plot shows the average number of breath cycles per minute for each subject in the two periods of data collection: Baseline, and Memorization period. A breath cycle is measured as the time between sequential maximum inhalations.

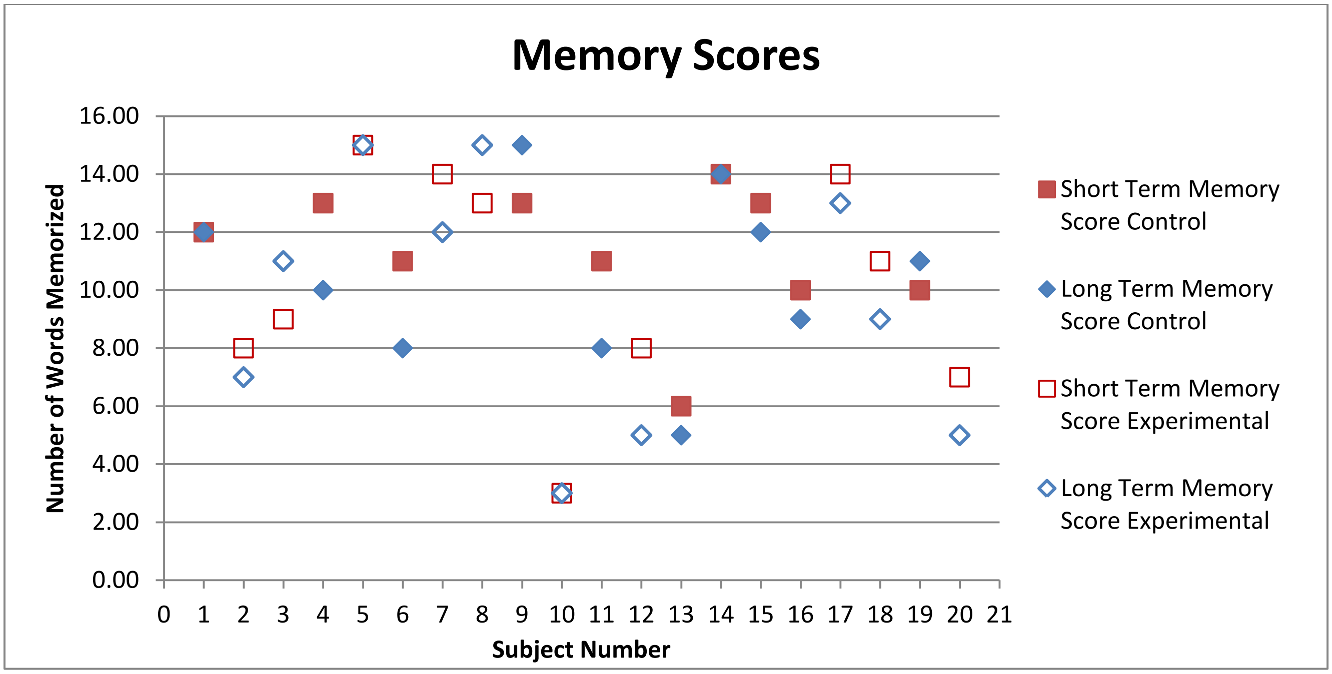


Figure 3: Scatter plot of Memory Scores

This plot shows Memory scores for each subject. Short term score was collected directly after memorizing words and long term score was one hour after the short term score. The Memory % score is the ratio of long term score to short term score.

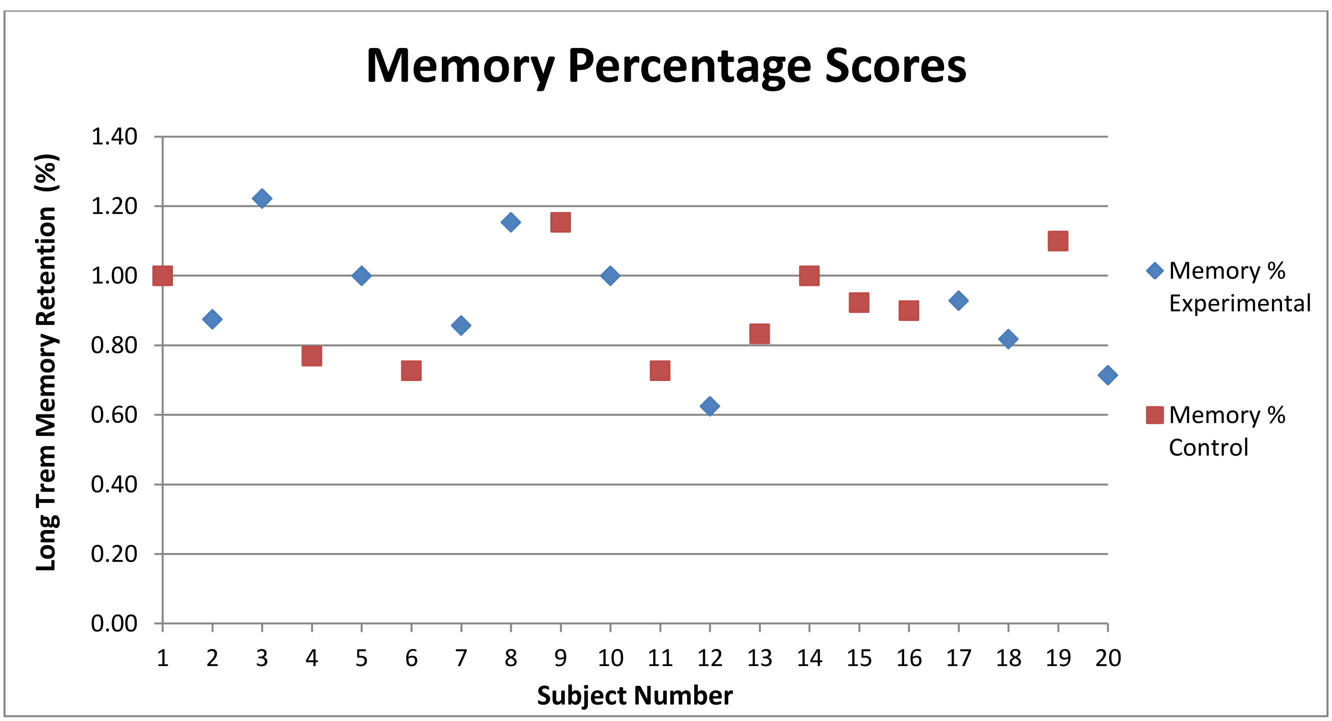


Figure 4: Scatter plot of Memory Percentage Scores

The Memory % score is the ratio of long term score to short term score. Short term score was collected directly after memorizing words and long term score was one hour after the short term score.

APPENDIX: Pilot study

A pilot study was performed at the request of the reviewers varying some of the methods of the original study. This was done to remove spatiotemporal memory cues and to reduce the amount of time given to perform the memory test. Also lessened was the amount of time given between short and long term memory tests, from 1 hour to 30 minutes. The 30 minute time was selected due to time constraints but still fits in the criteria of long term memory formation after learning.
In the pilot study, four subjects were used: two control and two experimental subjects. In further studies a larger sample size needs to be used. Physiological parameters measured were respiration rate, ECG and the memory test itself. To remove spatiotemporal cues that might have been aiding in memorization, the sixteen words were placed randomly and widely spaced apart on a letter-sized sheet of paper.
Based on the random order in which subjects wrote down the words they had memorized, any spatiotemporal cues that had assisted in memory formation in the main study had been removed. However in this pilot study there was no physiological response observed in the experimental subjects which indicates there was no activation of the stress response. This could be due to the fact that they were not exposed to the auditory stimulus long enough due to the decreased testing duration. We have demonstrated that these sounds are capable of eliciting a stress response as seen in the original study, so in future studies the subjects should be exposed to the stimulus for a longer time before the memory test begins.

Data follows:

Appendix Tables

Period	Variable	Mean	Standard Deviation
Baseline	Average BPM	23.70	0.00
	Average Heart Rate	77.66	5.05
	Short Term Memory Score	8.00	1.41
Memorization Period	Average BPM	18.92	3.09
	Average Heart Rate	93.82	1.89
	Long Term Memory Score	7.50	2.12
Comparison	Memory % (LTMS/STMS)	0.91	0.15

Table A1: Control Group Scores
This table lists the means and standard deviations for each variable collected for the control group in the pilot study.

Period	Variable	Mean	Standard Deviation
Baseline	Average BPM	20.97	2.77
	Average Heart Rate	83.31	12.42
	Short Term Memory Score	8.50	3.54
Memorization Period	Average BPM	17.27	4.98
	Average Heart Rate	87.34	11.37
	Long Term Memory Score	6.50	2.12
Comparison	Memory % (LTMS/STMS)	0.78	0.07

Table 2A: Experimental Group Scores
This table lists the means and standard deviations for each variable collected for the experimental group in the pilot study.