The effect of increased stress levels on memory test performance

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Abstract
The present study examines the effect of a sympathetic stressor on a short-term memory test, and changes in cortical Beta wave amplitude, which when present, indicate active cognition. While testing these variables, heart rate (HR) and blood pressure (BP) were also recorded to ensure that sympathetic stimulation occurred; control subjects were also used as a reference to ensure that stimulation was indeed caused by the intended stimulus. Two short-term memory tests were conducted; one under no stress, and another with an added sympathetic stressor. The changes between each recorded variable during these two tests were averaged across all subjects and analyzed for significance. Although results indicated a significantly increased sympathetic response overall (P < 0.05 for HR during recitation and BP), results for changes in Beta waves among all subjects were inconclusive (P > 0.05); therefore, the effect of stress on Beta wave activity has yet to be determined. However, it was concluded that increased sympathetic response had a negative impact on subjects’ short-term memory during this study, which may indicate that increased stress reduces performance on working memory tests.

Introduction
There is a prevailing “myth” on college campuses that one can induce stress by waiting to write a paper until the night before it is due or saving studying for the night before an exam and thus produce better results. Therefore, it was hypothesized that stress leads to increased performance on a short term memory test. According to the Netter textbook, the sympathetic nervous system mediates stress responses, such as the fight or flight response which is a reaction to fear, stress, or physical activity. Stimulation of the sympathetic nervous system results in an increase in HR, BP, bronchial as well as pupil dilation, sweating, and other changes in physiological processes. Throughout the experiment, it was expected that HR and mean arterial blood pressure (MABP) would increase with the presence of sympathetic stimuli. Thus, the presence of increased HR and increased MABP indicated that the body was in a stressed state. An electroencephalogram (EEG) was used to observe and analyze the subjects’ brain activity as they completed the two tests. An EEG records the summation of continuously generated electrical signals in the form of excitatory post synaptic potentials and inhibitory post synaptic potentials of neurons in the cerebral cortex. There are four frequency levels of cortical arousal that can be detected by the EEG but this experiment specifically observed and analyzed the amplitudes of Beta waves (which are active from 14Hz to 30Hz). An increase in Beta wave amplitude indicates a lower cortical arousal, due to the summation of synchronous brain activity. Thus, lower Beta wave amplitudes represents desynchrony (increased activity) in Beta waves and thus increased cortical arousal. More specifically, desynchrony in Beta waves indicates a behavioral or psychological state that demonstrates alertness, focus, problem-solving strategies, and/or high levels of environmental stimulation. Upon using the EEG, the change in the subjects’ individual Beta amplitudes between the stressed and unstressed state was analyzed in order to standardize the findings between subjects.

Materials and Methods
Thirteen subjects were recruited for the study (five males and eight females). Subjects were not randomly selected. Instead, subjects were chosen from a pool of students from the Physiology 435 class and age matched volunteers available at the time of testing. Prior to testing all subjects signed a consent form that identified any risks associated with the study. All subjects were hooked up to an EEG with a total of five electrodes. Electrodes were placed on the forehead, one on each temporal lobe and one over each parietal-occipital lobe. All of the electrodes were then covered with a snug hat or swim cap to enhance readings by increasing conductance. The electrode leads were connected to the iWorx EEG unit, which was connected to the lab computer and synced with the LabScribe2 program. Instructions were followed according to a
learning aid found at the system’s website. All subjects wore a pulse oximeter throughout the study to monitor and record heart rate. Blood pressure was also measured twice during the study per subject, one for each test. Blood pressure was taken by the same group member across all subjects to reduce inconsistencies and to provide the most accurate readings possible. The blood pressure was measured at the brachial artery and was recorded as the systolic, or maximum, pressure (SP) over the diastolic, or minimum, pressure (DP).

Subjects took two memory tests, each totaling approximately 60 seconds in length, during the study. For each test, the subject was given a piece of paper with thirteen clip art pictures on it and given twenty seconds to memorize as many pictures as possible. Different pictures were used for each test. Before subjects were tested a volunteer took both memory tests to ensure that both tests were of equal difficulty.

One test was taken in a relaxed (unstressed) state while the other was taken in a stressed state (a state of increased sympathetic nerve activity). During the unstressed test subjects were sitting in a comfortable chair in a room with a moderate noise level. During the stress situation the subjects were sitting in the same room in the same conditions but, to stimulate sympathetic nerve activity, were additionally required to place their hand in a bucket of ice water for the duration of the test (approximately 60 seconds). Temperature of the ice water was kept constant at 0 degrees Celsius. At the conclusion of each twenty second test the subject was to recite back the pictures they had memorized and the number of pictures memorized was recorded by the testing team. The order of the two tests was randomized. In order to ensure that one test did not affect the data of the next test the EEG and HR readings were given time to return to baseline prior to beginning the second test. EEG was recorded throughout both tests. Heart rate was measured with a pulse oximeter throughout the study and recorded during the unstressed memorization period, the unstressed recitation period, the stressed memorization period and the stressed recitation period. Blood pressure was recorded once during each test during the twenty seconds that the subject spent memorizing pictures.

Positive control subjects performed 25 jumping jacks to induce the sympathetic nervous system, and to produce the desired response of increased stress indicated by increased HR, BP and brain activity as indicated by EEG waves. This control produced the hypothesized results of increased HR and BP and could therefore be used in conjunction with the negative control of no relationship to analyze the experimental results. As a negative control one subject’s response was measured in the absence of a stressor which was achieved by performing the two memory tests in the absence of sympathetic stress stimuli, such as cold water.

**Statistical analysis.** Each subject served as their own control since measurements were taken in both the stressed and unstressed condition. This design allowed for standardization of the results to the change in heart rate, blood pressure and wavelength or frequency for each individual subject. This enabled comparison of the differences across subjects throughout the study. Data is represented as means ± standard deviation (SD). All differences between scores were then analyzed for significance (P < 0.05) using a paired difference t-test.

**Results**

**Heart rate and mean arterial blood pressure.** To determine whether the protocol actually induced the expected sympathetic nervous system stimulation, HR and arterial BP were measured during the subject’s memorization and recitation during the unstressed test and the stress test. Blood pressure readings, initially taken as a diastolic and systolic reading were converted to MABP using the equation: MABP = DP + 1/3(SP-DP). Heart rate during the recitation test and MABP increased (P < 0.05) during the stressed condition, thus suggesting increased sympathetic response. Change in HR between the stressed and the unstressed test during the memorization did not show statistical significance; although, the trend suggests that HR is increased during the stress test. Thus, it is expected that the data would be statistically significant with a larger sample size. Percent difference between the stress test and the unstressed test was then calculated for both HR and MABP (Fig. 1).

**Memory test scores.** Individual test scores during the stressed and unstressed condition were also compared using percent differences (Fig. 2), and then further analyzed as the difference in means between the stressed and unstressed state (Table 1). Scores were found to be significantly lower during the stressed condition than during the unstressed condition (P < 0.05).
**Beta waves.** Obtained Beta wave data was analyzed by taking the difference between the maximum wave value and the minimum wave value over a ten second reading during each portion of the test (memorization and recitation) and during both the stressed and unstressed condition. The difference between the values of the stress test and the unstressed test were than taken and analyzed. No statistically significant differences were found between the average absolute change in left Beta waves from the stressed to the unstressed condition during either the memorization or the recitation test (P > 0.05) (data not shown).

**Table 1. Changes in HR, MABP and memory test scores**

<table>
<thead>
<tr>
<th></th>
<th>HR(M)</th>
<th>HR(R)</th>
<th>MABP</th>
<th>Test score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=13)</td>
<td>(n=11)</td>
<td>(n=13)</td>
</tr>
<tr>
<td>Unstressed</td>
<td>70.8±7.3</td>
<td>78.5±11.0</td>
<td>90.6±9.4</td>
<td>8.3±1.5</td>
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<td>Stressed</td>
<td>74.0±7.0</td>
<td>85.7±11.1</td>
<td>94.4±9.5</td>
<td>6.8±1.5</td>
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<td>P-value</td>
<td>0.06195</td>
<td>0.00001</td>
<td>0.03256</td>
<td>0.00596</td>
</tr>
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</table>

Values are means ± SD.

Figure 1 Percent difference during the stressed state in comparison to the unstressed state measured as HR during memorization and recitation (A), MABP (B). Significant differences exist between each subject’s unstressed test and the stress test (P < 0.05) in HR during recitation and MABP. Trends also suggest a difference between the unstressed and the stressed test for HR during memorization, however these trends are not statistically significant. Note that subject number only indicates the number of subjects that had the given data collected; thus subject 1 in one graph does not necessarily correlate with subject 1 from another graph.

Figure 2 Percent difference in memory test scores during the stressed state in comparison to the unstressed state. Significant differences are shown between states (P < 0.05). Note that subject number only indicates the number of subjects that had the given data collected; thus subject 1 in one graph does not necessarily correlate with subject 1 from another graph.

**Discussion**

The purpose of this study was to test for a relationship between the response to a sympathetic stressor and performance, specifically in this experiment with regards to memorization. Of the three physiological responses observed, HR and MABP demonstrated significant increases during the stressed condition. However, EEG
Beta wave amplitude changes from the unstressed to the stressed condition were not statistically significant.

The sympathetic nervous system operates to mobilize the body’s functions for appropriate responses in times of stress, the “fight-or-flight” response. The HR is increased to supply the body with adequate amounts of blood, most importantly to the brain. This knowledge can account for the increase in HR in the stressed test when the body is undergoing physiological change from an outside source or a change induced internally\(^3\). The BP is a further result of the mobilization of appropriate amounts of blood in certain areas of the body when under stress and or perceived stress\(^3\). This is most clearly seen once again when comparing the unstressed test to the stress induced test. As expected, both HR and BP increased in this experiment and further illustrated the effects of the sympathetic nervous system on physiological functions. However, results did not support the hypothesis that performance in the form of memorization capacity would increase. In fact, the opposite effect was demonstrated; the number of objects memorized decreased on average when subjects were in the stressed condition.

Desynchronous Beta waves (smaller amplitudes) are indicative of the neuronal activity in the brain when an individual is alert/working, active, busy or anxious thinking, or actively concentrating. They are found symmetrically on either side of the head and are most evident frontally\(^3,4\). It is the active wave when individuals have their eyes open, and it is dormant when an individual’s eyes are closed. Beta waves are also active during passive movements, voluntary hand movements, and movement imagination\(^5\). Because these factors were not controlled during the experiment, unnecessary and excessive movements from the subjects may have affected the max-min amplitude recordings and contributed to the inconclusive changes between the stressed and unstressed readings. Other possibilities for the inconclusive Beta wave results include improper conductance and equipment malfunction.

Per reviewer’s request additional positive controls were tested (n=3) to show that the increase in sympathetic stimulation was due to increased stress levels and not the distraction of having a hand in ice water. The change in heart rate and Beta wave amplitude from the stressed test to the unstressed test was not significant in either the memorization or the recitation test (data not shown). Although not statistically significant, trends support the finding that after completion of jumping jacks MABP increased while memory test scores decreased (See Appendix Table 2). With a larger sample size it is expected that these trends would show significance and thus indicate that the effect found during the study was due to increased sympathetic stimulation and not the distraction of ice water.

Strengths of this experimental design include using each subject as his or her own control by taking the test in both the stressed and unstressed condition. Having each subject take both tests also allowed for analysis of the data using a paired t-test in place of ANOVA. This increases power, particularly in cases where the n is small. Another strength of this experiment is that the population of subjects tested is the same population upon which the hypothesis was based.

One limitation of this study is a small sample size as the subject pool was largely limited to the availability of students in Physiology 435. Another limitation of this study is that caffeine consumption or nicotine usage the morning of the study was not accounted for. The use of nicotine has been shown to have a stimulating effect on the central nervous system\(^1\). Similarly, caffeine has been shown to have a slight effect on Beta waves within the brain\(^3\). Other variables that were not accounted for include the amount of time the subject had been awake that day or if he/she had exercised prior to testing. Equipment difficulties also contributed to the list of possible limitations. For example, BP cuffs and stethoscopes were difficult to use in the lab and Korotkoff sounds were very difficult to hear. After the completion of the data collection it was found that one of the class EEG machines was producing faulty readings; however, because the machines are indistinguishable from one another it is unknown if this machine or the properly working machine was the one used to collect data for this experiment.

Future studies should aim to better control additional factors that can affect the sympathetic nervous system and brain activity. They should also analyze more than just the Beta wave from the EEG recordings in order to have a more complete data set. Future studies could also expand this study to a variety of populations (other than college students enrolled in a physiology class) or examine gender differences. Furthermore, other indicators of stress such as epinephrine or cortisol levels could also be measured to further investigate the relationship between stress and performance.

In conclusion, results did not support the hypothesis that increased stress will lead to increased performance on a short term memory test. From the results of this study it remains unclear how or if Beta wave amplitude changes from the unstressed to the stressed state. However, results did show that increased stress, as indicated by an increased sympathetic response, negatively impacted subjects’ short term memory test scores.
References


Appendix

Table 2. Changes in HR, MABP and memory test scores using jumping jacks to induce sympathetic stimulation

<table>
<thead>
<tr>
<th></th>
<th>HR(M)</th>
<th>HR(R)</th>
<th>MABP</th>
<th>Test score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=3)</td>
<td>(n=3)</td>
<td>(n=3)</td>
<td>(n=3)</td>
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<tr>
<td>Unstressed</td>
<td>77±13.5</td>
<td>98.7±7.8</td>
<td>89.1±8.7</td>
<td>9.7±1.5</td>
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<tr>
<td>Stressed</td>
<td>88.3±2.1</td>
<td>92.3±9.3</td>
<td>94.9±6.9</td>
<td>6.7±3.1</td>
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<tr>
<td>P-value</td>
<td>0.24388</td>
<td>0.45891</td>
<td>0.09644</td>
<td>0.09547</td>
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</table>

Values are means ± SD.