Key Words: Long Term Memory, Cognitive Recall, Stress, Cold Pressor

Brain Freeze: Can Physical Stress Induced By Low Temperature Reduce Memory Formation?

Margaret Brucker, Katlyn Orcutt, Tapan Sharma,

Matthew MacDonald, Anton Makhiboroda, Amber Zhou

University of Wisconsin - Madison

Abstract

While eyewitness memory continues to play a critical role in police investigations and court rulings, its validity has repeatedly come under question. Can we trust ourselves to recall critical information under stressful conditions? The association between stress and memory formation has long been a subject of debate. Inconsistencies in experimental findings may be due to experimental failure to create adequately stressful conditions. Environmental stressors, such as cold temperatures, have been shown to affect memory formation, and in turn cognitive recall. Our experiment uses a derivative of the cold pressor test to induce stress in participants. We hypothesized that stress induced by the cold pressor test should reduce long term memory retention. Forty subjects were randomly tested in control (n=20) and stress (n=20) experimental groups. The control group submerged their feet in room temperature water and the experimental group submerged their feet in cold water. Both groups were shown a crime scene video. We measured our participants stress state through testing heart rate, respiratory rate, and blood pressure. Ten minutes after experimentation, participants were asked to complete a memory test. Data from statistical testing revealed no significant difference between control and experimental groups.

Introduction

We are often surprised by the feebleness of our memories when asked to recall information obtained under stressful circumstances. Long-term memory (LTM) plays an essential role in ongoing learning for students but also has significance in other situations, including eyewitness testimonials, which often tend to come under question in court (Christianson, 1992). The aim of our experiment is to investigate the relationship between stress and memory formation so that we can provide conclusive support for or against the notion that stress impairs memory formation. Ultimately, our research will inform the justice system on the utility of eyewitness evidence in criminal cases.

Psychologists and neuroscientists have distinguished LTM as the retention of information or events over delays of a minute or more, in contrast to short-term or working memory that describe retention of events across delays of seconds (Ranganath and Blumenfield, 2005). Varying accounts of critical events may be the result of acute stress on the brain's ability to encode memories long-term (Kuhlmann, 2005). Physical or emotional stress on an individual at the time of a critical event may interfere with the fragile process of accurately encoding working memory, such as remembering the numbers seen on a license plate, into long term memory.

These inconsistencies in memory have been linked to the effect of stress and glucocorticoid levels on memory retrieval. Occurrence of physiological stress induces the secretion of glucocorticoids, a classic endocrine response to stress, that have been found to cause inhibitory effects on hippocampal neurons, thereby impairing LTM. Specifically, certain studies have shown the release of glucocorticoid hormone by the adrenal cortex in stressful situations to inhibit cognitive performance (Newcomer et al., 1994; Sapolsky et al., 2000). Research also shows that cold temperatures can lead to impaired learning, and thus cold temperatures can be one of the major stress factors associated with memory impairment. However, studies on the effects of acute stress on animals and humans have shown inconclusive findings, indicating that physical stress can both enhance and inhibit long term memory formation (Duncko, 2009).

This experiment is designed to study the effects of physiological stress on long term memory through a derivative of the cold pressor test. The cold pressor test, commonly used in research on stress, pain, and cardiovascular reactivity has been empirically validated by previous research. Our study includes a cardiovascular test to measure blood pressure and heart rate reactivity after immersion into ice water (Streff et al., 2010). Does physiological stress induced by a cold pressor test result in impaired long term memory formation? Will the stress group have a higher error rate after attempting to recall events that occurred simultaneously with the physical stressor? Our hypothesis was generated to gain a better understanding of how environmental factors affect learning.

Methods

Materials List

Consent form, three plastic bins allowing for full immersion of feet (ankle deep), ice, water, paper towels, glass thermometer, timer, video on laptop computer (raw crime scene footage, http://www.youtube.com/watch?v=_4s-3itJTps), memory quiz, heart rate monitor (Nonin Medical Inc., Pulse Oximeter Model No. 9843, Plymouth, MN), blood pressure cuff (Jobar International, Inc., Model No. HL168, Carson, CA), respiratory rate monitor (BIOPAC© Systems, Inc., Model No. SS5LB, Goleta, CA).

Participants

All participants were students from a midwestern university in the U.S. between the ages of 18 and 27. The students were randomly assigned to either the control group or the experimental (stress) group. There were 20 participants assigned to each group, with a total of 40 participants for the whole experiment. All participants signed a written consent form prior to experimentation.

Stress Test

The physical stress test included placing both bare feet in a tub of ice water (0°C.) To ensure all stress group participants' feet were the same temperature prior to the cold immersion, we placed their feet in room temperature water (23 °C) for one minute at the start of the trial. Immediately after one minute, feet of experimental group participants were placed into the ice water bath for one minute. This minute consisted of 15 seconds of waiting, followed by 45 seconds of crime scene viewing, and then the removal of the feet from the ice water right at the two minute mark. The control group participants immersed their feet in room temperature water (23 °C) for one minute before transferring to a second room temperature tub (23 °C), in order to keep consistent with the experimental group. During all stages and trials, physiological measurements were taken.

Measurements

Blood pressure, heart rate, and respiratory rate measurements were taken, as they are all indicators of stress. Measurements were taken per the procedural timelines seen in Figures 1 and 2. These measurements served as points of reference to compare participants' physiological states before, during, and after the examination to see how strongly cold stress influenced their physiology and, as a result, affected their memory formation. Heart rate, blood pressure, and respiratory rate readings were conducted on three participants before and after the participants ran up and down five flights of stairs to ensure equipment functionality and that these physiological values changed as expected to physiological stress on the body.

Memory Test

To test long-term memory performance, all participants were asked to recall specific details about the crime scene footage, ten minutes after experimentation. This was a one page written test consisting of questions of varied difficulty. The test was scored on points earned through correct responses. Each question had only one answer and any answer that deviated from the correct answer was counted incorrect. This type of testing served as a distinguishing tool between normal versus impaired memory recollection. A time period of ten minutes after witnessing the crime scene footage was selected based on average police response times to reported crime in the United States. The United States Bureau of Justice Statistics' latest report shows that in 58.6% of violent crime (robbery, aggravated assault, rape, sexual assault and simple assault) police response time across the United States averaged between five and ten minutes (United States Bureau of Justice, 2008). As our crime scene footage displayed a simple assault, we selected ten minutes as an adequate testing time for long-term memory, as this time appropriately represents the time frame in which a police officer would first ask a witness to recall information related to the crime. Our experiment complies with the policies and ethical regulations of the Journal of Physiology (Drummond, 2009).

Data Analysis

In order to analyze our data, we first defined parameters for stress based on heart rate, blood pressure and respiratory rate. Stress for each variable was defined as greater than or equal to a 10% increase in heart rate, greater than or equal to a 5% increase in systolic blood pressure, and greater than or equal to a 10% increase in respiratory rate. If a participant reached these threshold values for two or all three of these physiological variables (heart rate, blood pressure, and respiratory rate), then the participant was determined to have experienced physiological stress during the experiment. A 10% increase in heart rate was determined to show stress based on research that demonstrates that regardless of the time of day, heart rate increased between 8.75% and 17.5% during stress induced by a Trier Social Stress Test (TSST) (Kudielka et al., 2004). Additional research has shown that there is no statistically significant difference in biological stress indicators between TSST and cold pressor test (McRae et al., 2006). From this information, a 10% increase in heart rate was determined to be an appropriate threshold for determining participant stress. Research on systolic blood pressure increase in response to physical stress shows that participants' mean systolic blood pressure rose by 3.33% after one minute and reached a 10% increase by two minutes (Kurl et al., 2001). Because our systolic blood pressure readings were taken within the first minute of the stress test, we determined that a 5% increase in systolic blood pressure would adequately reflect participant stress. Research has shown that when subjects are immersed in cold water their respiratory rate increases by 5% at rest and up to 20% with high physical activity while immersed (Cooper et al., 1976). Because participants in our study had limited physical activity and were not fully immersed in cold water we determined that a 10% increase would appropriately indicate that the participant was physically stressed. Baseline heart rate was determined by averaging the heart rate taken at time 0 and 30 seconds. This was contrasted with the average heart rate of recordings taken at time 60 and 90 seconds to determine if threshold heart rate increase was surpassed during the experiment. Baseline systolic blood pressure was defined by the measurement taken at time 0 and contrasted with a blood pressure reading taken at time 60 seconds when the participant had been submerged in the second bin of water. Respiratory rate was averaged over the first minute that the participant's feet were submerged in the initial bin of water to determine a baseline breathing rate. This rate was then contrasted with the average breathing rate over the one minute of time in the second water bin. A Welch two-sample t-test was performed to determine if there was a significant difference in memory test performance between the control and experimental group. The null hypothesis of this test is that both control and experimental groups would perform equivalently on a memory test. A second Welch two-sample t-test was performed in order to decide if a significant difference in memory recall between participants in both the control or the experimental group who reached our definition of stress versus participants who did not experience physiological stress throughout the experiment. The null hypothesis of this second test is that participants who did not experience stress during the experiment would perform equivalently on a memory test.

Results

The initial equipment test with three participants after running five flights of stairs showed increases in heart rate (43.80%, 55.84%, and 178.40%), blood pressure (11.20%, 24.37%, and 7.20%), and respiratory rate (12.50%, 30%, and 233.30%). These results verify equipment functionality and predicted physiological responses to stress.

Heart rate readings determined that only two of the control group participants reached stress threshold while fifteen participants in the experimental group reached or surpassed 10% increases in heart rate (Figure 3). Six participants in both the control group and the experimental group reached stress threshold of a 5% increase in blood pressure (Figure 4). Respiratory rate measurements showed that nine participants in the control group and eleven in the experimental group reached 10% increase and were therefore determined to have been physiologically stressed (Figure 5). In total, fourteen participants were determined to have undergone stress based on the criteria that they reached threshold stress levels for two or more of the physiological measurements (Figure 6).

A Shapiro-Wilk test was performed on test score data to determine if the population was normally distributed. The null hypothesis of this Shapiro-Wilk test was that the data was normally distributed. This test indicated a p-value of 0.1965, (α=0.05). Thus, we failed to reject the null hypothesis and assume our data was normally distributed.

Memory test scores resulted in an average score of 36.250% for control group participants compared to an average of 34.750% for the experimental group (Figure 7).

Participants were then separated into stressed and unstressed categories if they showed physiological stress in two or more of the measurements regardless of whether they were in the control or experimental group. The average test score for stressed participants was 33.214% compared to the unstressed group average of 36.731% (Figure 8).

The first Welch two-sample t-test demonstrates that with a 95% confidence interval there is no statistically significant difference in memory test performance between the control and experimental groups ($t = -0.152$, $df = 36.527$, p-value = 0.88). Similarly, the second Welch two-sample t-test shows that with a 95% confidence interval there is no statistically significance in memory test performance between participants who experienced physiological stress versus those participants who did not display signs of physiological stress ($t = 0.9268$, $df = 26.157$, p-value = 0.3625).

The results on our written test, when compared between groups, did not indicate a significant difference in scores, suggesting that cold induced stress does not impair memory formation.

Discussion

Our results showed, contrary to our predictions, that memory formation is not impaired when people are under the influence of physical stress. We expected blood pressure, respiratory rate, and heart rate to be higher in the experimental group than in the control group, along with the experimental group to recall less information about the crime scene footage than the control group. This would have indicated we were able to induce physical stress on the experimental group and as a result, this group would score lower on the written test.

Our data does not support our prediction that memory formation is impaired under stressful conditions relative to a non-stressed control group. In contrast with our results, previous studies have shown that people under states of high emotional arousal, such as emotional stress or psychosocial stress, have an attenuated capacity of long term memory and working memory, respectively (Christianson, 1992; Kuhlmann, 2005).

The varying conclusions between our experiment and previous studies visualize the current ambiguous understanding of the association between stress and memory formation. Our results conclude that there is no linear relationship between the two events; however, the types of stress studied between the experiments discussed and their concluding results vary. While our experiment studied the relationship between physical stress and long term memory, Christianson (1992) connected emotional stress to impaired memory formation. From Christianson's study, emotional stress was defined as "a consequence of a negative emotional event, in which the person experiences a certain degree of stress or distress". It is possible that during our study, the emotional state of the subject had an effect on memory retention based on what emotions are evoked during the experiment by the video presentation or the induced pain. Similarly, Kuhlmann's (2005) study showed that psychosocial stress impairs memory development. Of the studies mentioned, Duncko et al. (2009) is the only group that investigated physical stress, using a cold pressor stress test, on its relation with working memory. As Christianson's review stated, we must place an emphasis on the importance of "type of event, type of detail information, time of test, and type of retrieval of information." Therefore, we cannot explicitly say that our results do not agree with those of the aforementioned studies because different types of stress and different types of memory were examined.

Another factor that could have caused discrepancies between our results and the other studies is the possibility of experimental flaws that gave rise to inconsistencies in our data. As the location of our ice bucket remained constant throughout our experimental trials, some of our participants may have anticipated which bucket their feet would land in as we moved them. This design of our experiment showed a possible source of error due to their anticipated stress, a classic example of the feed forward mechanism. Another factor to consider is the recent exposure of subjects to a long duration of cold winter climate. Tighter controls could have resulted in cleaner data. Had we conducted our experiment outside of the class lab room, in an isolated area, word of our manipulation might not have spread resulting in further anticipation. Finally, our biggest difficulty was with the respiratory rate monitor which would occasionally flat line despite being set up according to protocol.

In the future experiments, we recommend isolated sites to perform tests in order to make sure participants are blind coming into the experiment and to prevent any anticipation of stress. Furthermore, increasing the group sizes should reflect the normal population more accurately. Monitoring participants during the delay prior to memory testing would also be helpful in acquiring more accurate memory test results. Improved measurement precision as well as more accurate testing equipment would provide more authentic data and test results. Accuracy of measuring stress may be improved by quantifying salivary-free cortisol in subjects, if sufficient equipment is provided in the future. In order to clarify the relationship of cold stress on memory retention, we suggest further experimentation with an expansion of temperature ranges tested to detect possible thresholds for significantly altered memory retention. Furthermore, our cold pressor test may not have been adequate in simulating a situation stressful enough to induce memory impairment. In the future, we recommend using a global physical application of stress such as exposure to cold temperature throughout the body. A global stress response could have a much more significant impact on memory retention compared to localized stress. The global strain would increase the magnitude of somatosensory signaling; thus altering overall long term memory formation, as well as associated higher order cognitive function.

Long term memory plays an essential role in ongoing learning for students but also has significance in other situations, including eyewitness testimonials. A recent Supreme Court ruling in *Perry v. New Hampshire* held that due process rights do not require a preliminary inquiry into the reliability of an eye witness' testimony, preserving the crucial role of the eyewitness in America's legal system. While a witness may provide important insight into a case, inconsistencies in their testimonial can prove equally detrimental to a case. The inability of both our experimental and control groups to accurately recall answers on the memory test supports the notion that eye witness testimonies are not always reliable, stress notwithstanding.

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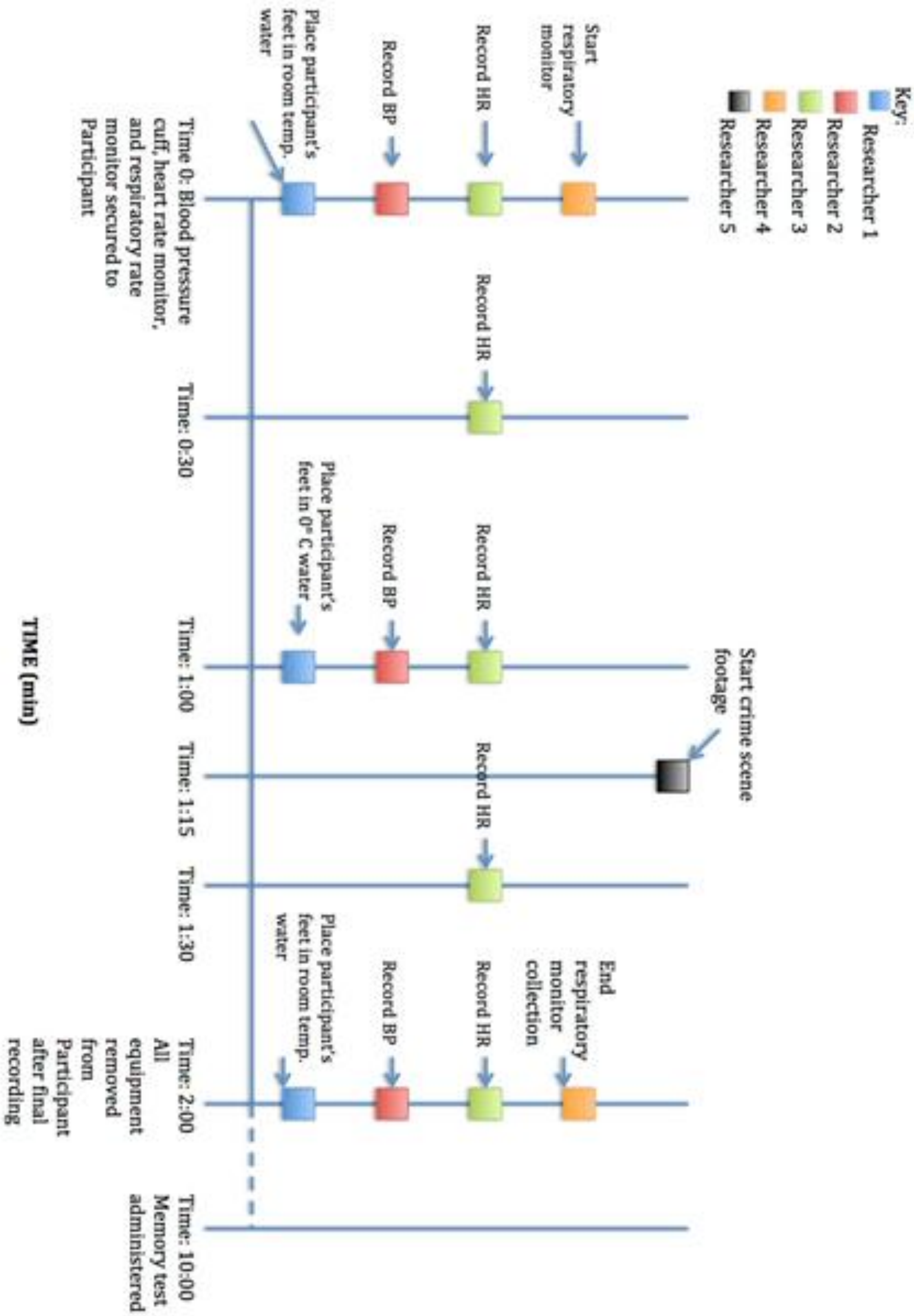


Figure 1: Procedural timeline for the experimental group

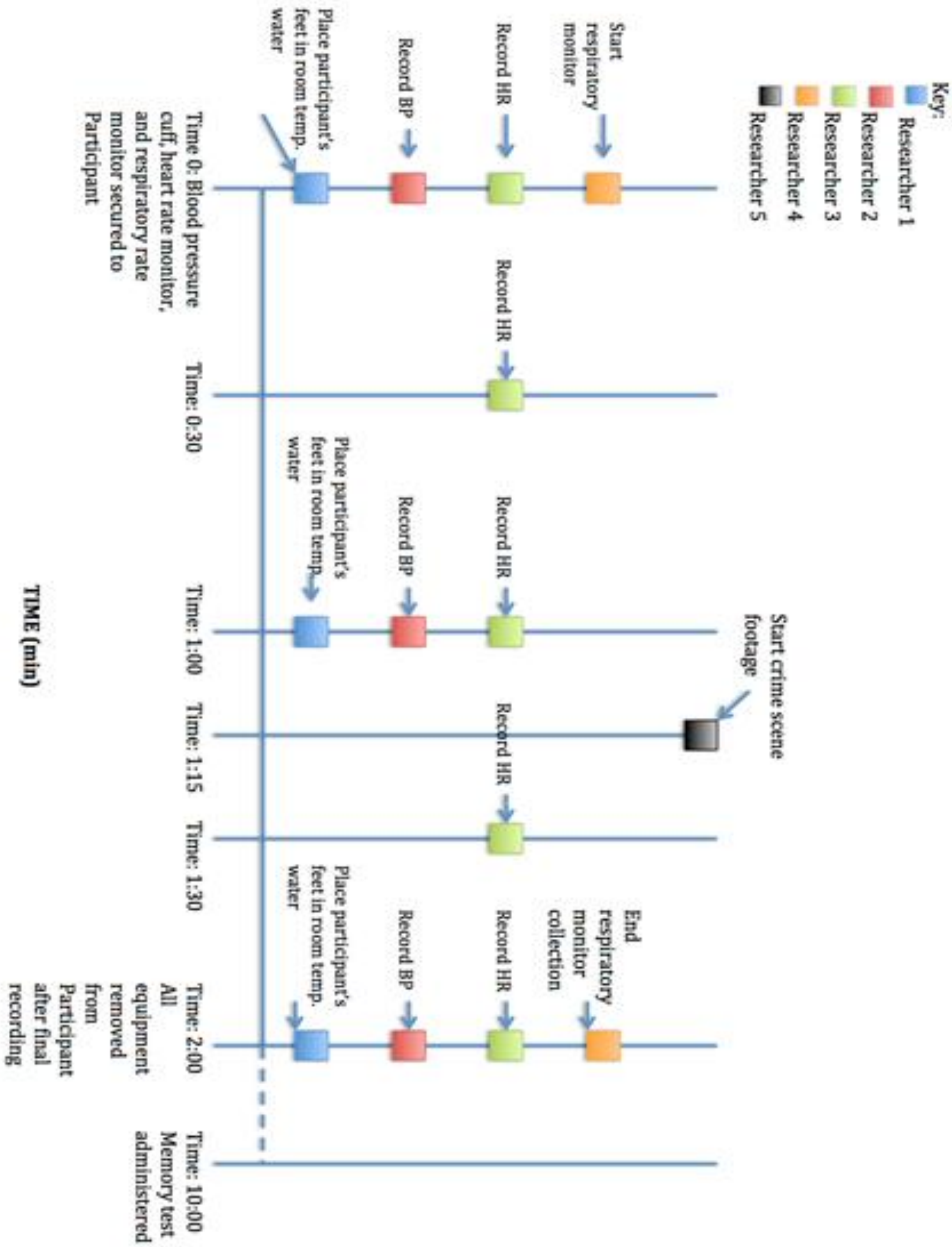


Figure 2: Procedural timeline for the control group

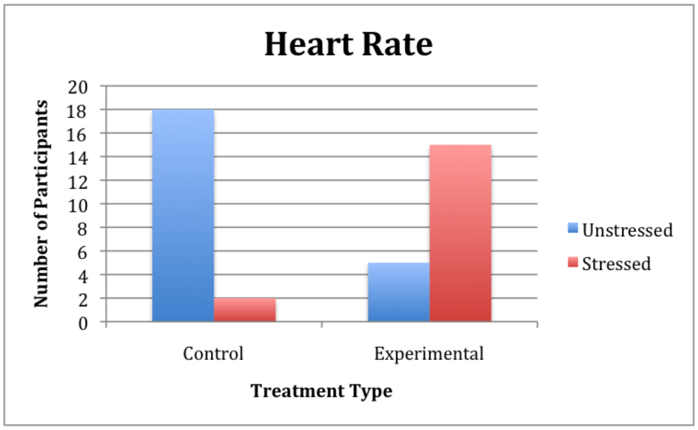


Figure 3. A higher number of participants in the experimental group (n= 15) reached stress threshold heart rate levels than in the control group (n= 2.)

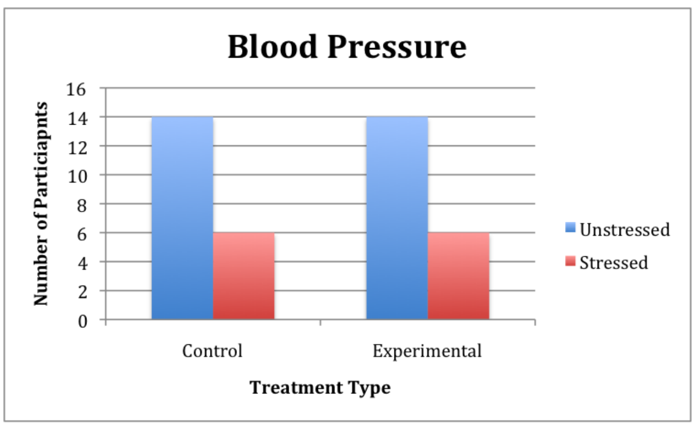


Figure 4. The same number of participants in both the experimental group (n= 6) and control group (n= 6) reached stress threshold blood pressure levels.

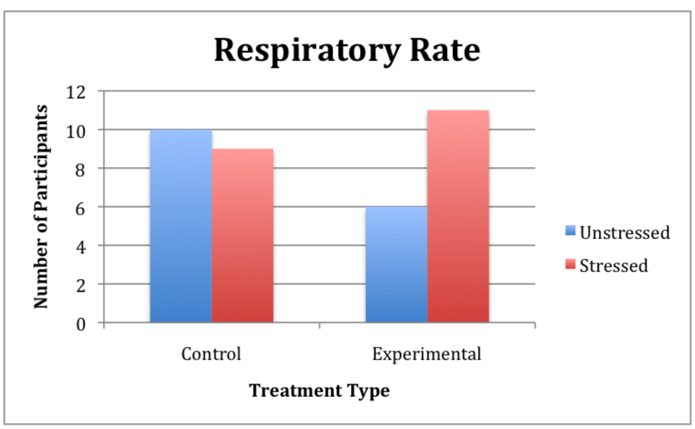


Figure 5. A higher number of participants in the experimental group (n= 11) reached stress threshold respiratory rate levels than in the control group (n= 9.)

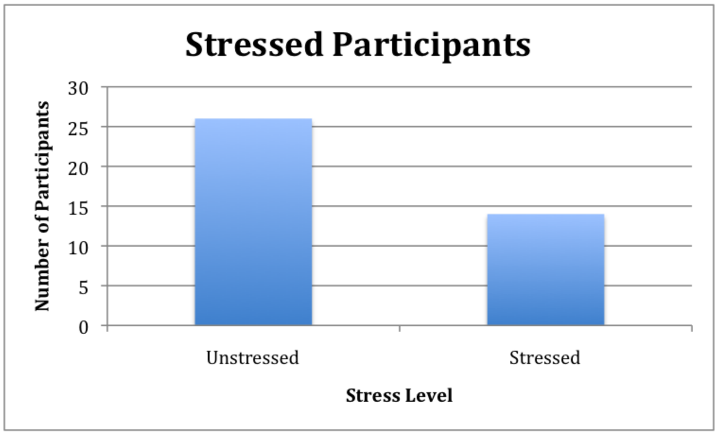


Figure 6. There were a higher number of unstressed participants (n= 26) in both experimental and control groups than there were stressed participants (n= 14.)

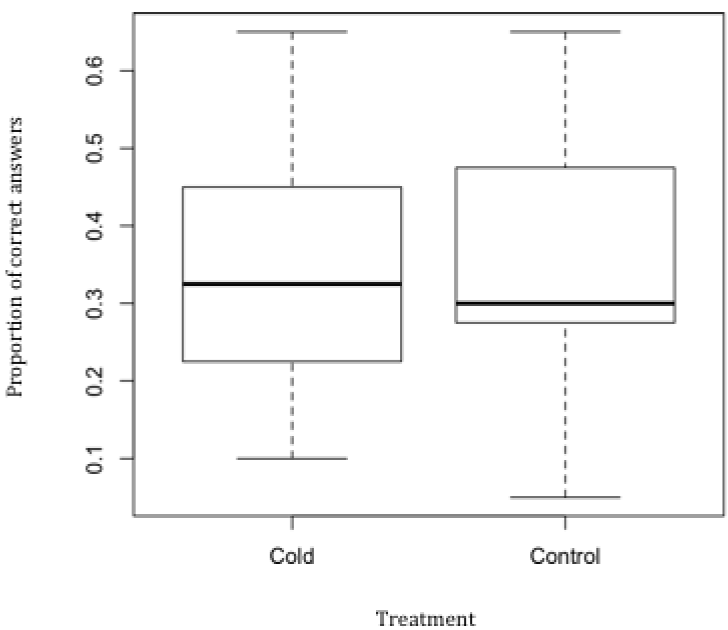


Figure 7. There is no significant difference in the average proportion of correct answers on the memory test between the experimental treatment (n=0.33) and the control treatment (n=0.3, t=-0.152, df= 36.527, p-value= 0.88.)

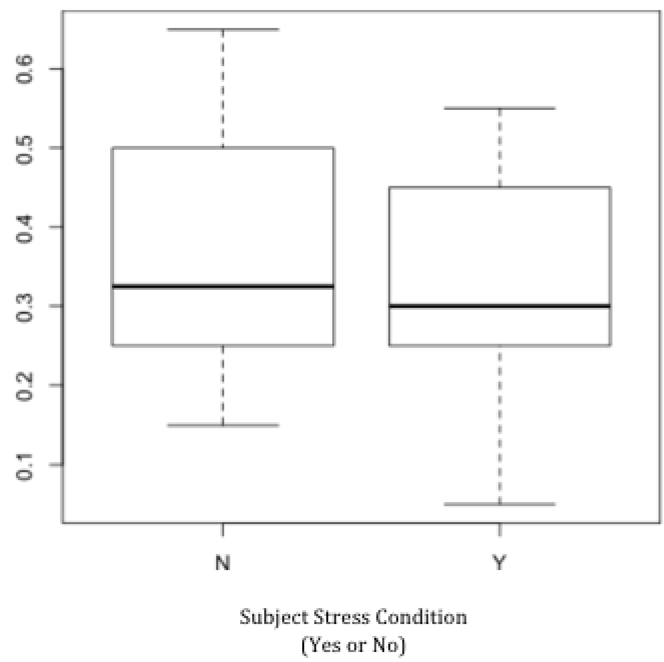


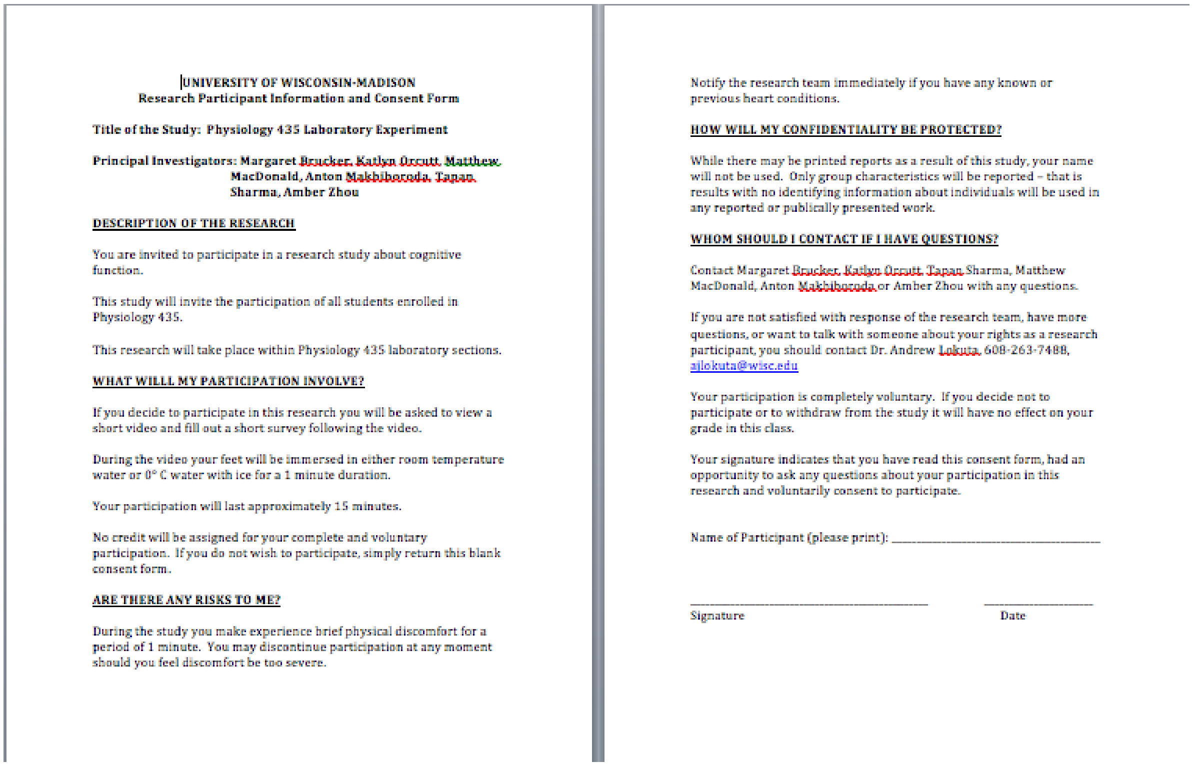
Figure 8. There is no significant difference in the average proportion of correct answers on the memory test between unstressed participants (n=0.33) and stressed participants (n=0.3, t=0.9268, df= 26.157, p-value=0.3625.)

Appendices

If you don't know the answer, please say "don't know".

1. Describe the color of the t-shirt of the person who threw the first punch.
2. What color top is the female customer wearing?
3. What is the gender of the cashier?
4. What kind of bottoms is the man who threw the first punch wearing?
5. What was the maximum number of people in the store?
6. What was the color of the liquid that had spilled during the fight?
7. What was the date of this video?
8. Did the person who first got punched punch back?
9. What color is the countertop of the store?
10. Did the victim have any items in his hand?
11. How many ethnicities are present in the video at one time?
12. Who calls 911?
13. How long is the person with the backpack in the video? If you did not see him, leave blank.
14. Describe the assailant's hair.
15. What purchase is made in the store during the fight?
16. Where is the victim hit with the first punch?
17. How many bystanders (excluding victim, assailant, and cashier) remain for the duration of the fight?
18. In addition to each other, who do both the victim and assailant explicitly talk to before the fight begins?
19. What is the hairstyle of the cashier?
20. What is the gender of the first person who runs out after the first punch is thrown?

Appendix A. Written memory recollection test that will be given to subjects after undergoing cold pressor test-derivative.



UNIVERSITY OF WISCONSIN-MADISON
Research Participant Information and Consent Form

Title of the Study: Physiology 435 Laboratory Experiment

Principal Investigators: Margaret Brucker, Katlyn Orcutt, Matthew MacDonald, Anton Makhiboroda, Tapan Sharma, Amber Zhou

DESCRIPTION OF THE RESEARCH

You are invited to participate in a research study about cognitive function.

This study will invite the participation of all students enrolled in Physiology 435.

This research will take place within Physiology 435 laboratory sections.

WHAT WILLL MY PARTICIPATION INVOLVE?

If you decide to participate in this research you will be asked to view a short video and fill out a short survey following the video.

During the video your feet will be immersed in either room temperature water or 0° C water with ice for a 1 minute duration.

Your participation will last approximately 15 minutes.

No credit will be assigned for your complete and voluntary participation. If you do not wish to participate, simply return this blank consent form.

ARE THERE ANY RISKS TO ME?

During the study you make experience brief physical discomfort for a period of 1 minute. You may discontinue participation at any moment should you feel discomfort be too severe.

Notify the research team immediately if you have any known or previous heart conditions.

HOW WILL MY CONFIDENTIALITY BE PROTECTED?

While there may be printed reports as a result of this study, your name will not be used. Only group characteristics will be reported – that is results with no identifying information about individuals will be used in any reported or publically presented work.

WHOM SHOULD I CONTACT IF I HAVE QUESTIONS?

Contact Margaret Brucker, Katlyn Orcutt, Tapan Sharma, Matthew MacDonald, Anton Makhiboroda or Amber Zhou with any questions.

If you are not satisfied with response of the research team, have more questions, or want to talk with someone about your rights as a research participant, you should contact Dr. Andrew Lokuta, 608-263-7488, ajlokuta@wisc.edu

Your participation is completely voluntary. If you decide not to participate or to withdraw from the study it will have no effect on your grade in this class.

Your signature indicates that you have read this consent form, had an opportunity to ask any questions about your participation in this research and voluntarily consent to participate.

Name of Participant (please print): ______________________

______________________ ______________
Signature Date

Appendix B. Consent form for subjects before testing.