

The Physiological Relationship of Sleep Duration with Blood Pressure, Heart Rate, and Reaction Time

Authored by: Justin Angles, Aria Kenarsary, Samantha Paddock, Zoey Schuck, Kaitlyn
Sonnentag

University of Wisconsin-Madison, Department of Physiology

Lab 601:Group 5

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ABSTRACT:

This experiment examined the relationship between sleep duration and systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, heart rate, and reaction time. We hypothesized that students who averaged fewer hours of sleep per night would have higher systolic, diastolic, and mean arterial blood pressure, faster heart rate, and slower reaction time than students who averaged more hours of sleep per night. In addition, we hypothesized that when the participants' average length of sleep varied from what they estimated getting on the first day of the study, their blood pressures, heart rates, and reaction times would be impacted. Finally, we hypothesized that external factors such as eating, exercising, consuming caffeine, and feeling hydrated would have no direct impact on reaction time. Participants' blood pressures, heart rates, and reaction times were measured on the first and the eighth day of the study, while a Google survey was used to record the self reports participants submitted, which indicated how many hours of sleep they slept each night. Results of the study demonstrated a significant correlation between the difference in sleep and change in systolic blood pressure. There was also a significant relationship recorded between day eight reaction time and perceived hydration.

INTRODUCTION:

During sleep, the brain actively sorts through information obtained during the day. Sleeping helps individuals stabilize knowledge and transfer knowledge from short term to long-term memories. Thus, one of the most important functions of sleep is consolidation of memories encoded when awake. Sleep and its various stages and components (i.e. Slow Wave Sleep and Rapid Eye Movement) play a crucial role in filtering memories and transforming them to long-term stores (Diekelmann, 2010). For this reason, getting the appropriate amount of sleep is vital for human beings. The American Academy of Sleep Medicine and Sleep Research Society reviewed over 5,000 scientific articles and determined that adults need to average at least seven hours of sleep per night to maintain optimal health (Watson *et al*, 2015). In addition, adults with an inadequate amount of sleep demonstrated a decrease in performance at work, an increase in errors of daily tasks, and an increase in daily accidents (Watson *et al*, 2015). Therefore, this study proposed that inadequate sleep is correlated negatively with physiological health. In

particular, our study examined physiological effects of moderate sleep deprivation in college students by measuring systolic blood pressure, diastolic blood pressure, heart rate, and reaction time and by calculating mean arterial blood pressure.

Insufficient sleep is a recurring phenomenon within the college and university population. According to research, college students are more likely to be sleep deprived than children and post-graduates because of numerous stress-provoking situations they face. In university students, stressful situations have been related to poor sleep quantity and quality (Galambos *et al*, 2009). College students are on the path to becoming more independent, and due to their lack of experience, they often face novel challenging situations that could be highly stressful. Thus, the combination of all these stressful challenges and work or school-related activities often force them to get less hours of sleep than they did in prior years of schooling (Galambos *et al*, 2009). Consequently, we believed that it was important to study the relationship of sleep duration in college students with five physiological parameters: systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, reaction time, and heart rate.

Blood Pressure:

For the last couple of years, scientists have hypothesized that there are links between sleep duration and cardiovascular health. One facet that many researchers have looked at is blood pressure (BP). A study from 2013 found that lack of sleep is associated with higher BP in children and adolescents (Au *et al*, 2013). In another study, Gangwisch and colleagues (2006) determined that there is a relationship between amount of sleep and hypertension in adults. Gangwisch and colleagues (2006) found that participants that reported sleeping five hours or less per night were found to be 60% more likely to develop hypertension than participants that

reported sleeping 6 to 8 hours per night (Gangwisch *et al*, 2006). The results demonstrated that short sleep duration correlates with an increased risk of developing high BP.

The link between BP and sleep duration has not been extensively researched in college students; unlike children and adults. Thus, we believed it was important to extend the previous research to other age groups to discuss an aspect of life (sleep duration) that could affect BP. This study aimed to bridge the gap between age groups and possibly provide evidence that shows sleep habits can affect overall cardiovascular health. We took each participant's systolic BP and diastolic BP on the first day of the study and one week later using an automatic blood pressure cuff. Then, we used the systolic and diastolic BP measurements to calculate mean arterial blood pressure (MABP) for day one and day eight.

Heart Rate:

Besides blood pressure, another facet of cardiovascular wellness is heart rate. The National Institute of Health (NIH) states that a healthy heart rate (HR) ranges from 60-100 beats per minute (bpm). In a 2011 study, researchers looked at how partial sleep deprivation in healthy male volunteers could lead to a decreased HR (Dettoni *et al*, 2011). They found that after five nights of sleep deprivation (less than five hours of sleep per night), there were no significant changes in HR or BP (Dettoni *et al*, 2011). In addition, the results demonstrated that acute sleep deprivation does not affect the HR of adult males. However, this 2011 study only researched 13 adult males and looked at acute sleep deprivation, four hours of sleep for five nights (Dettoni *et al*, 2011).

Our study focused on moderate sleep deprivation in college students rather than acute sleep deprivation. We believed that over longer periods of time, lack of sleep in college-aged students would show an increase in HR due to the negative cardiovascular effects sleep

deprivation is known to have. We also believe that having a sample size of more than thirteen individuals, in contrast to the Dettoni *et al* (2011), would allow us to obtain significant results.

Reaction Time:

Another measurement that has been studied in relation to amount of sleep is reaction time (RT). A study examined saccadic RT on a visual target to determine if sleep would improve this process (Gais *et al*, 2008). The results showed that when participants were allowed to sleep between RT assessments, their RT improved significantly (Gais *et al.*, 2008). A second test demonstrated that if sleep-deprived individuals were allowed to sleep after their first RT test, their second RT improved (Gais *et al.*, 2008). These results demonstrate the importance of adequate sleep for reaction times.

Since complete sleep deprivation over a short period of time has been determined to have a profound effect on reaction time, we investigated if sleeping less would also negatively affect an individual's' reaction time.

We hypothesized that college students who receive less sleep per night would have a slower reaction time, higher resting heart rate, and higher systolic, diastolic, and mean arterial blood pressure compared to their peers who received more hours of sleep per night. We further hypothesized that students who average less hours of sleep per night than what they originally predicted would have slower RT, higher systolic, diastolic, and mean arterial BP, and higher HR on day eight of the study compared to day one. Lastly, we hypothesized that external factors such as eating, exercising, and having caffeine before the measurement were taken, and feeling hydrated at the time the measurement was taken would have no direct impact on reaction time.

MATERIALS AND METHODS:

Participants consisted of 34 (19 females; 15 males) University of Wisconsin-Madison students enrolled in Physiology 435. The participants' ages ranged from 20 to 26 (\bar{x} =21.74, SD =1.08). Participants received no compensation for their time.

Before participation in the study, participants were required to read and sign a consent form that informed them that the research was focused on the relationship between amount of sleep and RT, systolic BP, diastolic BP, MABP, and HR. In addition, the consent form highlighted that participation would require an eight day commitment. This commitment consisted of a weeklong Google Form survey sent via email to all participants. This self reporting daily survey asked participants to state their names and how many hours of sleep they obtained each night of the study. The timeline for each participant is laid out in Figure 1. Pre-existing health conditions were not taken into account for this study due to lack of resources and time.

On day one of the study, participants completed a brief survey. Participants were asked to self report their name, sex, age, estimated average number of hours of sleep each night, and email address. In addition, participants were asked to self report whether or not they had exercised, eaten, consumed caffeine, and felt well hydrated that morning. The questions were asked in a yes/no format. These questions were asked to determine if these external factors had any direct impact on reaction time.

To obtain baseline measurements, participants' blood pressures (BP) and heart rates (HR) were measured simultaneously using an automatic blood pressure cuff (OMRON 10 series+, BP791IT, Omron Healthcare, INC. Lake Forest, IL, USA) on day one of the study. As a positive control, the experimenters tested the automatic blood pressure cuff to demonstrate the equipment was working properly. Participants' systolic and diastolic BPs were recorded in millimeters of

mercury (mmHg), and HRs were recorded in beats per minute (bpm). Blood pressure and heart rate were measured first to ensure they were not elevated due to the somewhat competitive nature of the RT test.

Following BP and HR recordings, reaction time was measured using BIOPAC Student Laboratory System (BSL 4 software, MP36 hardware, BIOPAC Systems, Inc., Goleta, CA, USA). To record each participant's reaction time, the BIOPAC Student Laboratory System, computer system (Dell Inspiron 530 computer), BIOPAC Hand Switch (SS10L, BIOPAC Systems, Inc., Goleta, CA, USA), and BIOPAC Headphones (OUT1, BIOPAC Systems, Inc., Goleta, CA, USA) were used. Prior to running the RT test, the BIOPAC device was calibrated for each participant as a positive control. Using this BIOPAC set-up, a random interval, dominant hand auditory RT test was performed for each participant. This specific auditory reaction test was used for data collection since it was the most accurate reaction time test available in the Physiology 435 laboratory. During each recording, participants were asked to sit down on a chair in front of the computer and wear the BIOPAC headphones. They were instructed to hold the BIOPAC hand switch in their dominant hand, close their eyes, and press the button on top of the hand switch when they heard an auditory stimulus played at random intervals. During each participant's' reaction test responses to each auditory stimulus were recorded in seconds and the average of their reaction time was calculated by the computer. The experimenters recorded the average reaction time for each participant.

On day eight of the study the participants' were again asked to self report whether or not they had exercised, eaten, consumed caffeine, and felt well hydrated that morning. Their HRs, RTs, and BPs were obtained using the same procedure as stated above and these were considered the treatment values.

Once all data was recorded, systolic and diastolic BP were used to calculate the mean arterial blood pressure (MABP) for day one and day eight for each participant. To calculate this, the following equation was used: $MABP = \text{diastolic BP} + \frac{1}{3}(\text{systolic BP} - \text{diastolic BP})$.

Statistical Analysis:

The data was analyzed with the assistance of a UW-Madison undergraduate statistician using R software. Using a linear regression analysis of the data, graphs were generated and used to depict the correlation between each parameter (RT, systolic BP, diastolic BP, MABP, and HR) on day eight of the study and average hours of sleep per night for a one-week period. Day eight measurements were used as opposed to day one measurements because we could analyze the day eight data against the week long sleep survey.

A second linear regression was generated to analyze the relationship between the difference (day eight - day one) in each parameter (RT, systolic BP, diastolic BP, MABP, and HR) and the difference of sleep. The difference in sleep for this study was calculated by subtracting the self reported, estimated amount of sleep participants stated in the survey they were given on day one of the study from the participant's average amount of sleep recorded throughout the week (recorded average hours of sleep - estimated average hours of sleep). We assumed that the self reported estimated average hours of sleep per night was an accurate value of each individual's average hours of sleep each night during a normal week. Therefore, we used this self reported estimate value as the participant's baseline.

Furthermore, ANOVA tests were performed to examine the relationships between RT and the external factors on day one and day eight of the study. The external factors included whether or not the individual had exercised, eaten, drank caffeine, or felt hydrated.

RESULTS:

Subject Characteristics:

There were 34 (19 females; 15 males) participants in the study whose ages ranged from 20-26 (\bar{x} =21.74, SD=1.08). The average hours of sleep per night ranged from 5.00 to 8.00 hours (\bar{x} =6.95, SD=0.69). The difference in sleep (average amount of sleep - estimated amount of sleep) ranged from -1.29 to 0.86 hours (\bar{x} = -0.02, SD=0.58).

Reaction Time:

The participants' day eight reaction time (RT) ranged from 0.176 to 0.362 seconds (\bar{x} =0.233, SD=0.041). The relationship between average hours of sleep per night and day eight RT showed no significant correlation (p -value=0.833) (Figure 2A). The change in RT from day one to day eight ranged from -0.066 to 0.067 seconds (\bar{x} =0.008, SD=0.029). The relationship between the difference in sleep and the change in RT showed no significant correlation (p -value=0.518) (Figure 3A).

From the day one measurements, there were no significant correlations between RT and exercising (p -value=0.2271), eating (p -value=0.4836), caffeine consumption (p -value=0.4211) or hydration (p -value=0.6184) (Figure 4A-D). From the day eight measurements, there were no significant correlations between RT and exercising (p -value=0.507), eating (p -value=0.333), or caffeine consumption (p -value=0.102) (Figures 5A-B, D). There was a significant correlation between RT and feeling hydrated on day eight (p -value=0.026) (Figures 5C).

Systolic Blood Pressure:

The participants' day eight systolic BP ranged from 94 to 134 mmHg (\bar{x} =112.88, SD=10.68). The relationship between average hours of sleep per night and systolic BP showed no significant correlation (p -value=0.235) (Figure 2B). The change in systolic BP from day one to day eight ranged from -19 to 23 mmHg (\bar{x} =0.09, SD=9.69). The relationship between the

difference in sleep and the change in systolic BP showed a significant correlation (p -value=0.018) (Figure 3B).

Diastolic Blood Pressure:

The participants' day eight diastolic BP ranged from 63 to 89 mmHg (\bar{x} =75.56, SD=8.39). The relationship between average hours of sleep per night and day eight diastolic BP showed no significant correlation (p -value=0.139) (Figure 2C). The change in diastolic BP from day one to day eight ranged from -27 to 17 mmHg (\bar{x} =-0.82, SD=9.92). The relationship between the difference in sleep and the change in diastolic BP showed no significant correlation (p -value=0.473) (Figure 3C).

Mean Arterial Blood Pressure:

The participants' day eight MABP ranged from 72.33 to 102.33 mmHg (\bar{x} =88, SD=8.16). The relationship between average hours of sleep per night and day eight MABP showed no significant correlation (p -value=0.124) (Figure 2D). The change in MABP from day one to day eight ranged from -13.33 to 19.67 mmHg (\bar{x} =0.52, SD=8.04). The relationship between the difference in sleep and the change in MABP showed no significant correlation (p -value=0.126) (Figure 3D).

Heart Rate:

The participants' day eight HRs ranged from 53 to 105 beats per minute (\bar{x} =72.53, SD=13.50). The relationship between average hours of sleep per night and day eight HR showed no significant correlation (p -value=0.642) (Figure 2E). The change in HR from day one to day eight ranged from -13 to 20 beats per minute (\bar{x} =0.18, SD=8.10). The relationship between the difference in sleep and the change in HR showed no significant correlation (p -value=0.619) (Figure 3E).

DISCUSSION:

Initially, we hypothesized that college students who averaged less sleep per night would have slower reaction time, higher resting heart rate, and higher systolic, diastolic, and mean arterial blood pressure compared to their peers who averaged more hours of sleep per night. However, the results of this experiment did not support this hypothesis. Participants who averaged less hours of sleep were found to have similar reaction time, systolic, diastolic, and MABP, and heart rate compared to participants who averaged more hours of sleep per night (Figure 2A-E). Even though these relationships were not significant, there was an observable downward trend for for systolic, diastolic, and mean arterial BP. These trends suggest that significant results could possibly be produced in future experiments if larger sample sizes are used to increase the range of average hours slept. Overall, the results demonstrated that average hours of sleep per night does not have a significant effect on participant's reaction time, resting HR, systolic, diastolic, or mean arterial BP.

We further hypothesized that students who averaged less hours of sleep per night than what they originally predicted would have slower RT, higher systolic, diastolic, and mean arterial BP, and higher HR on day eight of the study compared to day one of the study. All but one of these hypotheses were rejected. The results demonstrated that the difference in sleep caused no change in RT, HR, diastolic BP, or MABP on day eight compared to day one of the study (Figure 3A, 3C-E). However, a statistically significant correlation was found between the difference in sleep and the change in systolic BP (Figure 3B, p -value=0.018). This demonstrated that participants who reported sleeping more than they estimated on day one tended to have a decrease in their systolic blood pressure from day one to day eight. This relationship could have

been due to the participants sleeping more than normal over the week of the study, which could have caused their systolic blood pressure to lower.

Although a significant relationship was found between systolic BP and the difference in sleep, no significant relationship was found between diastolic BP and difference in sleep nor MABP and difference in sleep. This finding may suggest that heightened systolic BP due to inadequate sleep could be associated with the future risk of isolated systolic hypertension. Specifically, it is possible that reduced sleep duration may correlate with the hardening of arteries which can eventually lead to arteriosclerosis and isolated systolic hypertension. This relationship could also demonstrate that diastolic BP is less susceptible to fluctuations in sleep and that changes in diastolic BP requires a prolonged period of sleep deprivation to be elevated significantly.

Despite the fact that this study did not find a significant correlation between MABP and difference of sleep, a negative trend was observed (Figure 3D). The graph demonstrates that sleep duration increases, MABP decreases. A similar study with more participants could provide a larger range in the average sleep duration and reveal a significant relationship between the number of hours slept and MABP. This could be incredibly important to show how sleep can possibly affect MABP in college students, which would affect their long term cardiovascular health. One reason why the relationship between MABP and difference in sleep may not have been significant was because diastolic BP, which has more of a quantitative effect on the calculated MABP than systolic BP, did not show a significant relationship. However, the trend that was observed could be due to the fact that moderate sleep deprivation could be negatively impacting college student's MABP and, therefore, their future cardiovascular health. Previous research has shown that adequate amount of sleep is necessary to maintain optimal health

(Watson *et al.*, 2015) and that lack of sleep could lead to hypertension (Gangwisch *et al.*, 2006), so it is plausible that the amount of sleep one gets has an effect on one's MABP as well. Future studies need to take place in order to determine if the results can be replicated. If this result proves to be consistent throughout future studies, then the mechanisms that underlie this relationship should be further investigated.

In addition, we analyzed the data to see if there was any significant correlation between participant's RT and four external factors including whether or not they had exercised, eaten, consumed caffeine, or felt hydrated the morning of the study. The results demonstrated there was only a significant correlation between the participant's RT and how hydrated they felt the morning of day eight of the study (p -value=0.026). Participants who felt as though they were well hydrated tended to display quicker reaction times (Figure 5C). Participants who reported feeling less hydrated displayed slower reaction times. This result adds further support to previous studies showing that hydration status affects cognitive tasks as well as providing a new approach to study this relationship. Previous studies have shown that hydration plays an important role in the perceived tiredness and alertness (Szinnai *et al.*, 2005), and also indicated that there is a significant correlation between cognitive dysfunction and the severity of dehydration. Subjects under varying degrees of dehydration showed impairment in various cognitive tasks including mathematical ability, short-term memory and visuomotor function when their body fluid content decreased by 2% (Wilson *et al.*, 2003)

There is no consensus on the degree and duration of dehydration that would cause significant decrements in mental alertness and attention during various cognitive tasks. Furthermore, the exact underlying mechanism by which dehydration impacts cognitive performance is not thoroughly understood. However, we know that, unlike many other stressors,

dehydration is unique in a sense that it can change the amount of electrolytes that are available for neurotransmission in the brain and, therefore, can greatly alter neuronal activity (Lieberman, 2007).

There are various hormonal and cellular theories that have been proposed as how dehydration might lead to decreased cognitive performance. For instance, animal studies have identified neuronal mitochondrial damage, and glutamate hyper-transmission (resulting in excitotoxicity) in dehydrated rats (Wilson *et al.*, 2003). Furthermore, other studies demonstrated that there was an increase in cerebral nicotinamide adenine dinucleotide phosphate-diaphorase activity (nitric oxide synthase, NOS) with dehydration (Wilson *et al.*, 2003). NOS is an important enzyme found at most parts of the brain and is thought to play an important role in homeostatic preservation of cognitive function during dehydration (Wilson *et al.*, 2003).

The measurements taken on day one of the study yielded an insignificant relationship between RT and hydration status. Since day one and day eight produced conflicting results, additional studies investigating the relationship between reaction time and hydration should take place in order to determine whether or not there is a true relationship between hydration status and reaction time.

If this study were to be replicated, there are some major limitations that would need to be addressed. First, this study's population was limited to University of Wisconsin- Madison students enrolled in Physiology 435. Although this sample was unique, the sample was not a good representation of the general population of young adults in college. Future studies would require a larger, randomized sample to represent this population. Another limitation of this study was the inability of the experimenters to control the sleep duration of the participants. Since this study allowed participants to sleep normal hours, the data revealed average hours of sleep per

night ranged from 5.00 to 8.00 hours (\bar{x} =6.95, SD =0.69). Controlled sleep duration experiments could provide a wider range of data to analyze with the potential of more significant results.

Another limitation was that students were not asked about pre-existing health conditions that could have affected the amount of sleep one got, or the participant's' RT, HR, systolic, diastolic, and mean arterial BP. Some of these pre-existing health conditions that should be inquired about in future studies include but are not limited to sleep disorders, anxiety, depression, hypertension, congenital heart defects, and hearing problems. Furthermore, since participants were asked to self report their caffeine consumption, exercise participation, food consumption, and hydration in a yes/no format, the data was not comprehensive enough to be further analyzed. Further studies should either control for these variables or collect detailed measurements of participants' water, caffeine, and caloric intake. In addition, future experimenters should account for the duration, intensity, and type of physical activity each participant completed on the day of the study and the individual differences of participants in regards to these factors such parameters. These types of detail would allow future researchers to determine if differences in physiological measures were due to the amount of sleep the participants slept each night or other external factors.

Another important source of error in our study could be due to many uncontrolled variables that could have affected performance in the RT task. For instance we know that dehydration was correlated negatively with the participants' reaction time. There are many variables that could have affected the hydration of the participants that were not controlled for. For instance, caffeine is a diuretic and could have directly impacted the participants' hydration during the study, which in turn, could have affected their RTs. However, there were difficulties associated with the number of parameters that we could control for in our study. As it was mentioned previously, finding subjects that could meet all the criteria would have been a very

cumbersome task in the limited amount of time and resources that we had available to perform the study. This source of error could be why the results only demonstrated significance between RT and perceived hydration on day eight and not on day one.

According to statistical analysis, there was no significant relationship between average sleep duration per night and RT, systolic BP, diastolic BP, MABP, and HR. There was also insignificant evidence for any relationship between difference in sleep with the changes in RT, diastolic BP, MABP and HR. However, the results portrayed a trend that students who sleep more tend to have a lower MABP which could indicate future cardiovascular health. Our results also found a significant relationship between the difference in sleep and change in systolic BP, as well as perceived hydration and reaction time. However, more research needs to be conducted on these relationships to understand them. We believe that understanding the physiological effects of moderate sleep deprivation is important and that researching the link between these factors could provide greater insight on the overall health of the college-aged population.

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FIGURES AND LEGENDS:

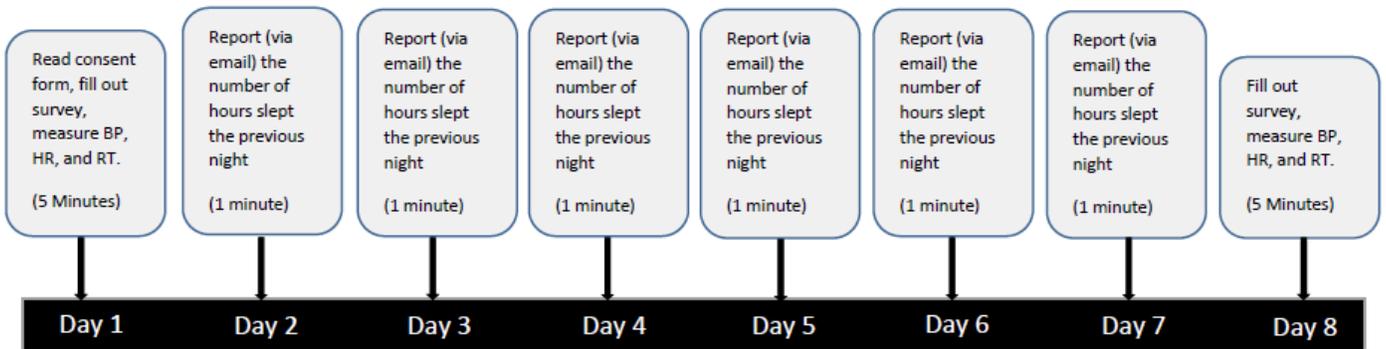


Figure 1: Timeline of the Study

Figure 1: This figure is a timeline of the study showing what each participant did each day and how much time was required each day.

Figure 2: Relationship Between average of hours slept vs Physiological Factors

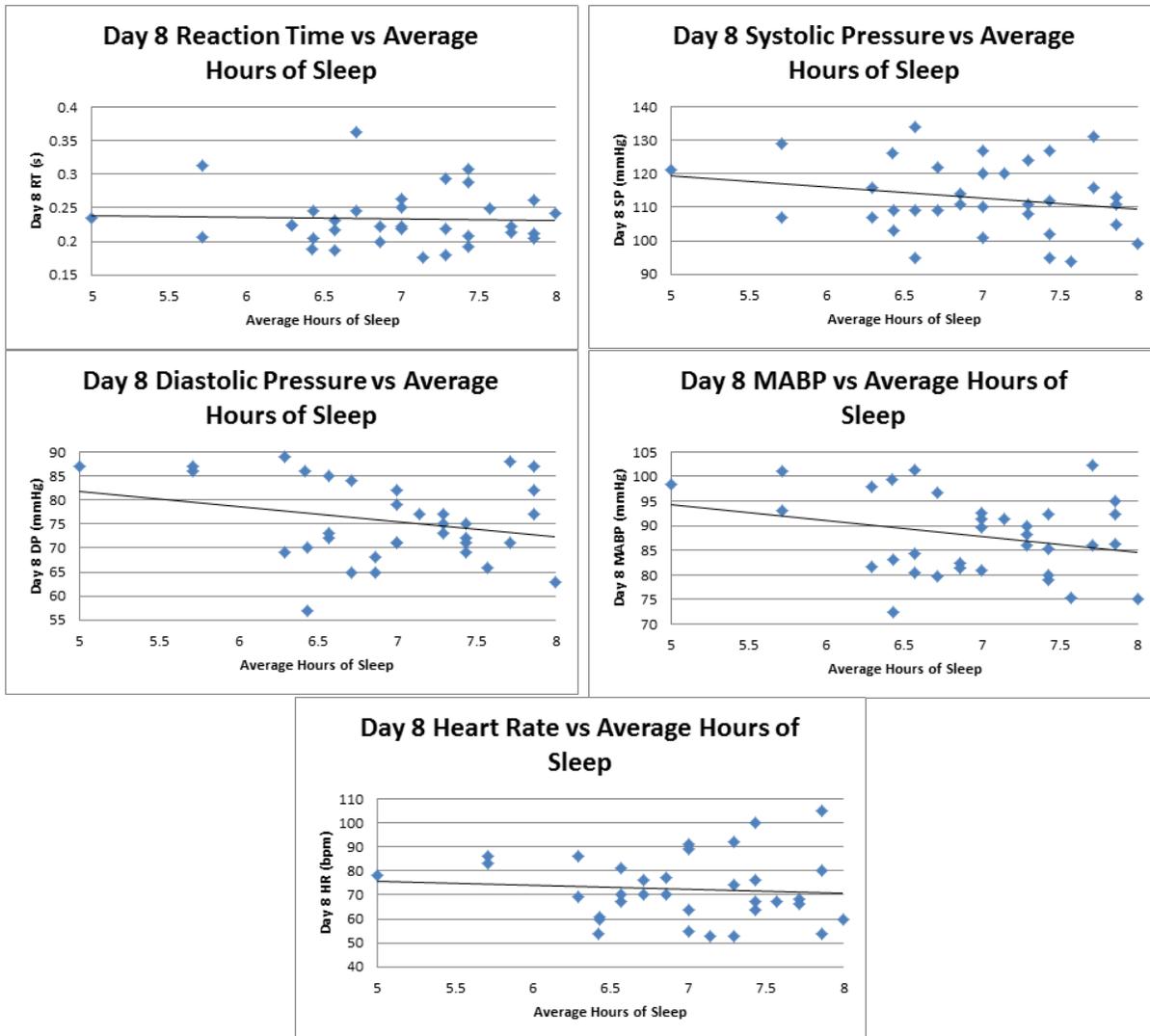


Figure 2 (A-E): These graphs show linear regressions of average hours of sleep and for physiological variables on day eight. The average hours of sleep per night ranged from 5.00 to 8.00 hours ($\bar{x}=6.95$, $SD=0.69$).

- Graph shows the relationship between day eight RT ($\bar{x}=0.233$, $SD=0.041$) and average hours of sleep. There was no significant relationship (p -value=0.833)
- Graph shows the relationship between day eight systolic BP ($\bar{x}=112.88$, $SD=10.68$) and average hours of sleep. There was no significant relationship (p -value= 0.235)
- Graph shows the relationship between day eight diastolic BP ($\bar{x}=75.56$, $SD=8.39$) and average hours of sleep. There was no significant relationship (p -value=0.139).
- Graph shows the relationship between day eight MABP ($\bar{x}=88$, $SD=8.16$) and average hours of sleep. There was no significant relationship (p -value=0.124).
- Graph shows the relationship between day eight HR ($\bar{x}=72.53$, $SD=13.50$) and average hours of sleep. There was no significant relationship (p -value=0.642).

Figure 3: Relationship Between Difference in Sleep and the change in Physiological Factors

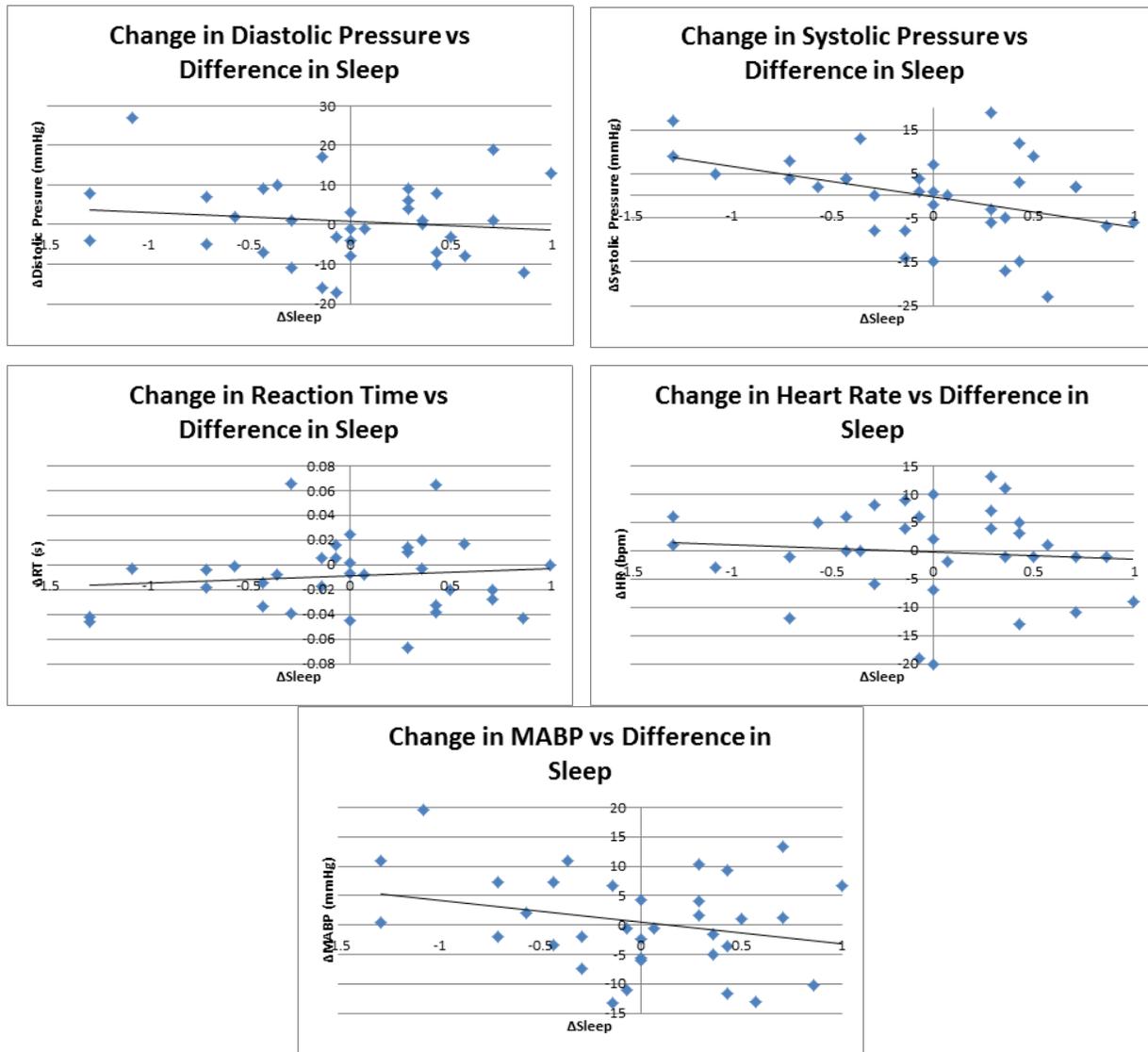


Figure 3 (A-E): These graphs show the linear regression of difference in sleep (average amount of sleep - estimated amount of sleep) and the change in the measured physiological variables. The difference in sleep ranged from -1.29 to 0.83 hours ($\bar{x} = -0.017$, $SD = 0.58$).

- The relationship between change in RT from day one to day eight ($\bar{x} = 0.008$, $SD = 0.029$) and difference in sleep. There was no significant relationship ($p\text{-value} = 0.518$).
- The relationship between change in systolic BP from day one to day eight ($\bar{x} = 0.09$, $SD = 9.69$) and difference in sleep. There was a significant relationship ($p\text{-value} = 0.018$).
- The relationship between change in diastolic BP from day one to day eight ($\bar{x} = -0.82$, $SD = 9.92$) and difference in sleep. There was no significant relationship ($p\text{-value} = 0.473$).
- The relationship between change in MABP from day one to day eight ($\bar{x} = 0.52$, $SD = 8.04$) and difference in sleep. There was no significant relationship ($p\text{-value} = 0.126$).

E) The relationship between change in HR from day one to day eight ($\bar{x}=0.18$, $SD=8.10$) and difference in sleep. There was no significant relationship (p -value=0.619).

Figure 4: Relationship Between Exercising, Eating, being Hydrated, or being Caffeinated and the Day One Reaction Time

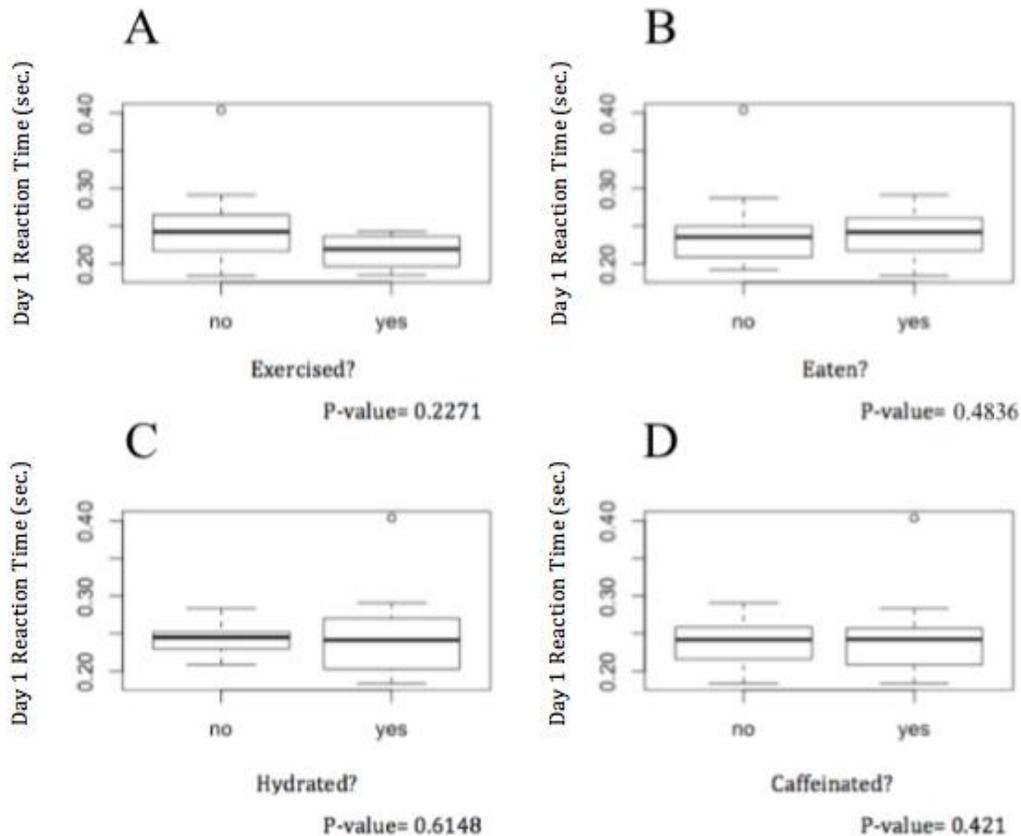


Figure 4 (A-D): These graphs show an ANOVA of exercising, eating, being hydrated, or being caffeinated and the day eight reaction time.

- A) This graph shows whether exercising the morning of the study affected the day eight reaction time. This relationship was insignificant with a p -value (0.227).
- B) This graph shows whether eating on the morning of the study had an impact on the day eight reaction time. This relationship yielded an insignificant p -value (0.484).
- C) This graph shows the relationship between hydration and the day eight reaction time, which yielded a significant p -value (0.615).
- D) This graph shows the relationship between caffeine and the day eight reaction time. This relationship yielded an insignificant p -value (0.421).

Figure 5: Relationship Between Exercising, Eating, being Hydrated, or being Caffeinated and the Day Eight Reaction Time

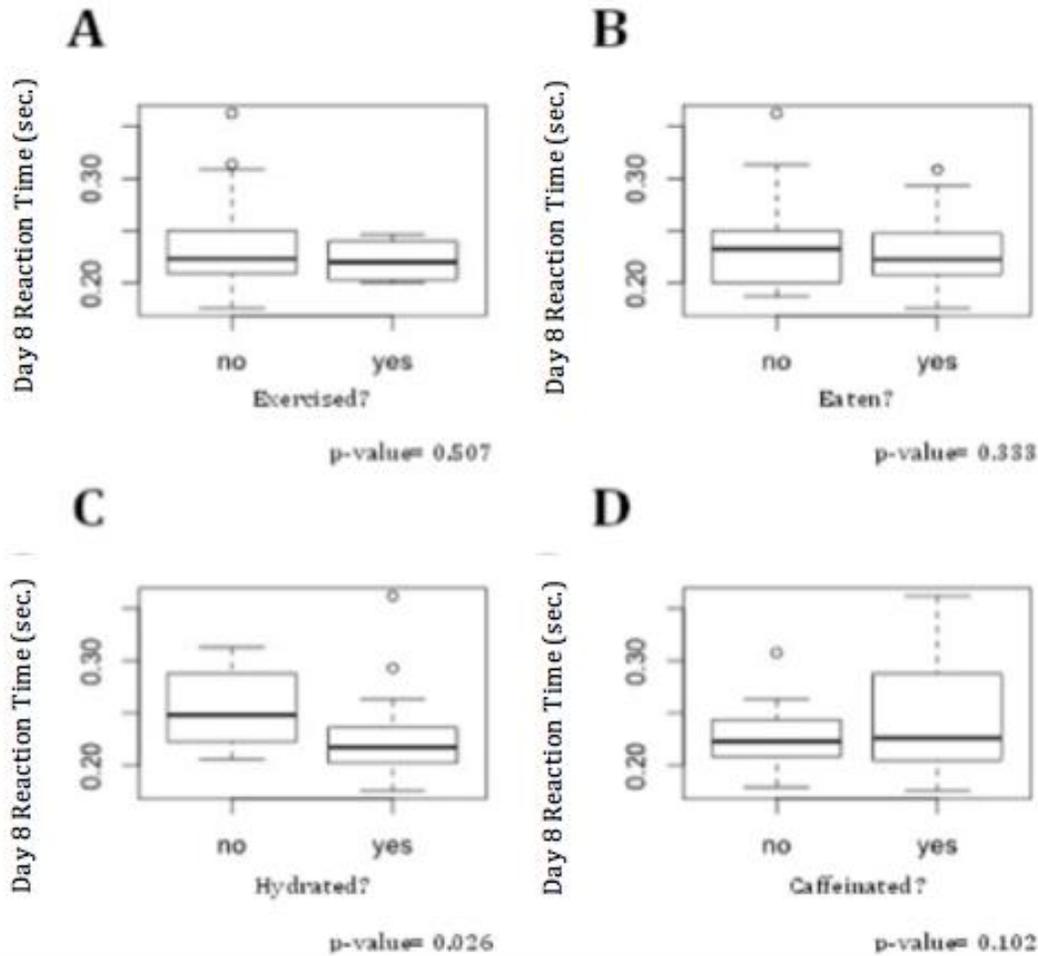


Figure 5 (A-D): These graphs show an ANOVA of exercising, eating, being hydrated, or being caffeinated and the day eight reaction time.

- A) This graph shows whether exercising the morning of the study affected the day eight reaction time. This relationship was insignificant (p -value=0.507).
- B) This graph shows whether eating on the morning of the study had an impact on the day eight reaction time. This relationship yielded an insignificant p -value (0.333).
- C) This graph shows the relationship between hydration and the day eight reaction time, which yielded a significant p -value (0.026).
- D) This graph shows the relationship between caffeine and the day eight reaction time. This relationship yielded an insignificant p -value (0.102).