Spring 2021

Analysis of Circadian Rhythm Irregularities on the Health of Shift Workers

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Honors Research Project

Submitted to

The Williams Honors College

The University of Akron

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Date: 4/19/21

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Abstract:

Circadian rhythms involve the natural 24 wake-sleep cycle that governs proper maintenance of biological sleep schedules. Circadian rhythm disruption has been associated with health issues such as diabetes, obesity, and mental health disorders such as bipolar and sleep disorders. In this study, a survey method was utilized in order to analyze specific population demographics to determine whether working day or night shifts predisposed individuals to health issues due to disruptions in their circadian rhythms. This study was also carried out to observe popular coping strategies among night shift workers. Of all the variables tested comparing the difference between day and night shift workers, all came back insignificant, with P values greater than the $\alpha = 0.05$. Results are as followed; Uninterrupted sleep ($P = 0.390$), average hours of sleep ($P=0.190$), weight ($P=0.270$), depression ($P=0.300$), and hopelessness ($P=0.051$). General trends were also developed based upon analyzing the data. There was a range of coping strategies employed by the night shift workers. However, the most popular strategy was for night shift workers to eat snacks during their respective shifts. In conclusion, the hypothesis posed for this study could not be supported based upon the results gathered.

Introduction:

A diverse range of organisms, from bacteria to mammals, have evolved under similar selective pressures to adapt to changes in time that accompany changes in the external environment. On a universal scale, organisms follow a complex network of endogenous timekeeping over twenty-four hours (Chan et al. 2012). Circadian rhythms, which symbolize biological, behavioral, and physiological oscillatory changes that an organism experiences every day, control this timekeeping cycle (Vitaterna et al. 2001). Internalized circadian “clocks" in cells of tissues from the central and peripheral nervous systems characterize circadian rhythms (Koronowski & Sassone-Corsi 2021). Zeitgebers are agents that adjust internal rhythms to the external environment, which substantiates the idea of biological clocks being the enforcers of the timekeeping system (Potter et al. 2016). The light-dark cycle is the standard zeitgeber for many kinds of diurnal organisms, but other cues such as temperature, exercise, diet, and other lifestyle choices impact circadian rhythms and can act to regulate potential phase delays or advances (Vitaterna et al. 2001). By following a pattern of the geophysical light-dark cycle, one’s circadian rhythms tend to activate and take place at similar times and rates daily.

Zeitgebers can disrupt the consistency between the environment and the internal clock system via exposure to a zeitgeber at an inappropriate time. For example, an individual can go through phase delays and advances based on light exposure during “darkness hours” or, in layman's terms, the “night” (Vitaterna et al. 2001). So, terms such as “night owl” or “early bird” are not far-fetched categories when defining an individual’s sleeping habits. Early birds tend to wake up early, thus, their circadian rhythms are “ahead of schedule” as they have experienced phase advances (Vitaterna et al. 2001). On the other hand, night owls tend to stay awake into the late hours of the night and end up waking up much later. As such, night owls tend to have their circadian rhythms occur later on as they go through phase delays. Night owls expose themselves to light during hours of the night when there should be only darkness (Vitaterna et al. 2001). Thus, rhythmicity may not follow a perfect twenty-four-hour period exactly, but a period around that amount of time (Vitaterna et al. 2001).

The timekeeping system that works to support circadian rhythms operates via complex crosstalk amongst the body on multiple scales. The circadian network operates via the entrainment of the timekeeping system every day through the light-dark cycle via light itself, an essential photic cue (Chan et al. 2012). The anterior hypothalamus of the brain holds the suprachiasmatic nucleus (SCN), the structure that requires synchronization (Chan et al. 2012). The SCN houses the “master clock” or the central circadian pacemaker, that light synchronizes to communicate the regulation of daily circadian rhythms (Fisk et al. 2018). The eye is significant in that it establishes phototransduction as photoreceptors can process light. Subsequently, the excitation of the photosensitive retinal ganglion cells (pRGCs) from electrical signals of photoreceptors can lead to the firing of action potentials to the retinohypothalamic tract (Fisk et al. 2018). The tract projects to the SCN and allows synchronization of the rhythms to the external environment (Fisk et al. 2018). Intricate modulation of this communication takes place as some of these pRGCs express the photopigment melanopsin, which provides additional receptivity for light and phototransduction (Fisk et al. 2018). The retinohypothalamic tract projects these electrical signals to the SCN as well as to SCN adjacent areas of the brain via neuronal synaptic transmissions (Jagannath et al. 2017).

The general mechanisms of the phototransduction of electrical stimuli from the pRGCs and retinohypothalamic tract to the brain rely on transcriptional regulation via neuronal relationships. Specifically, the mechanisms take place on a neuron-to-neuron basis in the brain to modulate the master clock of the SCN. The SCN neurons and their astrocytic counterparts influence the transcription of pertinent genes involved in the functionality of clocks (Koronowski & Sassone-Corsi 2021). These early genes assign rhythms to a twenty-four-hour period (Potter et al. 2016). Astrocytes also appear to have their own timekeeping system, which subsequently influences the neurons of the SCN (Koronowski & Sassone-Corsi 2021). During the day, glutamate release from astrocytic cells declines, which decreases the potential release of another neurotransmitter, GABA, from presynaptic neurons (Koronowski & Sassone-Corsi 2021). GABA is an inhibitory neurotransmitter, so postsynaptic neurons can become temporarily depolarized to increase internal concentrations of calcium (Koronowski & Sassone-Corsi 2021). Calcium concentration increases and initiates a cascade of events, which results in cAMP production, followed by the formation of CREB (Koronowski & Sassone-Corsi 2021). An important transcription factor, CREB (cyclic AMP response element-binding protein), binds to DNA in the SCN neurons to activate transcription of essential per genes (Koronowski & Sassone-Corsi 2021). The night does not have photic cues such as light, thus the mechanism has an opposite effect. Specifically, this response causes the promotion of astrocytic glutamate release, which influences presynaptic neurons to release GABA (Koronowski & Sassone-Corsi 2021). Since there is a higher concentration of GABA in the synaptic cleft, there is a higher likelihood of postsynaptic receptors binding GABA, which inhibits potential depolarization of postsynaptic neurons (Koronowski & Sassone-Corsi 2021). As such, calcium concentrations would not increase, which would not lead to cAMP production (Koronowski & Sassone-Corsi 2021). CREB would not go on to influence the transcription of genes necessary for master clock synchronization in the SCN (Koronowski & Sassone-Corsi 2021). A mechanism like this is necessary for assisting in the general entrainment of the master clock to the external environment by light every day (Koronowski & Sassone-Corsi 2021). However, this mechanism also works on fine-tuning the expression of genes that play a role in modulating aspects of circadian rhythms such as the phase and how long they will occur (Jagannath et al. 2017). Specifically, the increased expression of per1 and per2 genes leads to the increased amounts of PER1 and PER2 proteins (Jagannath et al. 2017). These proteins can influence phase advances and delays of the master clock during specific times of the day like morning or evening (Jagannath et al. 2017). Though this part of the timekeeping system seems long-lasting, the reality is that entrainment of any clock by light is temporary (Jagannath et al. 2017). Even if an organism were to stay in the same lighted conditions, the modulation of the clock by light from the environment would gradually decline (Jagannath et al. 2017). Mechanistically, CREB is not the only protein made in postsynaptic neurons during transmission between SCN neurons. CREB-regulated transcription coactivator 1 (CRTC1) controls upregulation of per genes (Jagannath et al. 2017). Beyond that, per genes are not the only genes that increase in expression from their transcriptional rates. The binding of CREB and CRTC1 also induces the increased expression of SIK1 (Jagannath et al. 2017). The SIK1 protein can deactivate CRTC1, thus inhibiting transcription of necessary per genes and decreasing light's modulatory influence on the master clock (Jagannath et al. 2017). As it appears, this mechanism can work similar to a negative feedback loop, as one of its own protein products can inhibit prior protein products to reduce transcription.

The SCN, which is the landmark referred to as the central pacemaker, coordinates circadian rhythms and their timing to molecular clocks in peripheral locations of the body (Chan et al. 2012). Molecular clocks in peripheral tissues such as the liver, lungs, adrenal glands, and other tissues, express pertinent genes necessary for circadian rhythms in a pattern on a temporal basis (Fisk et al. 2018). The SCN relays the coordination of specific circadian rhythms to the periphery through neuronal, hormonal, and other forms of communication (Fisk et al. 2018). Furthermore, other factors such as sleep patterns, energy metabolism, and glucocorticoids influence peripheral clocks (Jagannath et al. 2017). Circadian rhythms end up influencing patterns of gene expression of cellular clocks that make up that specific tissue based on interactions between transcription factors (Jagannath et al. 2017). Specifically, activated circadian-based transcription factors from zeitgebers and SCN input impact transcription factors unique to that tissue to modulate gene expression (Jagannath et al. 2017). Note that these mechanisms occur to modulate certain circadian rhythms during certain times of the day, considering “normal” rhythmicity (Jagannath et al. 2017). Interestingly enough, tissues isolated from the body, without coordination from the SCN, have showcased independent circadian control (Vitaterna et al. 2001). Being in constant light results in the desynchronization of the master clock to the external environment, but peripheral clocks still have a manner of carrying out a number of their rhythms (Koronowski & Sassone-Corsi 2021). There may be crosstalk between peripheral tissues as their rhythms occur, even in conditions of continuous light (Koronowski & Sassone-Corsi 2021). On the other hand, such an integrated system can be at risk if there is damage to the SCN, resulting in compromised neuroendocrine transmission to essential parts of the brain and the peripheral nervous system (Vitaterna et al. 2001). Also, lesions to this area can result in shifted phases of circadian rhythms of peripheral tissues (Koronowski & Sassone-Corsi 2021). This issue is not ideal as vital circadian rhythms could basically become dysfunctional, leading to a higher risk for serious disease and metabolic imbalances.

One might wonder how exactly a circadian rhythm forms from the synchronization of molecular clocks to zeitgebers, master clock coordination, and other cues. A molecular clock is representative of transcription-translation-based feedback loops (TTFLs) that rely on components vital to establishing and maintaining circadian rhythms (Jagannath et al. 2017). The primary feedback loop involves two transcription factors named “Circadian Locomotor Output Cycles Kaput” (CLOCK) as well as “Brain and Muscle Arnt Like-1 (BMAL1)" (Potter et al. 2016). The CLOCK genes influence the length of a circadian rhythm, while BMAL1 genes also assist in the maintenance of said rhythms (Chan et al. 2012). Both CLOCK and BMAL1 proteins will dimerize to form a complex, which will bind to the target DNA response element called the E-box in the promoter sequence (Jagannath et al. 2017). An E-box is a DNA control element found in the timekeeping system that has extensive input from tissue-based functionality to programmed cell death (Munoz et al. 2002). The upregulation of the transcription of clock genes "period" (Per1, Per2, Per3) and "cryptochrome" (Cry1 and Cry2) occur via this event (Jagannath et al. 2017). The period and cryptochrome proteins can dimerize into complexes that are capable of influencing other aspects of circadian genetics (Jagannath et al. 2017). These clock-controlled gene complexes play a role in regulating the transcription of many other clock-controlled genes (CCGs) (Potter et al. 2016). The temporal implications of CCGs are pertinent to the timing of biological functionality for tissues around the body, which uphold the ubiquity of circadian rhythms (Potter et al. 2016). The regulation of these CCGs is still under deliberation as some of them have E-boxes in their promoters, but some do not (Jagannath et al. 2017). The circadian system has developed fine-tune control through intricate crosstalk between various proteins to downregulate itself at set times. Casein Kinase 1 (CK1), has the ability to form a complex with PER proteins to send them for breakdowns, however, cryptochrome can bind to the dimeric complex to inhibit this occurring (Jagannath et al. 2017). The trimeric complex between PER, CK1, and CRY, when phosphorylated by other kinases, can travel and repress the transcription of period and cryptochrome genes via repression of the CLOCK-BMAL1 dimer in the nucleus (Potter et al. 2016). The concentration of the trimeric complex reaches its apex at dusk, but its nadir at dawn (Jagannath et al. 2017). Thus, such a mechanism promotes not only the circadian rhythms that occur in a target tissue but the period of length in which the rhythms are active in that tissue (Jagannath et al. 2017). The primary TTFL embodies a negative feedback pathway that is willing to downregulate its own transcriptional rate when the time calls for it. Influence over the primary TTFL can also come from other TTFL in the timekeeping system that provides further regulation and fine-tuning. For example, a secondary TTFL involves nuclear receptors ROR**α** and REV-ERB**α** and their ability to regulate BMAL1 transcription (Jagannath et al. 2017). Specifically, these receptors can bind to ROR DNA response elements in the promoter region of BMAL1 (Jagannath et al. 2017). Such factors bind to the promoter at strategic times for BMAL1 concentration to peak at dawn and reach its lowest concentration at dusk before rising again the next morning (Jagannath et al. 2017). Such regulation impacts the rate of transcription of the CLOCK-BMAL1 dimer that later influences the activation of CCGs, which coordinate local tissue physiology and behavior at peak hours.

From mental disorders to the stress response coordinated by the hypothalamus-pituitary-adrenal (HPA) axis, circadian influence propagates through various forms and paths. For example, there is considerable circadian influence over metabolic pathways, but misalignment and other factors can result in metabolic imbalances that lead to disease. One such disease is obesity, which can potentially lead to an individual having Type II Diabetes Mellitus (T2DM). Circadian misalignment amongst individuals, based on reports, does have an influence over glucose metabolism in the sense that they exhibited aspects such as insulin resistance, a hallmark of obesity (Huang et al. 2011) What's more, the cited reports also looked at effects on those with diabetes and found that misalignment decreased degrees of glucose tolerance and insulin secretion (Huang et al. 2011). What initiates such metabolic imbalance comes from several initiators, one of the most recognizable factors being sleep disruption (Potter et al. 2016). In particular, several pathways studied propose sleep disruption leading to obesity and symptoms of obesity. For example, sleep disruption promotes circadian misalignment, which impairs the gut microbiome in a way that can lead to hyperglycemia, insulin resistance, and obesity (Potter et al. 2016). On the other hand, sleep dysfunction can promote dysregulation of endocrine communication, impairment of adiposity, and a greater propensity for feeding (Potter et al. 2016). These effects are in sync with a greater risk for obesity as well as hyperglycemia and insulin resistance (Potter et al. 2016). So, the question remains as to why circadian misalignment is so common when an easy solution is to maintain a healthy lifestyle. Having good habits, from sleeping habits to eating habits, is a good pattern of behavior to implement throughout life, but circumstances can push humans into harmful misalignment without any choice. For example, diseased states such as Alzheimer’s can cause misalignment on a biological level from physiological impairment and genetic mutations in circadian genes (Potter et al. 2016). However, environmental and behavioral implications such as light-dark cycle disruption and feeding-fasting cycle disruption promote circadian misalignment in circumstances involving jet lag and shift work (Potter et al. 2016).

Society within the scope of the twenty-first century, in the US in particular, is at a greater risk for circadian rhythm disruption, and the negative implications that come with it. Inappropriate exposure to light to large portions of the population results from progress in advanced technology like smartphones (Fisk et al. 2018). Not just from technology, but a rise in industry and business have called for a large proportion of the workforce to work unnatural night shifts (Fisk et al. 2018). Thus, circadian alignment has become more of an active challenge, while shift workers have become a subsection of the population that is at risk (Koronowski & Sassone-Corsi 2021). In contrast to what one considers normal, people that work night or early morning shifts have opposite rhythms compared to normal circadian rhythms (James et al. 2017). Specifically, workers that work the "night shift" period, times ranging from the night to the early morning, are active during times when they should be asleep and when their alertness is usually at its lowest points (James et al. 2017). Workers that are working the "night shift" period or that must work under rotation shifts have to adapt to constantly alter their sleep schedules (James et al. 2017). Under such strain, circadian misalignment is not only a high possibility, but the degradation of their health becomes a possibility too (James et al. 2017). From one's mental health to one's physical well-being, an array of diseases such as metabolic syndrome, diabetes, cancer, cardiovascular disease, and other health-related issues can become very real (James et al. 2017). With current and future research prospects, this study hopes to highlight risks related to circadian rhythm misalignment from shift work. Hopefully, this study can also educate readers interested in circadian rhythms and how they influence our daily lives. I hypothesize that night shift workers will get less sleep, will have more interrupted sleep, will have a propensity to be overweight, will feel more depressed, and will feel more hopeless more often than day shift workers. A research question posed for this study, on the other hand, is if there are any popular coping strategies employed by night shift workers to reduce stress and increase productivity before, during, and after their respective shifts.

Methods:

Participants:

This study recruited a total of 39 participants, 20 of whom were a part of the "day shift" group, while 19 were a part of the "night shift" group. Participants were recruited primarily from hospital settings, which do involve shift work. However, some participants were recruited outside of healthcare and were involved with other industries and careers that required shift work. The age range of participants was from 19 to 71 years. The average age to expect from a participant in the "night shift" group is around 31 years of age. The average age to expect from a participant in the "day shift" group is around 40 years of age. There was a total of 10 males in this study, while there were 29 females. Of the "night shift" group, there were exactly 15 females, while there were four males. Of the "day shift" group, there were exactly 14 females, while there were six males.

Materials:

For the purposes of this study, participants were expected to complete a survey. The survey, with all questions and items asked, was made by Sahil Rajan on a survey coordinating and data collection software known as *SurveyMonkey*. Participants all filled out the same survey, which was created to gather knowledge on health-related aspects between both groups. There was a total of 18 questions asked in the survey. However, target variables from the survey that were assessed to address the main hypothesis included sleep, weight, depression, and hopelessness.

The variable "sleep" was addressed through two items, which were if participants had uninterrupted sleep during their sleeping periods and what their average amount of sleep was per sleeping period. In order to address the item "uninterrupted sleep," a specific question was asked. "Would you say that you get uninterrupted sleep following your work shift?" was specifically asked of all participants, regardless of what group they were in. Participants could answer by designating either "Yes" or "No." In order to address the item "average sleep" a specific question was asked. "How many hours would you say, on average, you get per 24-hour period?" was specifically asked for all participants, regardless of what group they were in. Participants were given the chance to type in a number that best represented how many hours of sleep they received. In order to address the variable "weight," a single item involving "weight interpretation" was asked as a single question. "Do you consider yourself to be overweight?" was asked for all participants, regardless of what group they were in. Participants could answer by designating either "Yes" or "No." The next variable, depression, was addressed through a single item involving "depression interpretation," which was asked through a single question. "Do you struggle with depression?" was asked for all participants, regardless of what group they were in. Participants could answer by designating either "Yes" or "No." The last variable, helplessness, was addressed through a single item involving "hopelessness interpretation," which was asked through a single question. "Based on the following, please answer each item accordingly: Feelings of hopelessness?" was asked to all participants, regardless of what group they were in. This particular question was a Likert item, so there were multiple choices participants could choose from. Participants could choose from five choices, which were never (1), rarely (2), sometimes (3), often (4), and always (5) that best represented how often they felt hopeless.

A separate research question, not included in the hypothesis, was explored in this study. This research question asked if there were popular coping strategies that specifically night shift workers used. To address this aspect of the study, a single question was posed for only the participants in the "night shift" group. "Before, during, and/or after the night shift, what strategies do you employ to deal with work- induced stress and increase productivity?" was asked and the night shift participants could choose as many or as little as they would like. There were many coping strategy choices to choose from. Please refer to ***Table Two*** of the **Results** section for clarification of these choices.

Procedure:

Before being able to complete the survey, participants were educated on this study. Participants were told that the study was designed to analyze circadian rhythms and to showcase health risks of potential circadian misalignment through populations that carry out shift work. Once participants were briefed on the study, who could participate in this study, and other key pieces of information, they were electronically asked to read and sign an informed consent document. Participants were free to or not to agree to this study, depending on their choice to or not to consent to their involvement in the study without any penalties whatsoever. Participants that consented were directed to the electronic survey. From their designated choice asking them what shift position they worked, participants were assigned into either the "night shift" group or the "day shift" group. All participants simply took around 10 minutes to complete the survey on their own time without any experimental manipulation. After completing the survey, participants were thanked for their cooperation.

Results:

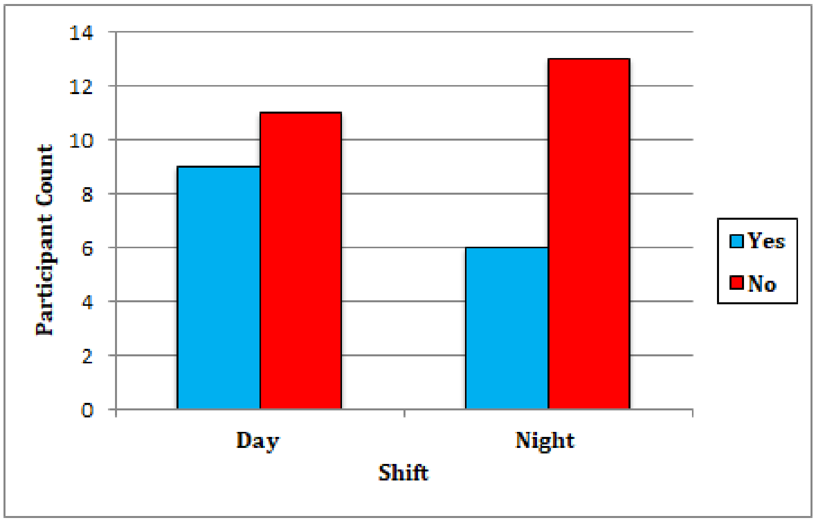


Figure One: **A graphical representation of participant responses on whether they designated "Yes" or "No" to if they got uninterrupted sleep during their respective sleeping periods.**

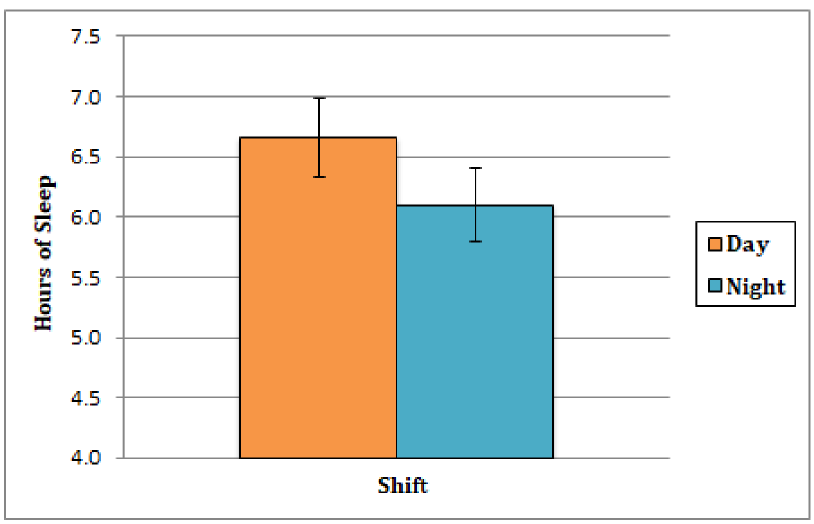


Figure Two: **A graphical representation of the average amount of sleep received between day shift and night shift workers based on participant responses.**

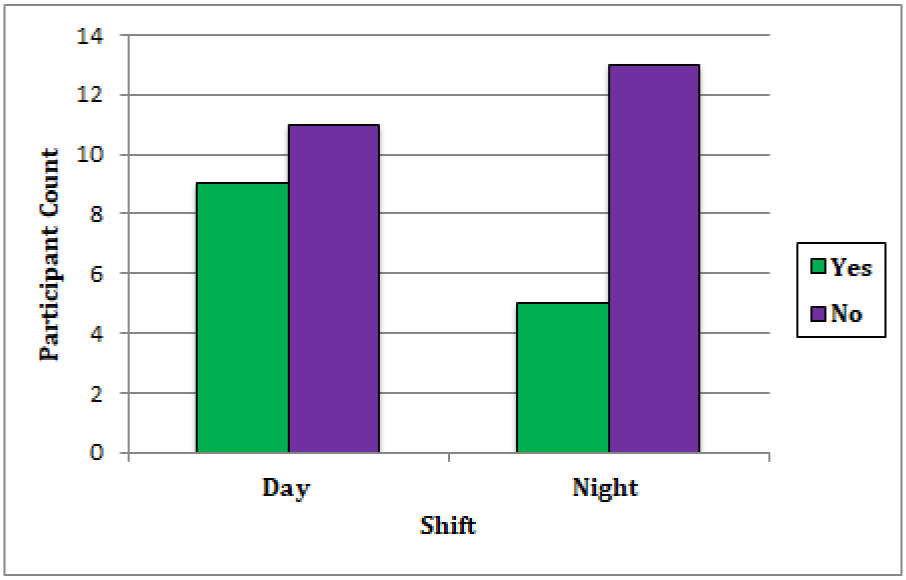


Figure Three: **A graphical representation of participant responses on whether they designated "Yes" or "No" to if they considered themselves "overweight."**

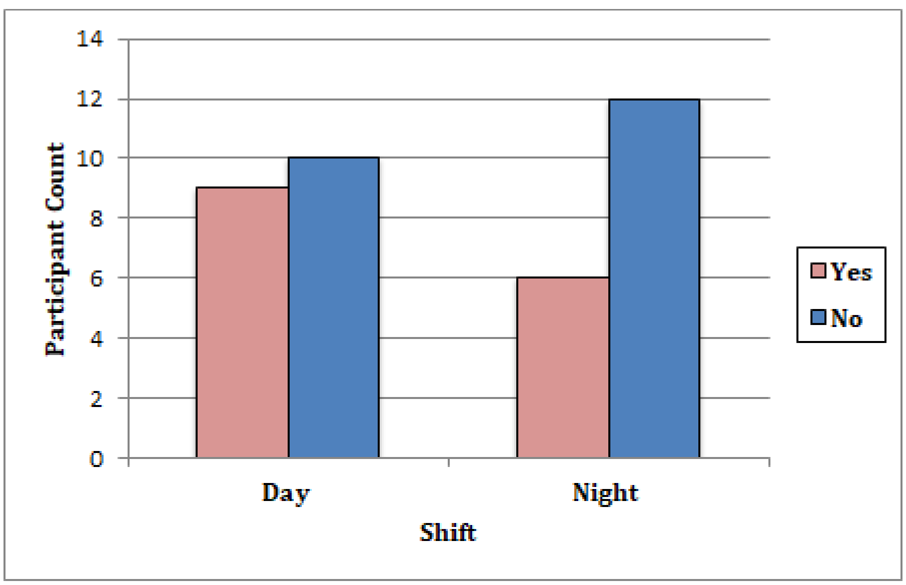


Figure Four: **A graphical representation of participant responses on whether they designated "Yes" or "No" to if they consider themselves "struggling/suffering from depression."**

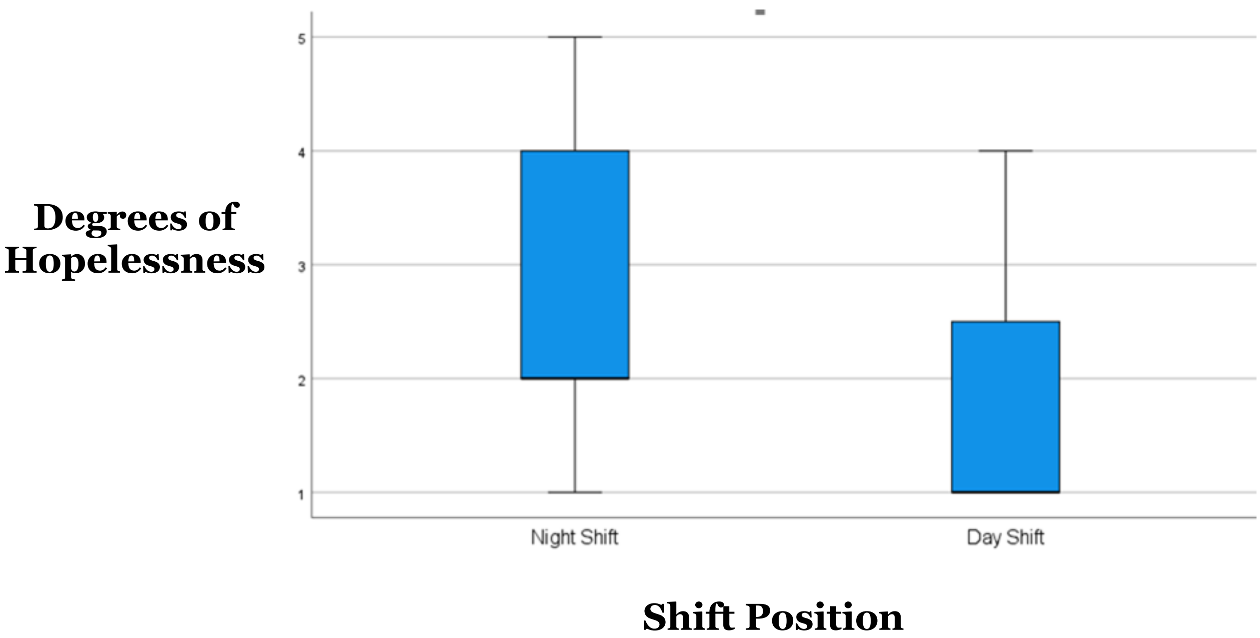
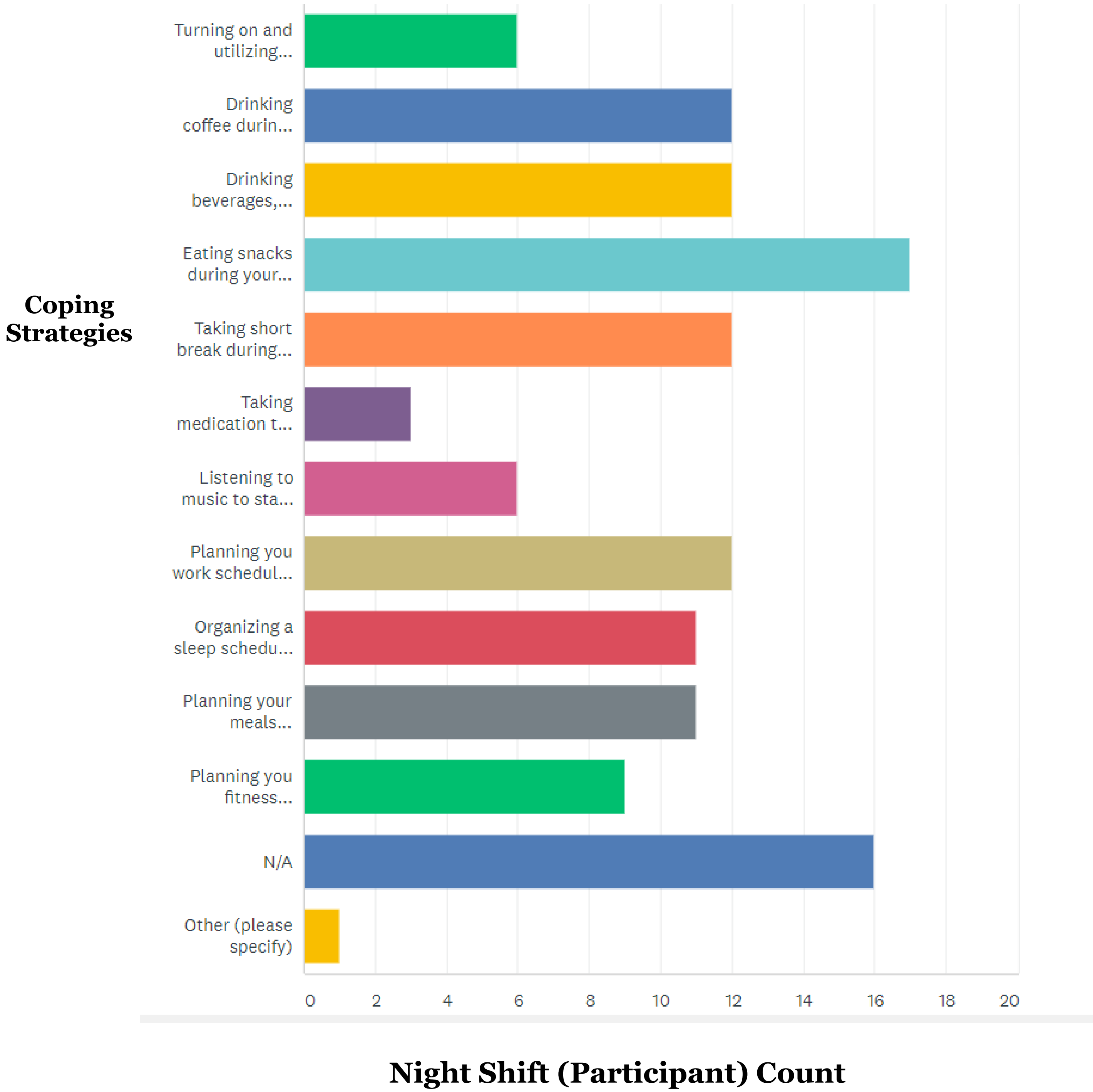


Figure Five: **A graphical representation of a boxplot showcasing the range of responses in regard to "feelings of hopelessness" between participants of the day and night shift groups. The degrees represent how often participants believe they felt hopeless. These degrees include "Never" (1), "Rarely" (2), "Sometimes" (3), "Often" (4), and "Always" (5).**

Variable Assessed Between Day Shift vs. Night Shift	Statistical Test Conducted	P-value Calculated
Uninterrupted Sleep (Figure One)	Chi-Square test	0.390
Average Hours of Sleep (Figure Two)	T-test	0.190
Weight (Figure Three)	Chi-Square test	0.270
Depression	Chi-Square test	0.300
Hopelessness	Mann-Whitney U Test	0.051

Table One: **A tabular representation of the variables addressed to support or not support the hypothesis of this study. The table showcased the appropriate statistical test, along with the determined P-value, for each respective variable.**



<u>Figure Six:</u> **A graphical representation of participants from the night shift group choosing potentially more than one coping strategy. Selections by a night shift worker specifically represents the way(s) in which that individual deals with stress and productivity in the**

context of before, during, and/or after their shift work. Please refer to *Table Two* for a clearer tabulation of what the strategies are, the number of night shift workers who selected for those strategies, and other key pieces of information. The option "N/A" is not a strategy, but a choice that day shift workers were expected to designate. Day shift workers also could have ignored the question completely as this part of the survey was directed for night shift participants to address only.

Coping Strategies	Number of Night Shift Participants who Selected the Strategy	Extra Information to Consider
Utilizing lights during shift	6	X
Drinking coffee during shift	12	X
Drinking beverages, juice, soda, and/or any other drinks that are not coffee during shift	12	X
Eating snacks during shift	17	X
Taking short breaks during shift	12	X
Taking medication to stay awake	3	X
Listening to music to stay awake	6	X
Planning your work schedule with respect to your preferences (scheduling when and/or how many days you will work your respective night shift(s))	12	X
Organizing a sleep schedule with respect to your	11	X

schedule (planning when you will sleep and/or for how long during your respective sleeping period)		
Planning your meals with respect to your schedule (scheduling times to eat and/or what you will eat)	**11**	**X**
Planning your fitness with respect to your schedule (scheduling when and/or how much exercise you will do)	**9**	**X**
Others (Specify)	**1**	❑ **This option offered the participant the opportunity to share their unique strategy that was not listed.** ❑ **The single participant that selected this option specified their strategy, which is mentioned below.** ❑ **"I am on a shift rotation. I switch from day to night shift weekly."**

Table Two: **A tabular representation clearly characterizing each coping strategy described in this study, the number of night shift participants that selected for each respective strategy, and other pieces of information.**

Figure One represented if participants in either the night shift or day shift group had uninterrupted sleep or not during their respective sleeping periods. A chi-square statistical test was conducted, and a subsequent P-value was calculated to be 0.390. Thus, there was not a significant difference between potentiality of uninterrupted sleep between day shift and night shift workers. ***Figure Two*** represented the average hours of sleep received between day and night shift workers during their respective sleeping periods. A T-test statistical test was conducted, and a subsequent P-value was calculated to be 0.190. Thus, there was not a significant difference between the average hours of sleep received between night and day shift workers during their respective sleeping periods. ***Figure Three*** represented if participants in either the day or night shift group considered themselves overweight or not. A chi-square statistical test was conducted, and a subsequent P-value was calculated to be 0.270. Thus, there was not a significant difference between the propensity of being overweight between workers of the day and night shift groups. ***Figure Four*** represented if participants in either the night shift or day shift group considered themselves struggling with depression. ***Figure Five*** represented how participants in either the night shift or day shift group rated their feelings of hopelessness. A chi-square test and Mann Whitney U test were conducted for depression and hopelessness, respectively. As a result of such tests, P-values for depression and hopelessness variables were 0.300 and 0.051, respectively. Thus, there was not a significant difference for struggling with depression between night shift and day shift groups. Also, there was not a significant difference between the ratings of hopelessness between the night shift and day shift groups.

Discussion, Limitations, & Future Prospects:

To restate, the purpose of this study was to investigate the influence of circadian rhythms on the health-related risks between day shift workers and night shift workers. Furthermore, this study explored the idea of popular coping strategies, which were employed by night shift workers. Though significant differences were not found between both groups in relation to all the variables tested, there were interesting patterns and trends that the data displayed. However, future studies with larger samples sizes and a more diverse range of participants can determine if the conclusions are meaningful. Discussions on these patterns in relation to prior research can provide insight into what could make up further studies into circadian rhythms.

Night shift workers ended up having higher chances of having interrupted sleep and had, on average, less amount of sleep than their day shift counterparts. Such conclusions make sense as prior research has proposed concepts and models to explain the unnatural sleeping patterns of night shift workers. Sleep physiology is a major part of the alignment or misalignment of a worker's circadian rhythms. One can define the difference in a worker's sleep-wakefulness via the "two-process" model of sleep (Wickwire et al. 2017). This model represents two elements, sleep and wakefulness, and their regulation based on homeostatic pressure and circadian alert signaling respectively (Wickwire et al. 2017). Homeostatic pressure and circadian alert signaling increase as the day carries on when one is working, but both signals fall when one falls asleep (Wickwire et al. 2017). However, the normal degrees of signaling would be problematic for people who work during the night shift. Increasing levels of circadian alert signaling, which promotes wakefulness, would make it difficult for night shift workers to sleep during their sleeping periods (Wickwire et al. 2017). Decreasing circadian alert signaling during the night would not only negatively affect night shift workers and their performance on the job but could lead to injury (Wickwire et al. 2017). Such a pattern of irregularity can take a toll on the health of night shift workers over time. For example, shift work-related disorders can exacerbate or even develop other common health problems in the long term for night shift workers (Wickwire et al. 2017).

Another health-related variable assessed between day and night shift workers was weight. Generalized trends from the data showcased a greater proportion of day shift workers believed they were overweight compared to night shift workers. These findings differ with consideration to prior research conducted on the weight of night shift workers. For example, previous studies have defined relationships on night shift work with pathology such as dyslipidemia and diabetes mellitus (Brum et al. 2020). In addition, other works have highlighted a directly proportional relationship between greater BMI with a higher number of night-shift workers than day shift workers (Brum et al. 2020). Metabolic consequences can also attest to greater weight in night shift workers such as a reduction in satiety, an increase in appetite, less insulin sensitivity, and other modulating factors (Brum et al. 2020). The study conducted by Brum et al. (2020) investigated shift work and its connection to obesity and found that night shift workers presented higher levels of being overweight, being obese, and getting fewer hours of sleep than day shift workers.

Along with the physical health risks that can come from circadian dysfunction, the investigation explored the emotional and psychological side as well. Specifically, the variables assessed included both depression and feelings of hopelessness. The general trends from the data were surprising among both variables. Hopelessness is a symptom of depression, yet the trends exhibited among both variables were opposite. Day shift workers appeared to showcase higher levels of struggling with depression than night shift workers. However, it was night shift workers who felt more hopeless more often than day shift workers. As per research, there seems to be an association between shift work and mental illness in the long term due to the psychological stress that comes with irregular schedules (Chellappa 2020). Night shift work was consistent with greater risks for depression and anxiety (Chellappa 2020). Though the exact mechanisms for such assessments are ambiguous, circadian rhythm misalignment can regulate mood, which can make one more susceptible to their mental health degrading (Chellappa 2020). An fMRI study between circadian misalignment and brain activity showcased unfavorable regulation of activity in areas such as the frontal cortex and amygdala (Chellappa 2020). Such areas are important to the contextualization and regulation of mood, but activity was lower during times when night shift workers were active (Chellappa 2020). On the other hand, sleep loss, as mentioned, is a common implication of night shift work and has additive effects on circadian-related mood dysregulation (Chellappa 2020). Specifically, sleep deprivation can lower reactivity and lower mood (Chellappa 2020).

With all the results considered, the hypothesis cannot be supported. Some of the trends seen from the data, from both sleep variables to the ratings of hopelessness, were not shocking. However, other variables such as weight and depression were opposite to what one expects. Research cited also provided evidence of studies conducted that reached conclusions opposite to the conclusions reached from this study. So, why were the data and subsequent conclusions different? One reason was that the night shift workers who participated in this study were, on average, much younger than the day shift workers who participated in this study. Please refer to ***Supplementary Figure One*** from the **Appendix** section for insight on the ages of participants. Based on Figure Three, day shift workers reported greater levels of believing they were overweight than night shift workers. This statement is understandable when considering the common idea that biological aging can alter one's metabolic rate such that weight loss is harder and fat accumulation is easier. Another possible reason is that the night shift workers happened to adopt coping mechanisms to adapt to their circadian misalignment and keep themselves healthy. Most of the participants came from the hospital setting, so coping strategies in the healthcare industry, which is a twenty-four-hour business, is common. For example, a study conducted by Savic et al (2019) explored the most common coping strategies employed by night shift nurses in hospitals. This study emphasized four main themes to represent both positive and negative strategies employed by nurses. Health practices, social and leisure, cognitive coping strategies, and work-related coping defined a range of coping strategies analyzed from this study (Savic et al. 2019). Under health practices, strategies such as eating, relaxation, and sleep were common methods employed to handle their shifts (Savic et al. 2019). For social and leisure, one of the most common strategies, social support, lowered stress, while upholding well-being (Savic et al. 2019). The third theme, cognitive coping strategies, described nurses incorporating spiritual and mindful techniques, which lowered emotion demand and stress (Savic et al. 2019). Lastly, under work-related coping, strategies such as taking breaks, influencing scheduling rostering, and controlling workload were positive (Savic et al. 2019). Specifically, these strategies provided these night shift nurses time to recuperate to handle feelings of helplessness, which also reduced stress (Savic et al. 2019). Such coping strategies investigated in this study were strictly used by participants in the night-shift group. The research question posed had night shift workers choose strategies that they employed to reduce stress and increase productivity before, during, or after their shift. From this study, there was a range of popular coping strategies, such as night-shift workers drinking coffee and other beverages, taking short breaks, and planning their work schedules. However, the most popular strategy determined from this study was that night-shift workers would tend to eat snacks during their shift. Why such insights are important is because night shift workers have to use these strategies to cope with "being on the clock" at unnatural hours. Not only that, but industries that have grown to require night shift employment must consider the ramifications of circadian irregularities on their employees' health. The workplace must form protocols to enforce these strategies and build necessary support systems for night shift workers. By doing so, these workers can meet the demands and high stress that comes with their jobs and adapt more effectively to these conditions. In the context of this study, these night shift workers have been employing these strategies for a long period of time. Thus, such coping strategies could have had a positive impact on the way they handle their health, on a physical, emotional, and psychological level. Considering those implications, it is clear how the data showcased that fewer night shift workers considered themselves struggling with depression than their day shift counterparts.

There were, however, a few limitations that resulted in the data and conclusions being less accurate than they could have been. One limitation was that the recruitment period conducted for this study was too short. As a result, the sample size was too small, with only thirty-nine participants. Having a small sample size resulted in a reduction of this study's power statistically and further increased the chance of error. Another limitation was that the participants that participated were not diverse enough in terms of their status in the workforce. Almost all of the participants recruited came from hospital settings in the healthcare industry. Night shift work involves different kinds of professions other than medicine. These different professions include law enforcement, transportation, and food industries (Wickwire et al. 2017). Had shift workers, both day and night, came from different settings, there would have been greater variation in their skills, coping strategies, backgrounds, etc. Beyond that, an emphasis on more variation in age, race, and other personal characteristics would have also added to the diversity as well. Having a diverse participant pool would have better represented the entire population of shift workers that are currently in the workforce. If someone were to replicate this study in the future, there should be a longer period of time dedicated to recruiting participants from a variety of industries, which would effectively provide a larger and more diverse sample size of shift workers. Keeping these notions in mind would provide a group that is far more representative of shift workers in the workforce.

Circadian rhythm-related research continues to raise questions and such research does not appear to be halting any time soon. Greater insight into circadian mechanisms and concepts have provided means to understand disease on another level. For example, there appear to be connections between the circadian system and neurodegenerative disorders. Though such connections are not fully understood during this time, people with neurodegenerative disorders showcase circadian rhythm disruption at a more severe level than people with simply normal misalignment (Leng et al. 2019). When considering patients with Alzheimer's disease, they showcase much higher levels of sleep irregularity, reduced amplitude of circadian rhythmicity, and phase delays (Leng et al. 2019). Alzheimer's patients are likely to suffer from irregular sleep wake-sleep disorder, where they have periods of sleep during the day but are awake for long periods during the night (Leng et al. 2019). On the other hand, people diagnosed with Parkinson's disease have common circadian-related issues such as sleep-wake irregularities (Leng et al. 2019). A main question that investigators should explore is if circadian-based dysfunction results in neurodegeneration? Circadian-based dysfunction plays its role before the symptoms of such diseases become noticeable and prevalent (Leng et al. 2019). Post-mortem studies have showcased loss of essential neurons in the SCN for people who suffered from

Alzheimer's disease (Leng et al. 2019). From a separate viewpoint, circadian rhythm sleep disorders are some of the most common implications in such neurodegenerative diseases (He et al. 2019). Circadian dysfunction in such disorders involves dysfunction on the mechanistic level, more or less, in people with neurodegenerative diseases. Specifically, circadian clock mutations can cause abnormal glutamic signaling (He et al. 2019). Sleep loss, associated with such disorders, is linked to abnormal metabolic functioning, memory impairment, mental health degradation, and other factors (He et al. 2019).

Circadian influence is revolutionizing aspects of the medical world based on its role in therapeutics, treatments, and essential drug use. Treatments such as cognitive-behavioral therapy (CBT), light treatment, and interpersonal & social rhythm therapy, all correspondingly attempt to regulate the circadian system to a normal, set schedule to reduce misalignment (Hong & Zhang 2019). Evidence has supported interpersonal & social rhythm therapy effects on reducing feelings of hopelessness and levels of depression, among other factors, for people struggling with MDD (Hong & Zhang 2019). However, an area of interest that does not have as much clarity is circadian influence over pharmacokinetics and drug metabolism. Pharmacokinetics involves processes evaluating drug absorption, distribution, metabolism, and excrement in and from the body (Musiek & FitzGerald 2013). There is support towards circadian influence at every phase of this process, which can regulate the efficacy of target drugs. For example, drug absorption works through many bodily and physiological parameters like pH, but some control does come from circadian influence. The activity of the stomach by circadian clock genes regulating ghrelin levels and further influence of clocks on gut physiology plays important parts in the absorption event (Musiek & FitzGerald 2013). Gut physiology includes the expression and regulation of essential transporter proteins, many of which exist under circadian clock control (Musiek &

FitzGerald 2013). Another example of circadian influence in this system is through drug excretion. Studies using mice support the idea of circadian clock genes having influence over renal components such as channels and transporters that can regulate filtration and urine flow (Musiek & FitzGerald 2013). Additionally, drugs are also more likely to flush out of the body if urinary pH is high, which is regulated by circadian adjacent systems (Musiek & FitzGerald 2013). The relationships that are still ambiguous between circadian rhythms and therapeutic drugs should continue to influence future-directed studies. More discoveries in this field could uncover more pathways that can act via the circadian system to administer drugs more smoothly.

From all the research that the future holds for circadian rhythms, industries have taken note of ways to assist their night shift workers. As mentioned, night shift workers are at a higher risk of developing circadian-related sleep-wake disorders (SWD) that can influence their health in a negative way. So, how can organizations, industries, and businesses make changes in and out of the workplace to help their employees? Well, goals are oriented by higher-ups to recognize issues that come from shift work, prevent these problems, and cultivate healthy patterns of behavior (Wickwire et al. 2017). Industries try to extrapolate and analyze assessments that consider a worker's ability to do their duties, attending to employees at a higher risk for SWD compared to others, and providing paths for help (Wickwire et al. 2017). Such help includes providing workers with healthcare regarding their sleeping patterns or providers that can put them on the right path (Wickwire et al. 2017). However, to truly reach a symbiotic relationship, employers and employees must form a partnership, a bridge built on communication and space in order for the workplace and the workers to function properly. Businesses should assess risk for jobs in the context of work-related schedules, and the environment where workers will do their jobs (Wickwire et al. 2017). Such risks and other needful assessments should open the eyes of potential candidates for the job (Wickwire et al. 2017). If work-related accidents do occur from shift workers under supervision from employers, both parties should engage in conversations and actions to determine the cause and work on avoidance of such issues for the future (Wickwire et al. 2017). With that in mind, standard protocols and procedures should address shift workers and their issues, so that risk is minimized (Wickwire et al. 2017). The workplace should provide enough space to shift workers so that they can understand their limitations and regulate themselves accordingly. Meaning, shift workers should make their own schedules and take necessary breaks during their shifts (Wickwire et al. 2017). Giving shift workers these powers can increase satisfaction, increase the quality of work, and may reduce the risks of SWD (Wickwire et al. 2017). In relation to the workplace itself, giving shift workers the opportunity to work in areas that are well lit and under appropriate temperatures can also have positive outcomes (Wickwire et al. 2017). With more of the workforce becoming aware of circadian-related risks, shift work has been and continues to inspire many necessary conversations. With greater knowledge of the circadian system at play endogenously, industries can create influential policies to protect their workers from injury and harm. Circadian rhythms are revolutionizing the way we as a society discuss normalcy and what that means in the scope of our internal biology. The push towards understanding more about circadian rhythms aligns with our desire to reduce the hurdles that come with the human condition.

Works Cited

Chan, M., Spieth, P. M., Quinn, K., Parotto, M., Zhang, H., & Slutsky, A. S. (2012). Circadian rhythms: From basic mechanisms to the intensive care unit. Critical Care Medicine. 40: 246-253.

Chellappa S. L. (2020). Circadian misalignment: A biological basis for mood vulnerability in shift work. The European Journal of Neuroscience. 52: 3846–3850.

Fisk, A. S., Tam, S. K., Brown, L. A., Vyazovskiy, V. V., Bannerman, D. M., & Peirson, S. N. (2018). Light and Cognition: Roles for Circadian Rhythms, Sleep, and Arousal. Frontiers in Neurology. 9: 1-18.

He, S., Zhang, X., & Qu, S. (2019). Glutamate, Glutamate Transporters, and Circadian Rhythm Sleep Disorders in Neurodegenerative Diseases. ACS Chemical Neuroscience. 10:175–181.

Hong, W., & Zhang, Q. (2019). Biological Rhythms Advance in Depressive Disorder. *A*dvances in Experimental Medicine and Biology. 1180: 117–133.

Huang, W., Ramsey, K. M., Marcheva, B., & Bass, J. (2011). Circadian rhythms, sleep, and metabolism. The Journal of Clinical Investigation. 121: 2133-2141.

Jagannath, A., Taylor, L., Wakaf, Z., Vasudevan, S. R., & Foster, R. G. (2017). The genetics of circadian rhythms, sleep and health. Human Molecular Genetics. 26: 128-138.

James, S. M., Honn, K. A., Gaddameedhi, S., & Dongen, H. P.A. V. (2017). Shift Work: Disrupted Circadian Rhythms and Sleep - Implications for Health and Well-Being. 3: 104-112.

Koronowski, K. B., & Sassone-Corsi, P. (2021). Communicating clocks shape circadian homeostasis. Science. 371: 1-10.

Leng, Y., Musiek, E. S., Hu, K., Cappuccio, F. P., & Yaffe, K. (2019). Association between circadian rhythms and neurodegenerative diseases. 18: 307-318.

Munoz, E., Brewer, M., & Baler, R. (2002). Circadian Transcription. The Journal of Biological Chemistry. 277: 36009-36017.

Musiek, E. S., & FitzGerald, G. A. (2013). Molecular Clocks in Pharmacology. Handbook of Experimental Pharmacology. 217: 243-260.

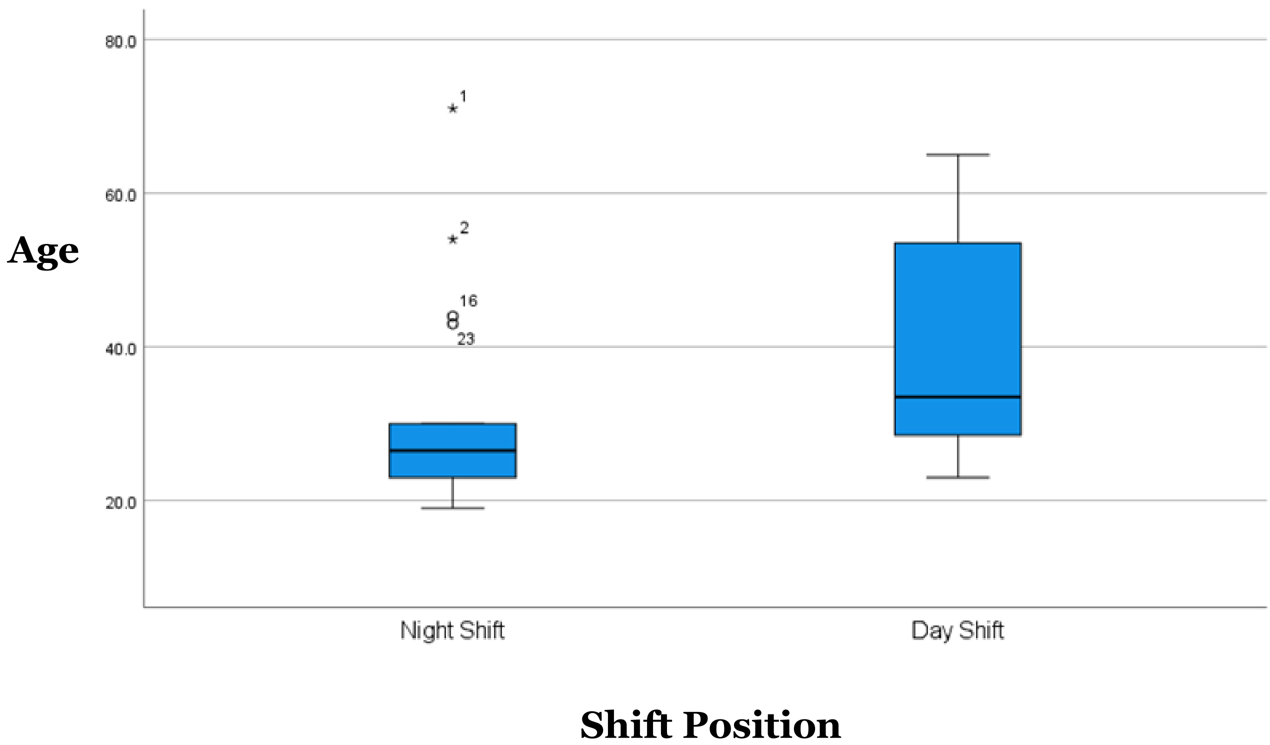
Potter, G. D., Skene, D. J., Arendt, J., Cade, J. E., Grant, P. J., & Hardie, L. J. (2016). Circadian Rhythm and Sleep Disruption: Causes, Metabolic Consequences, and Countermeasures. Endocrine Reviews. 37: 584-608.

Savic, M., Ogeil, R. P., Sechtig, M. J., Lee-Tobin, P., Ferguson, N., & Lubman, D. I. (2019). How Do Nurses Cope with Shift Work? A Qualitative Analysis of Open-Ended Responses from a Survey of Nurses. International Journal of Environmental Research & Public Health. 16: 1-17.

Vitaterna, M. H., Takahashi, J. S., & Turek, F. W. (2001). Overview of Circadian Rhythms. Alcohol research & health: The Journal of the National Institute on Alcohol Abuse and Alcoholism. 25: 85–93.

Wickwire, E. M., Geiger-Brown, J., Scharf, S. M., & Drake, C. L. (2017). Shift Work and Shift Work Sleep Disorder: Clinical and Organizational Perspectives. Chest. 151: 1156–1172.

Appendix:



<u>Supplementary Figure One:</u> **A graphical representation of a boxplot showcasing the range of ages of participants who make up the night shift group and the day shift group.**