Role of Dim Artificial Light at Night (dALAN) on Body Weight Percentage Increase of *Mus musculus*

Stephanie A. Babinec

Spring Valley High School, Columbia, SC

Light pollution at night is a growing issue in many suburban and urban settings, commonly referred to as artificial light at night (ALAN). Many studies have been conducted as to how the intensity or wavelength of this lighting can disrupt the circadian rhythm but none have evaluated how the timing of this light could affect it. It is hypothesized that those that chronically experience dim artificial light at night (dALAN) after biological day will lead to a more pronounced disruption in the metabolic system and therefore will cause an increased level of weight gain. Mice were used as test subjects and were split into four groups: 1) 12 light, 12 dark (L:N); 2) 12 light, 4 dim, 8 off (L:D:N); 3) 12 light, 4 off, 4 dim, 4 off (L:N:D:N); and 4) 12 light, 8 off, 4 dim (L:N:D). The weight of these mice was tracked weekly to obtain the necessary data. Theis data were then analyzed for percent body weight increase and an ANOVA was run, obtaining a p-value of 0.000053. A Scheffe test was then run, finding a significant difference between L:N and L:D:N, L:N and L:N:D, and L:D:N and L:N:D:N. These results support that chronic dALAN exposure can lead to increased percent body weight changes. Future studies can further examine the possibilities as to why this is.

Introduction

The prevalence of obesity worldwide has almost tripled since 1975. More than 1.9 billion adults were overweight in 2016, with 650 million of them being obese (WHO, 2017). Obesity-related conditions, such as cardiovascular disease, type 2 diabetes, and strokes, are among the top preventable causes of death. Obesity further contributes to being an economic burden on the healthcare system. However, obesity, and the associated diseases, can be prevented through certain lifestyle changes.

Sleep deprivation is strongly related to the development of obesity and metabolic diseases. Sleep is when the metabolism is able to regulate itself, and the lack of it can lead to serious issues (Marsís Vargas et al., 2019). However, millions worldwide do not get adequate sleep to maintain a healthy level of function for their metabolism. Furthermore, many take place in work or activities that take place when the body naturally promotes sleep. This misaligned metabolic system is associated with non-homeostatic food intake, reduced sleep, and irregular sleep schedules (McHill & Wright Jr, 2017).

One leading cause of sleep deprivation is artificial light. The most common form of this is light pollution. 99% of the population of the United States and European Union live in areas where the night sky is brighter than the no-impact status set by the International Astronomical Union, which is 10% brighter than the natural sky brightness at night (Chepesiuk, 2009). However, artificial light is also experienced inside the home, from room lights to phone screens to night lights.

Literature Review

Circadian Rhythms

A circadian rhythm is an almost 24-hour pattern that is independent of external factors such as body temperature, mood, and sleeping patterns (Baron & Reid, 2014). It is present in most mammals, and evolutionarily followed the pattern of light-dark in time with the biological day and night (Vitaterna, 2001). Although the rhythms will continue to cycle without a biological day/night, light is the most important environmental factor to train the internal 24-hour circadian rhythm to the external 24-hour cycle of Earth (Marsís Vargas et al., 2019). However, the circadian rhythm can become disrupted, such as by light during a dark phase, a term referred to as circadian misalignment. Circadian misalignment refers to a lack of synchrony with the internal and external circadian rhythms, leading to health issues such as sleep deprivation, insomnia, metabolic dysfunction, and higher risks of cardiovascular disease (Baron & Reid, 2014).

The circadian clock occupies two nerve cell clusters referred to as the suprachiasmatic nuclei or the SCN. This is located near the base of the brain near the anterior hypothalamus and is the main controller of the internal circadian rhythm. A strong association between circadian rhythms and metabolism has been established, and therefore a misalignment within the circadian rhythm is apt to skew the metabolic processes (La Fleur et al., 2001).

Metabolism

Metabolism is the range of biological processes that occur within the body, and is, in simpler terms, the amount of energy needed for the body to maintain itself. The metabolism helps control a range of things, including glucose metabolism, food intake, weight gain, and irregular appetite. Throughout sleep, the lower the metabolic rate, the better the body recovers from the damage dealt in the wake period (Sharma & Kavuru, 2010). However, when sleep deprivation occurs, an abnormal energy balance is created that then leads to altered glucose metabolism, irregulated appetite, lower energy expenditure, and increased insulin resistance (Knutson et al., 2007). A study by Kiegel, Leproult, and Van Cauter explored the possible impact of sleep debt on metabolic and endocrine function and found that glucose tolerance was lowered. Another study conducted by Arble et al. found that eating during the sleep period instead of the wake period can have negative effects. In the experiment, mice that consumed food during the sleep period gained twice as much weight in the same time period as compared to mice that ate during the day (Arble, 2009).

Light

Light is a component of life that almost all animals rely on to survive. Naturally, for most residents within the continental United States, approximately 12 hours of daylight is followed by 12 hours of night. But, with the advancement of technology, artificial light is able to be experienced throughout both the daytime and the nighttime hours, both residentially and industrially (Mendoza, 2021). Even though exposure to light is considered beneficial throughout the day, during the night, exposure to light can have harmful effects. This light is considered ALAN, or artificial light at night, and is measured in lux. Lux translates to lumen per square meter, or more commonly, the intensity of light to the human eye. ALAN within the household can range from 200 to 2000 lux. However, this typically included ceiling lights, floodlights, and other room-

brightening methods. Light from TVs, mobile phones, and nightlights are typically far dimmer, and range between 0-25 lux. This is referred to as dim artificial light at night (dALAN). Although significantly dimmer, human circadian rhythms are still responsive to this low level of light (Duffy & Czeisler, 2009). Other mammals, such as mice, are also stimulated by this light.

Humans and mice receive light signals through the retina. The SCN receives information from the retina, which helps entrain the circadian rhythm to light and dark cycles. Specifically, ipRGC's, or intrinsically photosensitive retinal ganglion cells, are responsible for the circadian connection. These cells are spread across the retina, project to the SCN, and contain melanopsin, a photopigment (Fleury et al., 2020). When light is perceived, the ipRGC's projections reach the SCN and stimulate it, along with parts that deal with the metabolic system. Because of this, a connection between ALAN and changes in the metabolic processes can be presumed. One study shows evidence of this by exposing rats to blue ALAN for an hour during a 12-hour nighttime cycle and then giving the rats a choice between regular chow or a high-fat high sucrose (HFHS) diet. Male rats exposed to the blue ALAN exhibited an increased intake of the HFHS diet and showed a higher glucose response (Marsís Vargas et al., 2019). This experiment also supports the idea that the wavelength of light affects the circadian rhythm. A study conducted by University of Nevada researchers used zebra finches to determine if 3000K or 5000K lights affected bodily conditions, food intake, and other metabolic functions. It was shown that the cooler temperature light, or 5000K, results in elevated glucocorticoids, or elevated stress levels (Alaasam et al., 2018). Another experiment conducted exposed human subjects to either 450 nm or 555 nm light on a 6.5-hour phase delay. It was concluded that melatonin suppression was higher in the 450 nm light, or the cooler light. This further supports the circadian disruptions that TV and phone screens could be causing, as they are blue light-based, and therefore in the most impactful range of the spectrum.

The timing of ALAN and dALAN is also important to the impact on the function of mice. (Duffy & Czeisler, 2009). Mice that were exposed to constant bright light exhibited a reduction in the circadian rhythm, a fluctuation in food intake, and an increase in glucocorticoids. Furthermore, mice exposed to dALAN experienced a shift in the timing of food intake but the food intake was not affected (Fleury et al., 2020). Fonken et al. (2014) showed that when restricting food intake to only nighttime in mice that are subjected to dALAN, the established dLAN gain in body weight was prevented. However, neither experiment explored the possibility of subjecting the mice to dLAN at altering times throughout the night.

Therefore, this study hypothesized that if mice are exposed to dALAN at varying times throughout the night, they will differ in food intake and therefore body weight due to non-homeostatic eating based upon circadian misalignment. Specifically, a group that experiences a light cycle and then a segmented dark-dim-dark cycle would experience the largest percentage changes. Based upon the results, a well-educated conclusion could be implemented to participate in better lifestyle choices. To do this, the mice were organized into four groups: 1) 12 light, 12 dark (L:N); 2) 12 light, 4 dim, 8 off (L:D:N); 3) 12 light, 4 off, 4 dim, 4 off (L:N:D:N); and 4) 12 light, 8 off, 4 dim (L:N:D). They were monitored daily and their weights were recorded weekly.

Methods

Humans would have been the preferred test subject as it would most accurately model human behavior. However, ethical and institutional constraints did not allow this to happen. Instead, *Mus musculus* were used with the supervision of a qualified scientist. Mice and rodents were used in many experiments relevant to circadian rhythm studies (Fleury et al., 2020); (Fonken et al., 2014);(Marsís Vargas et al., 2019), and have been used in translational studies on human physiology. Again, due to time and financial limitations, *Mus musculus* was the most suitable option available. Due to a limited number of mice, eleven mice per group were used for a total of 44 mice.

Mice are social creatures and therefore perform closer to "normal" within experimentation by being housed with other mice. Originally, previous research determined that the experiment should only use female mice in order to avoid violence as a significant factor within the male mice, as male mice tended to behave more violently when placed around other males and females (National Research Council, 2011). The first 15 mice received were all females, and randomly sorted into each group. However, due to a limited supply, the remaining mice received were a mix of both females and males. Initially, this was not considered a limiting factor and again, mice were randomly sorted into each group. However, it was immediately apparent that the male mice were behaving aggressively towards both the female mice and the other males, as a large increase in bite marks and squeaking was reported. Therefore, it was necessary for the safety of the mice to group the male mice together. A qualified scientist provided assistance as necessary to reorganize these mice. Male mice are clearly distinguishable from female mice the older they get, but the method is simple: males will visually appear to have a larger protrusion from the hind view and customarily appear bigger and exhibit more aggressive behavior. These male mice were grouped together into group L:N:D:N.

Setup

Four cages were used to ensure ample space for each group of eleven mice. Since the mice used were albino, they are more sensitive to phototoxic retinopathy and therefore should not experience lights above 325 lux or risk damaging their eyes (National Research Council, 2011). Due to this, bright lights were in the range of 250-260 lux measured with a lux meter at cage level. Bright lights run on a 12-on 12-off schedule, set for 0700-1900 on and 1900-0700 off. Cages were then equipped with dim lights that were in the range of 10-15 lux at cage level, roughly the lux level of a cell phone. These dim lights were set to timers in three cycles: 1) four hours dim light, eight hours off (L:D:N); 2) four hours off, four hours dim, four hours off (L:N:D:N); and 3) eights hours off, four hours dim (L:N:D). The fourth group was kept as a control, and only experienced 12 hours of light and 12 hours of dark, or (L:N). Within each cage, mouse houses were provided as a place for shade and shelter. Each cage received bedding, a mouse house, a chew stick, a water bottle, and a food tray. Food pellets were used to give mice a way to grind their teeth as well as provide sustenance. Four mL of pineapple juice was incorporated into 500 mL of water as adding small amounts of sugar to water reduces discomfort and encourages mice to stay hydrated (Litwak, K. et al., 2015).

Husbandry

Fifteen square inches is required per mouse in order to maintain a comfortable environment. Therefore, cages 20.25" x 10.5" were used with a maximum of eleven mice per cage to ensure proper space was given. Glass aquariums were utilized as cages, as they are easy to clean and deodorize as well as have a solid floor, whereas a mesh floor can lead to injuries in the feet and hindlimbs due to becoming trapped in the openings. Paper-based bedding was used due to high absorption and soft, larger, fibrous fragments, which mice tend to prefer and which cause the least amount of irritation (Frohlich, 2021). Cleanouts were administered biweekly, with a bedding and necessary materials change every Wednesday and a full bleach cleaning every Sunday. A soap-bleach solution was used with hot water to thoroughly disinfect and then rinsed off multiple times to remove harsh chemical fragrances.

Mice require a relatively warm, stable temperature in order to remain comfortable in their environment (Litwak et al., 2015). The temperature in the mice rooms was kept between 70^{o}-85^{o} in accordance with the aforementioned research. The room experienced little to no disturbance

throughout the day, and was kept secluded from heavy foot traffic, noises, and vibrations which may cause impaired cognitive and behavioral function in the mice. It was also ensured that the only light the mice received was experiment-induced in order to limit inconsistencies between the groups.

Handling

Marking**:** Since each mouse's weight is tracked individually, the mice must be marked as they were grouped together. Ear tagging was the preferred method of identifying animals, however, due to financial/time constraints, an alternative method was adopted: food coloring. Food coloring marks last between 1-2 weeks and produces a safe, temporary identification mark for albino/light-colored mice (Cadillac, 2006). Non-gel food coloring was discovered to work the best and was applied via q-tip. Blue and green were ultimately used to mark the mice as preliminary trials indicated that red caused heightened aggression between the mice. Directly after marking, the mice were held away from the other mice until the food coloring had set so that the marks did not become indistinguishable.

Weighing: The mice were individually weighed weekly within the experiment via a scale that tracked to milligrams. A small box, such as a nail box, can be used to hold the mice throughout the weighing process, and tape can be administered if necessary. As the mice are prone to scurry around which changes the weight on the scale, waiting until they are settled down is crucial to obtain an accurate weight. To transfer mice from the scale to the cage, it may be necessary to pick them up. The easiest method for this is to pick them up by their tails, which is commonly practiced throughout research laboratories (Litwak et al., 2015). However, this can be stress-inducing and if necessary, an open hand or a small tube that a mouse is likely to climb into can be used for transport.

Procedures for Death

Since live vertebrates are being used in this experiment, procedures for handling death are extensive. If a vertebrate dies while in the care of the researcher, it is required that the body of the vertebrate be submitted for a necropsy to DHEC immediately. In the case of this experiment, the qualified scientist was a veterinary pathologist and could perform the necropsy quicker and easier than submission to DHEC would allow. For both mice that died, this procedure was followed and the pathology reports are found below (See Appendix B-C).

Procedures for the euthanization of mice neonates allow cervical dislocation for up to 10 days of age. This was performed on all pups birthed throughout the experiment. For the adult mice, proper euthanization and disposal measures were followed by the qualified scientist.

Data Collection

The importance of well-organized data collection sheets is highly stressed throughout this experiment. Twice a day, documentation of the upkeep of the mice is required. Digital or paper collection is acceptable, although paper collection was found to be more feasible. Daily data sheets should include the time of day checked upon, amount of food/water remaining/administered, temperature, and area for notes for each day. Weekly weigh-in sheets should include at least one row for each mouse for weight collection and necessary remarks.

Results

Experimentation initially provided eleven trials per group. However, during experimentation, this number varied. Mice, particularly males, have a tendency to a higher level of violence than female mice do. In group L:N:D, two mice died during experimentation and their respective results were nullified. A necropsy was performed on both mice, and it was shown that in mouse #001, the resulting diagnosis was intestinal nematodiasis, or pinworms (See Appendix B). These pinworms were found in the large intestine, are common in laboratory mice, and are typically insignificant unless they develop to a severity that could be fatal. In mouse #002, the diagnosis was ultimately pneumonia. This led to only nine mice being considered within this group.

Furthermore, mice become sexually reproductive within six to eight weeks of birth. This would not present an issue had the mice been organized by male and female correctly. However, two mice were incorrectly identified as females and kept in the control group, or L:N. As these mice got pregnant, this led to wildly skewed data as body weight highly fluctuates during pregnancy. Therefore, any mice that showed signs of pregnancy were excluded from the final data used for the control group. This left only five mice able to be used from this group.

Analysis

To evaluate the body weight change in the mice, a body weight increase percentage was calculated for each mouse using the collected data (See Appendix 1), as shown in the table below. After this calculation, it was unnecessary to keep the labels that identified the mice from one another as that was only needed to accurately track their weights.

Figure 2 shows that L:N experienced the most body weight percentage changes, with a high of 55.7%. However, L:N also had the largest range of percentages, with a low value of 18.1%. Furthermore, it shows that L:D:N had the lowest body weight percentage changes, with the lowest value being 0.31% and the high being 16.4%. L:N:D:N averaged 22.2% change, however, the data was compacted, and the range was only 20, as compared to L:N at 37.6.

To test for a significant difference, statistical analyses were conducted. A one-way ANOVA was run with H_o: all mean percent body weight increases are the same and H_1: at least one mean is different from the rest. The ANOVA ran with an alpha value of 0.05 and received an F value of 2.76 and a p-value of <0.01. (Table 3). The rejects the null hypothesis and proposes a significant difference between the means.

Therefore, a post hoc analysis was run. Due to different sample sizes, a Scheffe test was used. The Scheffe test indicated a significant difference between 1 (L:N) and 2 (L:D:N), 1 and 3 (L:N:D), and 2 and 4 (L:N:D:N). Although a significant difference was found between groups 2 and 4, it was excluded due to the fact that it was not between the control group, or group 1.

Discussion

The purpose of this research was to determine if varying times of dim light in a 12L:12N schedule significantly affected *Mus musculus* body weight and therefore if controlling exposure to light throughout the night could help reduce the chance of obesity. This study contained three groups exposed to differing times of dim light throughout the night and a control group and was designed to measure body weight as an indicator of increased food intake and metabolic dysfunction. The hypothesis was partially supported, with there being a significant difference between the

control groups and the dALAN groups, just not where originally hypothesized.

The significant differences occurred between 1 (L:N) and 2 (L:D:N) and 1 and 3 (L:N:D), as the third significant difference got excluded due to the difference not being near the control group. Neither of these groups was hypothesized to have the most significant bodyweight percentage increase, yet both did have it, whereas L:N:D:N did not. However, based on the collected data, the control group had the highest body weight percentage increase. Therefore, these results imply that the groups that experienced dALAN experienced less bodyweight percentage increase than the control group.

These results can be attributed to experimental design limitations. As previously addressed, the control group only contained five trials, whereas L:N:D:N contained nine, and the other two contained eleven. Originally, all mice planned to be used for the experiment were female, as they were less likely to fight and restrict food from one another (National Research Council, 2011). However, supply shortages proved this impossible, and 13 of the 44 mice received ended up being male. Male and female mice being grouped together showed to be ineffective, and eventually, the male mice were grouped together as L:N:D:N. Two male mice were incorrectly sorted and were placed into the control group L:N. As the gestation period for mice is fairly short, the female mice began getting pregnant. This skewed the results as this was abnormal weight gain, and all pregnant mice were excluded from the results. Again, due to supply limitations, the mice received were nocturnal, or their circadian rhythm would be the opposite 12 hours of a human. However, other studies have used nocturnal mice in behavioral research involving food, and it is shown that a nocturnal mouse's circadian rhythm is able to mimic that of a diurnal mouse (Hut, 2011).

In the future, diurnal mice should be used to more accurately test for the consequences of dALAN exposure. Furthermore, these mice should all be of one gender, preferably female, to minimize fighting and eliminate the chance of pregnancy. This would help curb the inaccuracies in food intake due to mice acting territorially. Another solution to these issues would be the keep each mouse separately, a method not imposed in the experiment due to supply limitations. This would eliminate all fighting and food intake could be monitored individually. However, mice are social creatures, and this could lead to emotional or psychological issues that affect feeding.

Although no other studies have specifically identified which time of light affects body weight most significantly, it has been shown that experiencing light during the night does affect the circadian rhythm set in mice and humans (Pauley, 2004). Intensity and color of the light experienced affect the circadian rhythm more/less harshly as well. These can be further explored in future research. The possibility of different wavelengths or intensities of light at these specific times could also be considered, due to a lack of research in this area. Furthermore, a look into whether the gender of mice affects the food intake could be researched, as this experiment showed that there was a significant difference in the timing of light on bodyweight percentage increase. Finally, while measuring weight gain is a simple indicator of metabolic function, this experiment did not consider how dALAN affects the physical aspect of mice. In the future, locomotor activity could be tracked as a better indicator of the full realm of the effects of dALAN

These results help establish guidelines for better sleeping habits and ultimately could help eliminate a factor in unhealthy weight gain. As stated before, most Americans live in an area where they experience light pollution daily, and the use of electronics only further heightens experiencing dALAN. Even though the results of this experiment had some sources of uncertainty, it can still be acknowledged that experiencing chronic ALAN can result in unhealthy weight gain. By reducing this exposure, many Americans can begin to lead healthier lives.

Notes and References

World Health Organization. (n.d.). *Obesity and overweight*. World Health Organization. Retrieved October 29, 2021, from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight laboratory animals (Tenth, pp. 1–14). essay, Animal Welfare Institute.

Masís-Vargas, A., Hicks, D., Kalsbeek, A., & Mendoza, J. (2019). Blue light at night acutely impairs glucose tolerance and increases sugar intake in the diurnal rodent Arvicanthis ansorgei in a sex-dependent manner. *Physiological Reports*, 7(20), e14257. https://doi.org/10.14814/phy2.14257

McHill, A. W., & Wright, K. P., Jr (2017). Role of sleep and circadian disruption on energy expenditure and in metabolic predisposition to human obesity and metabolic disease. *Obesity Reviews : An Official Journal of The International Association for the Study of Obesity*, 18 Suppl 1, 15–24. https://doi.org/10.1111/obr.12503

Chepesiuk R. (2009). Missing the dark: health effects of light pollution. *Environmental Health Perspectives*, 117(1), A20–A27. https://doi.org/10.1289/ehp.117-a20

Baron, K. G., & Reid, K. J. (2014). Circadian misalignment and health. *International Review of Psychiatry* (Abingdon, England), 26(2), 139–154. https://doi.org/10.3109/09540261.2014.911149

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(2), 85–93.

La Fleur, S. E., Kalsbeek, A., Wortel, J., Fekkes, M. L., & Buijs, R. M. (2001). A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. *Diabetes*, 50(6), 1237–1243. https://doi.org/10.2337/diabetes.50.6.1237

Sharma, S., & Kavuru, M. (2010). Sleep and metabolism: an overview. International *Journal of Endocrinology*, 2010, 270832. https://doi.org/10.1155/2010/270832

Knutson, K. L., Spiegel, K., Penev, P., & Van Cauter, E. (2007). The metabolic consequences of sleep deprivation. *Sleep Medicine Reviews,* 11(3), 163–178. https://doi.org/10.1016/j.smrv.2007.01.002

Arble, D. M., Bass, J., Laposky, A. D., Vitaterna, M. H., & Turek, F. W. (2009). Circadian timing of food intake contributes to weight gain. *Obesity* (Silver Spring, Md.), 17(11), 2100–2102. https://doi.org/10.1038/oby.2009.264

Mendoza J. (2021). Nighttime Light Hurts Mammalian Physiology: What Diurnal Rodent Models Are Telling Us. *Clocks & sleep*, 3(2), 236–250. https://doi.org/10.3390/clockssleep3020014

Duffy, J. F., & Czeisler, C. A. (2009). Effect of Light on Human Circadian Physiology. *Sleep Medicine Clinics,* 4(2), 165–177. https://doi.org/10.1016/j.jsmc.2009.01.004

Fleury, G., Masís-Vargas, A., & Kalsbeek, A. (2020). Metabolic Implications of Exposure to Light at Night: Lessons from Animal and Human Studies. Obesity (Silver Spring, Md.), 28 Suppl 1(Suppl 1), S18–S28. https://doi.org/10.1002/oby.22807

Alaasam, V. J., Duncan, R., Casagrande, S., Davies, S., Sidher, A., Seymoure, B., Shen, Y., Zhang, Y., & Ouyang, J. Q. (2018). Light at night disrupts nocturnal rest and elevates glucocorticoids at cool color temperatures. *Journal of Experimental Zoology*. Part A, Ecological and integrative physiology, 329(8-9), 465–472. https://doi.org/10.1002/jez.2168

Fonken, L. K., & Nelson, R. J. (2014, August 1). Effects of light at night on circadian clocks and metabolism. *OUP Academic*. Retrieved September 10, 2021, from https://academic.oup.com/edrv/article/35/4/648/2354673.

National Research Council. (2011). Terrestrial Animals. In Guide for the Care and Use of Laboratory Animals (Eighth, pp. 42–54). essay, National Academy of Sciences.

Litwak, K., Liss, C., Tilford, D., & Reinhardt, V. (2015). Mice. In Comfortable quarters for laboratory animals (Tenth, pp. 1–14). essay, Animal Welfare Institute.

Frohlich, J. (2021, December 15). Mice and rats as pets - exotic and laboratory animals. *Merck Veterinary Manual.* https://www.merckvetmanual.com/exotic-and-laboratory-animals/rodents/mice-and-rats-as-pets

Cadillac, J. D. V. M. (2006, December 1). Animal Identification Systems used for mice. *The Jackson Laboratory.* https://www.jax.org/news-and-insights/2006/december/animal-identification-systems-used-for-mice#

Hut, R. A., Pilorz, V., Boerema, A. S., Strijkstra, A. M., & Daan, S. (2011). Working for food shifts nocturnal mouse activity into the day. *PloS One,* 6(3), e17527.

https://doi.org/10.1371/journal.pone.0017527
Pauley S. M. (2004). Lighting for the human circadian clock: recent research indicates that lighting has become a public health issue. *Medical Hypotheses*, 63(4), 588–596. https://doi.org/10.1016/j.mehy.2004.03.020
Centers for Disease Control and Prevention. (2021, June 7). *Adult Obesity Facts*. Centers for Disease Control and Prevention. Retrieved October 29, 2021, from https://www.cdc.gov/obesity/data/adult.html.
Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *Lancet* (London, England), 354(9188), 1435–1439. https://doi.org/10.1016/S0140-6736(99)01376-8

Figures and Tables

Figure 1: Experimental Design Diagram

Title of the Experiment
Role of Dim Artificial Light at Night (dALAN) on Weight of Mus musculus

Hypothesis
It is hypothesized that if mice are exposed to dALAN at varying times throughout the night, they will differ in food intake and therefore body weight due to non-homeostatic eating based upon circadian misalignment.

Independent Variable
Timing of exposure to dALAN

Levels of Independent Variable	12L:12N	12L:4D:8N	12L:4N:4D:4N	12L:8N:4D
Number of Repeated Trials	11	11	11	11

Dependent Variable
Weight of each mouse

Control Group
The control group is the 12L:12N group, which only experiences 12 hours of light and 12 hours of night.

Constants
Some constants included the brand of food administered, how much food was administered, how much water was given daily, cage setup, type of bedding used, size of cage, intensity of light, type of light used, temperature, and how often cleanouts were.

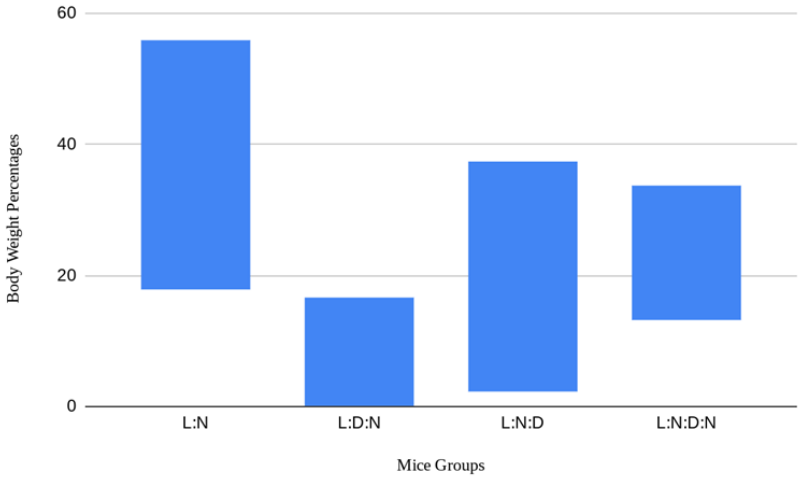

Figure 2. Candlestick Chart of Body Weight Percentage Changes of L:N, L:D:N, L:N:D:N, and L:N:D

Table 1. Percent Body Weight Increase In Each Mouse

L:N	L:D:N	L:N:D	L:N:D:N
54.40758294	16.42178047	11.43076333	25.78292578
27.73919753	16.01700921	12.8	28.62789385
18.08403361	0.3172085646	2.501737318	33.5106383
55.69904548	4.262542437	20.36964215	13.42342342
29.06494703	10.15943641	5.087440382	17.69704073
	9.152035861	33.28865058	23.22175732
	7.993079585	9.337860781	17.59868421
	8.318457694	16.96806464	25
	10.9940288	10.79069767	15.02911208
	9.839650146	37.18297101	
	5.294530154	15.71115974	

Table 1 displays the percent body weight increase in each mouse per group. It is not necessary to have individual mouse labels anymore as that was only a way to identify them from one another as to be able to track their weights accurately.

Table 2. ANOVA Tables per Group

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2902.1928	3	967.39762	10.619344	0.0000530	2.9011195
Within Groups	2915.1255	32	91.097674			
Total	5817.3184	35				

The tables above contain the ANOVA results when a one-way ANOVA was run on each test group. All groups returned a p-value less than 0.05, meaning that the null hypothesis was rejected.

Table 3. Scheffe Test

Data Combinations	F'	F x (k-1)	Interpretation
1 Vs 2	29.62	8.71	Significant
1 Vs 3	16.72	8.71	Significant
1 Vs 4	7.72	8.71	Non Significant
2 Vs 3	2.93	8.71	Non-Significant
2 Vs 4	9.51	8.71	Significant
3 Vs 4	2.13	8.71	Non-Significant

Appendix

L:N:

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
LH BL	21.10	22.94	25.75	26.89	27.97	32.58
RH BL	29.69	34.18	48.99	41.23	39.52	50.33
LS BL	25.92	28.99	30.90	31.11	32.52	33.11
RS BL	29.75	32.84	34.66	35.65	36.03	35.13
2D BL	17.81	18.91	22.13	23.69	23.91	27.73
LH GR	28.52	33.48	47.14	37.90	36.12	52.36
RH GR	24.24	28.80	42.40	34.33	33.04	43.85
LS GR	21.71	22.46	24.38	26.89	25.97	28.02
RS GR	20.53	21.12	21.07	22.94	27.60	39.67
2D GR	26.40	29.02	38.90	34.67	34.72	42.98
NM	25.85	26.92	32.85	48.67	32.52	32.73

The yellow highlight denotes excluded data. This data was excluded because these mice got pregnant and gained large amounts of body weight, which was not conducive to accurate data.

L:D:N:

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
LH BL	23.14	25.18	24.88	24.70	25.95	26.94
RH BL	28.22	31.27	31.56	30.46	31.80	32.74
LS BL	25.22	26.27	24.47	25.00	24.64	25.30
RS BL	26.51	26.78	26.16	25.47	26.10	27.64
2D BL	26.97	26.50	26.88	27.74	29.10	29.71
LH GR	26.77	28.70	28.27	29.04	29.75	29.22
RH GR	28.90	29.51	28.71	29.90	30.36	31.21
LS GR	28.01	27.69	29.14	31.60	31.20	30.34
RS GR	28.47	30.62	31.93	31.16	31.00	31.60
2D GR	27.44	28.03	26.91	27.43	27.76	30.14
NM	28.52	29.49	29.70	28.79	30.01	30.03

L:N:D:N:

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
LH BL	26.07	26.25	26.95	26.59	26.13	29.05
RH BL	25.00	27.11	27.62	28.40	26.95	28.20
LS BL	28.78	28.38	28.18	27.88	28.72	29.50
RS BL	25.43	23.44	21.44	24.21	28.40	30.61
2D BL	31.45	31.92	31.25	31.40	31.93	33.05
LH GR	22.38	25.53	28.59	29.03	30.92	29.83
RH GR	29.45	29.59	29.77	30.53	31.53	32.20
LS GR	25.99	27.52	28.33	29.24	29.17	30.40
RS GR	21.50	22.90	23.47	23.25	23.39	23.82
2D GR	22.08	25.08	27.69	28.04	28.48	30.29
NM	22.85	24.31	25.92	25.38	26.20	26.44

L:N:D:

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
LH BL	23.31	26.82	27.02	26.31	27.96	29.32
RH BL	16.21	-	-	-	-	-
LS BL	17.71	20.14	20.04	21.08	22.63	22.78
RS BL	28.20	31.53	32.13	34.32	35.50	37.65
2D BL	-	-	-	-	-	-
LH GR	33.30	34.70	36.57	38.18	36.77	37.77
RH GR	34.13	35.16	37.94	39.77	39.79	40.17
LS GR	28.68	31.36	30.42	30.77	31.94	35.34
RS GR	30.40	31.84	31.93	33.64	32.14	35.75
2D GR	27.32	29.08	29.66	30.63	33.16	34.15
NM	27.48	30.18	30.43	30.00	29.60	31.61