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Effect of ambient temperature on recovery of surgically instrumented Sprague-Dawley rats

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Effect of Ambient Temperature on Recovery of Surgically Instrumented Sprague-Dawley Rats

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I. Acknowledgements

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III. Abstract

Based on current guidelines for housing rodents after surgical instrumentation, rodents may be housed at temperatures that hinder surgical recovery and cause thermal stress– room temperature (21°C) being one of them. Rodents are often housed at room temperature since this temperature is easy for humans to be caring for them. However, if recovering rodents are thermally stressed, experimental results will be confounded. To address this issue, Sprague-Dawley rats were surgically instrumented with radiotelemtry thermoprobes (Data Sciences, #TA-10F40) to monitor their core temperatures, then housed at one of five temperatures to assess the effect ambient temperature had on multiple aspects of their recovery from surgical instrumentation. Previous studies have found that when rats, which have not gone undergone surgical instrumentation, can choose their ambient temperature they choose 27°C (Pham-Le and Brown 2012). Knowing this, rats recovering from surgery may benefit from a slightly warmer temperature than their preferred temperature since heat was lost to the environment from the exposing of body cavities during surgery. In this project it was hypothesized that rats recovering at slightly warmer temperatures (30°C and 33°C) than their preferred temperature of 27°C would recover more quickly than rats recovering at colder temperatures (21°C and 24°C). To test the hypothesis, various aspects of recovery were assessed among the groups such as survival rate, return to a normal core temperature (36.5°C), return to pre-surgical weight, food consumption, and water consumption. It was found that rats in the 27°C group had a higher survival rate (80%) than the rats at the warmer groups, which partially rejects the hypothesis. Surprisingly, there was no trend in the effect of Tamb to the time to return to a normal core temperature of 36.5°C. The statistical analysis for the return to pre-surgical weight, food consumption, and water consumption were not accomplished due to time constraints from COVID-19. The statistically tested data suggests that mild heating may not facilitate surgical recovery as hypothesized. Although, accurate conclusions cannot be made because the sample sizes were too small and further statistical analysis must be done. In the future, larger samples sizes will be obtained to gain more clarification on the effect temperature has on surgical recovery.

IV. Introduction

Summary of problem

Current housing guidelines suggest that Sprague-Dawley (SD) rats be housed at a temperature within the range of 20°C to 26°C (Guide 2011). Rats are normally housed at room temperature (~21°C), since this is a comfortable temperature for humans to care for them. However, previous work in this lab has found that when rats are allowed to choose their own ambient temperature (Tamb) they choose 27 °C (Pham-Le and Brown 2012). This behavior of rats to choose a warmer temperature, one that it outside of current guidelines, suggests that rats are more comfortable at higher temperatures than what is required by current housing guidelines.

SD rats are often surgically instrumented with devices to measure physiological processes such as core temperature (Tc), motor activity, heart rate, etc. Surgery greatly affects normal thermoregulation. When anesthetics are used to surgically instrument rats, not only does the invasive surgical instrumentation lead to the loss of body heat by exposing body cavities to the environment, but anesthetics also cause a lowering of body temperature by deactivating thermoregulatory control mechanisms which leads to hypothermia (Prudian et. al 1997). Normal Tc is 36.5 to 38C in unstressed SD rats, but can greatly decrease following invasive surgery. “Small mammals can experience a decrease of 4 to 10 °C in core body temperature during only 15 to 20 min of general anesthesia, resulting in marked hypothermia” (Taylor 2007). Therefore, various external heating sources are used to help mitigate the surgically induced hypothermia. “The extremely large body surface area to weight ratio of small animals renders them particularly prone to heat loss during surgery, which must be compensated for with external heating devices” (Roehl et al. 2011). How long, and to what degree that heating should be maintained to facilitate recovery, remains unexplored but could be a significant factor in facilitating surgical recovery by helping to minimize thermal stress. Since the body temperature of a rat is low after surgery, a warmer ambient temperature beyond current guidelines may be beneficial for a rat's recovery in that it could help the animal recover to a normal Tc more rapidly, and thereby reduce the thermal stress in which an animal is exposed after surgery.

Unfortunately, rats are often returned to their home cage and environment shortly after surgery for recovery. This often means the rats are housed at ~21°C, which is comfortable to personnel caring for the animals and is within current guidelines, but well below their preferred temperature. The result is that rats recovering from surgery at room temperature are cold stressed and spend their metabolic energy thermoregulating in addition to recovering from surgical stress. A rat can appear to be fully recovered after surgical instrumentation based on appearance and mobility, but they can be experiencing significant metabolic stress that affects basic metabolism, circadian rhythms, and other physiologic variables that are not easily observed externally, which confounds experimental outcomes. Using thermally stressed rats for experiments will make it difficult to distinguish whether the results were caused by thermal stress or the experiment itself. Observing the detailed behavioral and thermoregulatory responses of a rat during its recovery is a simple way to monitor their recovery progress, but other measures should be taken to ensure that they are fully recovered.

In this study it is proposed that warming rats by housing at 30°C or 33°C for an extended period of time (1 week) after surgery will facilitate recovery while housing rats at cooler temperatures (21°C or 24°C) will hinder that recovery. Food and water intake, body weight changes, and alterations in Tc will be closely monitored for signs of recovery in each group. Findings in each Tamb group will be compared to rats housed at 27°C (control group) because that is their preferred Tamb when unstressed.

Overview of Thermoregulation and the Role of Tamb in Housing of Rats

The thermal neutral zone (TNZ) is the range of Tamb at which rats are using minimal metabolic energy to thermoregulate (Gordon 1993). It is marked by the lower critical temperature (LCT) and the upper critical temperature (UCT), which in SD rats is 22°C and 27°C respectively (Gordon 1993). When a rat is housed outside of these Tamb it will spend more metabolic energy thermoregulating. Since the room temperature at which rats are often housed (21°C) is below the LCT for rats, rats are forced to spend their metabolic energy on generating heat to maintain core temperature (Tc). If this occurs after surgery, rats are spending metabolic energy regulating their normal Tc instead of recovering from surgical stress. This may prolong their recovery from surgery. Conducting lab experiments with rats at temperatures within the TNZ however allows for the closest approximation of the normal, unstressed physiologic response. Therefore, the results from an experiment will not be confounded by thermal stress factors.

The brain normally coordinates thermal homeostasis responses despite numerous alterations in environmental temperatures. The preoptic area (POA) of the hypothalamus, the thermoregulatory control center in mammals, activates thermo-effector mechanisms in a multitude of ways to bring Tc back to set point when mammals are exposed to extreme external temperatures. The POA does this by taking information in from the body's temperature sensors and comparing the Tc to the thermoregulatory set point, then determining what changes need to take place. Exposure to temperature extremes outside of the TNZ result in behavioral, physiological and morphologic changes (Gordon 1993). If the ambient temperature where rats are housed is below the LCT or above the UCT, the Tc may be forced outside of the normal range requiring the POA to coordinate metabolic and behavioral changes to thermoregulate.

When Tc is below the LCT, shivering thermogenesis can bring the core temperature back to set point by the shivering of skeletal muscle, which leads to the oxidation of fatty acid reserves in adipose tissues (Vaillancourt et al. 2009). However, non-shivering thermogenesis is a more common automatic response to cold stressed rodents. This process involves the activation of brown adipose tissue located in the interscapular region on a rat, which helps generate heat by making ATP production less efficient (Himms-Hagen 1984). Also initiated by the POA, the vasoconstriction of the vasculature in the rat's tail brings blood closer to their core to limit heat loss to the environment. Behaviorally, rats will burrow, or minimize their surface area by huddling their posture, to prevent heat loss when they are cold (Gordon 1993). When the ambient temperature is above the UCT, the POA will activate thermo-effectors that vasodilate vasculature in the rat's tail to facilitate heat loss. Rats will also sprawl out their bodies to lose heat to the environment or lick their fur to spread saliva on their skin, which when it evaporates, helps to lower Tc (Hainsworth 1967).

The outcome of biomedical experiments in the whole animal often depends on the thermal environment and can be greatly influenced by endocrine mechanisms as well (Romanovsky et. al 2002). For example, stress hormones like cortisol, epinephrine, thyroid hormone, and overall sympathetic outflow of the nervous system are increased during stress. Each of these would increase thermogenesis and many would affect cardiovascular and respiratory function too. The hypothalamus-pituitary-adrenal gland axis (HPA) is activated during times of stress and releases stress hormones that can be measured in animals. In rats it was found that when exposed to mild heat stress (32˚C) for one week, there was a significant increase in adrenocorticotropic steroids and Cortisol, which are the end product of the HPA activation (Wang et al. 2015). For cold stress on the other hand, it was found that rabbits exposed to moderate cold stress temperatures had an increase in thyroid hormone, presumably to increase their core temperature (Brown-Grant et al. 1954). These stress hormones are often considered as the most classic and important indicators in studying stress (Wang et al. 2015). "Currently there is a lack of understanding of how the laboratory environment affects animal physiology and behavior, particularly as it might effect disease states. Given the evidence on how stressors affect physiology, efforts to model human physiology in animal models must consider animal stress as a confounding factor" (Gaskilll and Garner 2017). If experiments are conducted in an environment where the animal is not thermally stressed then the data collected from the animal would be less affected by the compensatory neuroendocrine responses to it, and therefore the data collected would be more reliable and repeatable. While others suggest that rats recover adequately at a wide range of temperatures, we predicted that rats would recover more quickly at 27˚C compared to lower temperatures still within the TNZ. It is possible that higher heat conditions actually might facilitate a faster recovery of normal body temperature.

Significance

Since rats are often used as models to understand the etiology behind health and disease, it is essential that experimental data gathered from them be from animals that are in their normal physiological state and not affected by undue stress outside of their experimental parameters. This is why governing bodies that oversee animal research (OLAW – Office of Lab Animal Welfare and local IACUC- Institutional Animal Care and Use Committees) created guidelines for surgical recovery of lab animals. The guidelines by the IACUC suggest that lab animals in surgical recovery be provided with supplemental fluids, mild external heating (heating pad), ventilation, and constant monitoring after anesthesia until independent movement is observed

(Office of Laboratory Animals Welfare 2002). However, the guidelines do not speak to how long recovery should be after initial movement is observed or when animals are available for experimentation. Perhaps extended mild heating, or even housing at mildly warm Tamb facilitates recovery. Additionally, current housing guidelines allow for housing at Tamb below the LCT and therefore allow for cold stress to affect a rat's recovery. If data from this study suggest that current animal care guidelines allow for housing rats at a Tamb which cause stress, then it would be beneficial to change the housing temperature guidelines for SD rats to limit this thermal stress and thereby facilitate surgical recovery in these animals.

Hypothesis

It is hypothesized that rats housed at their preferred Tamb of 27°C will be less stressed and therefore recover to a normal Tc (36.5°C) more quickly, regain body weight quicker, and survive better, than rats recovering at lower Tamb such as 21°C and 24°C. Alternatively, housing rats at 30°C and 33°C after surgery will facilitate recovery from surgery even more quickly than rats in any other group.

Experimental Design

To better understand which temperature is best for recovery from the surgery the rats were housed at one of a select number of Tamb for at least a week after surgery. To this end, rats were randomly assigned a treatment group where they would be housed in one of five different Tamb (21°C, 24°C, 27°C, 30°C, 33°C) (Table 1). The 27°C group acted as a control group because SD rats prefer this temperature normally. The 21°C group is believed to be in a cold stressed environment although it is technically within the recommended animal care guideline for housing rats. The 24°C group is within the TNZ but is predicted to be a mild cold stress for rats that are recovering from surgery. The 30°C group was slightly above the UCT and is believed to be a mild heat stressor, while the 33°C group acted as a non-life-threatening heat stress. The rats within each group were all monitored for their return to a normal core temperature (36.5°C). This value was chosen because it is considered the low end of the normal Tc in a rat (Gordon, CJ 1996). Since the combined thermal and surgical stress also put the rat's survival at risk, their survival rate for the week of recovery was also monitored.

Table 1. Experimental Groups for Ambient Temperature

Tamb	**Type of Stress**	**Experimental Question**
21°C	Typical Room Temp / mild cold stress	Does housing rats at normal room temperature cause a cold stress which hinders surgical recovery?
24°C	Within the TNZ	Does housing rats at Tamb within their TNZ beneficial for surgical recovery?
27°C	Preferred Ambient Temp (control group) and within the TNZ	Do housing rats at their preferred Tamb affect recovery? 27°C is the preferred Tamb therefore this is the control group.
30°C	Above UCT/ mild warm stress	Does mild warming facilitate surgical recovery?
33°C	Above UCT /warm stress	Does a warm stress facilitate or hinder surgical recovery?

V. Methods

Animal Environment Pre-Surgery

In the JMU Bioscience building Vivarium, rats were housed in polycarbonate cages (50 x 26.8 x 36.4 cm) with shredded corn-cob bedding before surgery. Rats remained at an ambient temperature (Tamb) of 25 ± 1°C, 40 ± 5% relative humidity, with a 12:12 hr L:D cycle (lights on at 0700h). Laboratory rodent chow (Harlan Teklad) and water was provided *ad libitum*.

Surgical Instrumentation

Male and female Sprague Dawley (SD) rats were used in this project because they are a commonly used strain and much of their behaviorial response to surgical and thermal stress are established. The SD rats used in this project must have been at least 7 weeks old and weigh between 225 grams to 385 grams. Preliminary studies in our laboratory have shown that the rats that meet these two requirements are an adequate size for experimentation, and that these rats should be strong enough to withstand the thermal and surgical stressors placed on them. The rats were first anesthetized using a binary mixture of ketamine and xylazine (75 mg/kg ketamine and 15 mg/kg xylazine intraperitoneally). Paralube ointment was glazed over the animal's eyes after the anesthesia was achieved to prevent eye dryness. The rat was prepared for surgery by shaving the abdomen and dorsal skull (as part of a separate surgery done at this time for a different project) and both areas were scrubbed with iodine solution. The animal was placed on a heating pad to maintain Tc at or above 31°C therefore limiting hypothermia during surgery. This pad was covered by a sterile drape on top of an absorbent pad (20"x20"). Another sterile drape with a hole was placed over the rat's incision site so that only the abdomen with the incision was exposed. Then, using aseptic techniques, a 1-inch incision was made at the linea alba of the rectus abdominus to insert a 0.75" x 1.25'' x 0.25" radiotelemetry thermoprobe (Data Sciences, #TA-10F40). This allowed measurement of Tc non-invasively. The muscle of the abdomen was sutured back together using 3.0 silk sutures, and the skin was stapled to close the wound. A sterilized 20-gauge stainless steel cannula (26mm) was sterotaxically implanted into the brainstem and secured in place with 3-4 bone screws and cranioplastic cement as part of the separate project which began after the rat's full recovery from surgery. To prevent debris from clogging the cannula and limit risk of brain infection, the open end of the cannula was capped with a 4mm section of PE90 tubing. To conclude the surgery, rats were injected with saline (1ml/150g) intraperitoneally to replace any fluid that was lost via heat evaporation during surgery. Rats were checked for adequate anesthesia every 10 minutes throughout the surgery via the toe-pinch reflex. All methods and materials have been approved by the JMU IACUC and follow the NIH guidelines for animal care.

<u>Recovery Period</u>

Post-surgery, rats were housed individually with the same pre-surgical housing conditions, aside from the Tamb which was maintained at a set level per experimental group for this project. Rats were also supplied with ibuprofen in their water supply (0.2mg/ml) for the duration of their recovery. Oral administration of analgesia is preferred to injection because there is no handling induced hyperthermia and stress as is the case with injection. Furthermore, oral analgesia has been shown to be more effective than opioids at pain relief (Blaha and Leon 2005). In addition, it assures that this analgesic was ingested, which is a problem in other methods of oral analgesia administration. Based on preliminary studies a rat drinks 38.2ml of water per day. This means the rats ingest about 7.6mg of ibuprofen per day.

The rat's body weight, food consumption, and water consumption were measured around the same time each day. These measurements were important for determining their return to pre-surgical weight and determining if there was any correlation between these measurements (dependent variable) and different Tamb groups (independent variable), which is mentioned in the next section. Since rats can mask pain, it may not be evident when a rat is experiencing pain after surgical instrumentation. The most evident sign of pain in a rat will be reduced food and water consumption (Perret-Gentil 2015). Studies show that approximately 1 week is required for rats to recover following the surgical implantation of radiotelemetry transmitters into the abdomen (Greene at al. 2007). One week is essential for surgical recovery because surgical instrumentation of rats under anesthesia can affect the return to normal heart rate, core temperature, and activity for up to 7 to 10 days following surgery (Prudian et. al 1997). Once a rat had returned to pre-surgical weight, demonstrated a normal circadian rhythm in Tc (increased Tc at night and lower during the day), and recovered for at least one week, it was declared fully recovered. Preliminary studies done in the lab have shown that most rats have recovered by day 6-7. However, upon rare occasion some rats can take up to 14 days to meet these recovery requirements.

Experimental Design:

To explore the effect of Tamb on surgical stress, rats were randomly placed in one of five different Tamb groups for the duration of their recovery. Warm air circulators were affixed to the cage's top, under the HEPA filter, to maintain the Tamb within ± 1°C of the set Tamb (Figure 1). The five experimental groups for Tamb were 21°C, 24°C, 27°C, 30°C, or 33°C so that various intensities of heat and cold stress could be analyzed (Table 1).

Figure 1. Cage Top Warmer. The gray piece on top of the cage is the warm air circulator, which lies below the HEPA filter. It maintains Tamb within strict limits for the duration of the recovery period.

Each rat cage rested on a receiver that was calibrated to the rat's thermoprobe. The receiver measured the rat's Tc every five minutes for the duration of recovery. The Tc was recorded on the lab computer using specialized software (Data Science International DataQuest 2.0). Since rats are nocturnal and follow a 12:12 hr L:D cycle (lights on at 0700h), it was expected that the Tc would be highest during the hours of 7pm-7am, and lowest from 7am-7pm. These times were chosen to correlate to the normal light:dark phases in the vivarium where the light phase is from 7am-7pm. When viewing the Tc of the rat, peaks during the middle of the dark phase and troughs during the middle of the light phase were expected for a normal, cycling circadian rhythm

<ins>Data Analysis</ins>

The recovery data of each rat, which includes its body weight, food consumption, water consumption, and Tc was compiled into a cumulative file in Excel and organized into the five Tamb groups. Data was compared between each Tamb group to determine if it affected recovery time. To assess whether Tamb influences survival rate, the number of rats that survived to full recovery were counted in each Tamb group. If rats did not survive past day 1 of surgical recovery it was assumed that it was because of surgical stress or a surgical complication, and not because of the temperature stress, therefore these rats were excluded from the survival rate calculations. It was planned that a Kaplan-Meir analysis would have been used to determine survival rate (Table 2). Unfortunately, due to JMU's response to the Corona virus, which involved closing campus, further detailed statistical analysis could not be completed because of the lack of access to the Bioscience building where the raw data resides. Instead, a simple calculation of survival rate was done by dividing the number of rats alive after day 7 with the total number of rats that entered the study after day 1.To further asses the effect of Tamb on Tc, the mean Tc for each Tamb group was compared at 24 hours of recovery (7:00pm of Day 2 of recovery) using a One-Way ANOVA test with Tukey's post hoc analysis and significance set to $p \leq 0.05$.

To assess the body weight fluctuations of rats over the recovery period, a Two-Way ANOVA test was used (day of recovery and Tamb as factors). Since the rats chosen for each temperature group did not all start at the same pre-surgical weight, the percent control change in body weight was calculated in order to determine the body weight fluctuations over one week of recovery. Therefore, the mean body weight percentage drop from pre-surgical weight was compared each day of recovery among each Tamb group.

Lastly, the mean daily food and water consumption were compared between each Tamb using a Two-way ANOVA test (day of recovery and food/water intake as factors). All tests were done using Sigma Plot V11.0 and the p value for the standard of significance was set at ≤ 0.05 for all statistical comparisons.

VI. Results

One of the main goals of this study is to determine if the Tamb affects the ability of a surgically instrumented rat to fully recover from the surgical stress. One of the fundamental measures of recovery is the ability to survive the combined thermal stress and surgical stress for the full week of recovery. Table 2 outlines the survival rate of rats in each Tamb group. Surprisingly, the rats with the highest survival rate were those in the 21°C group (82%) and those with the lowest were housed at 30°C (70%) and 33°C (63%). Normally, a Kaplan-Meier analysis would be done to determine actual statistical significance between groups, if any. However, due to JMU's response to COVID-19, and the subsequent closing of labs and access to data, tools to analyze it, and time to do that analysis, these aspects of the project could not be explored.

Table 2. The Effect of Ambient Temperature on Survival Rate.

Tamb Group	Number of rats that survived to full recovery	Number of rats that did not survive to full recovery	Total rats per group	Full Recovery Rate (%)
21°C	9	2	11	82%
24°C	8	3	11	73%
27°C	12	3	15	80%
30°C	16	7	23	70%
33°C	12	7	19	63%
Total Rats			79	

Core temperature was monitored for the effect of Tamb on both short- and long-term changes. Figure 2 displays the mean Tc of each Tamb group during the week following surgical instrumentation. At first glance the data do not seem significantly different. Each rat starts with an incredibly low Tc near 33°C– 34°C after surgery as expected. As anesthesia wears off over the next several hours, they begin to thermoregulate on their own and Tc stabilizes. Then, their circadian cycling of Tc begins to become apparent at ~day 3 (72 hrs) after surgery in most groups. Determining differences in the onset of circadian cycling in each Tamb group would be a valuable addition to this project in the future. It is not part of this project due to time constraints. In the 24 hours following surgery however there may be a subtle difference in how the mean Tc in each Tamb group returns to a normal level (Figure 3). Rats housed at 24°C and 33°C seemed to reach a normal Tc (36.5°C) the quickest while the moderate temperature groups (27°C and 30°C) seemed to take the longest. Again, more animals need to be included in the study and statistical analysis will need to be done before any official conclusions can be made.

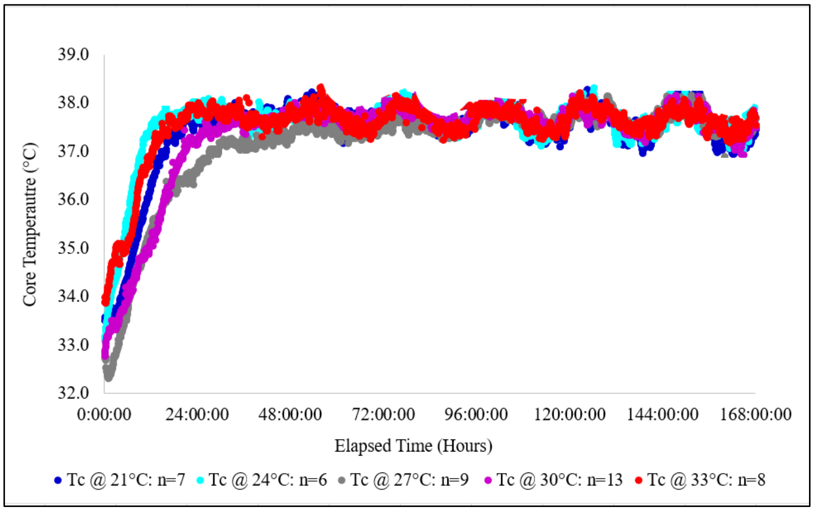

Figure 2. Fluctuations in Tc among each of the Five Tamb groups over the course of 7 days After Surgery. Tc of rats were tracked for at least one week after surgery. A circadian rhythm seems to appear at ~day 3 (72 hrs) after surgery. Minor differences in Tc between the Tamb groups may exist before day 3 of recovery time.

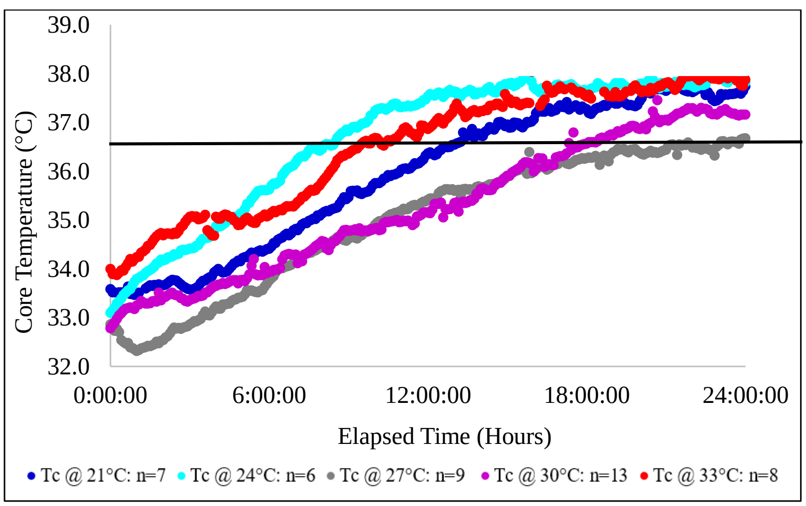

Figure 3. Effect of Tamb on Tc Recovery in the 24 Hours After Surgery. Tc of rats were tracked for at least a week after surgery. By isolating the data in the first 24 hours after surgery some minor differences may be observed.

The decrease in rats' body weight from pre-surgical levels was predicted after surgery, but the extent of that variation was unknown (Figure 4). After surgery, the body weight of rats in each Tamb group continued to drop until ~ day 3 of recovery, then each of the rats returned to their pre-surgical weight on or before day 7. Rats in the 27°C and 24°C groups actually reached pre –surgical levels on day 6 while the other groups were recovered by day 7.There also appears to be differences in weight fluctuations between the 24°C and 30°C groups in how much weight they lost although the variance in each group is rather large so definitive conclusions will have to wait until more animals and proper statistical analysis can be done.

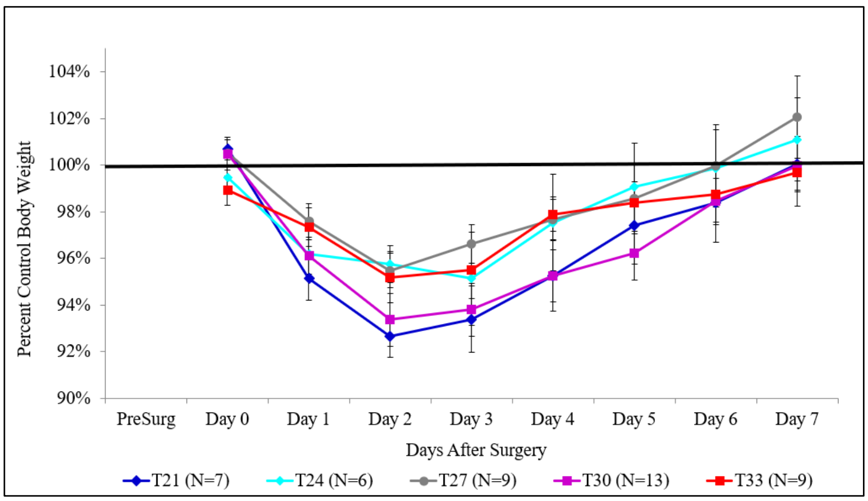

Figure 4. The Effect of Tamb on Restoration of Body Weight to Pre-surgical Levels. "Pre-Surg" is weight of rat before surgery whereas "Day 0" is body weight of the rat after surgery. Some minor differences may be observed between the 24°C or 21°C groups and the others.

The food and water consumption of rats during recovery gradually increases over one week of recovery (Figures 5 and 6). Each data point represents the total food/water ingested for that single day. So in each graph the rats progressively ate and drank more than the previous day until those values leveled off near the end of the recovery week. On day 6 of recovery there seems to be a difference in food consumption between the 33°C and 21°C Tamb groups. Water consumption increases daily in all groups until it levels off at the standard water intake of rats, which is ~40g/day. Again, further statistical analysis of these differences will be accomplished in the future as well as obtaining a larger sample size.

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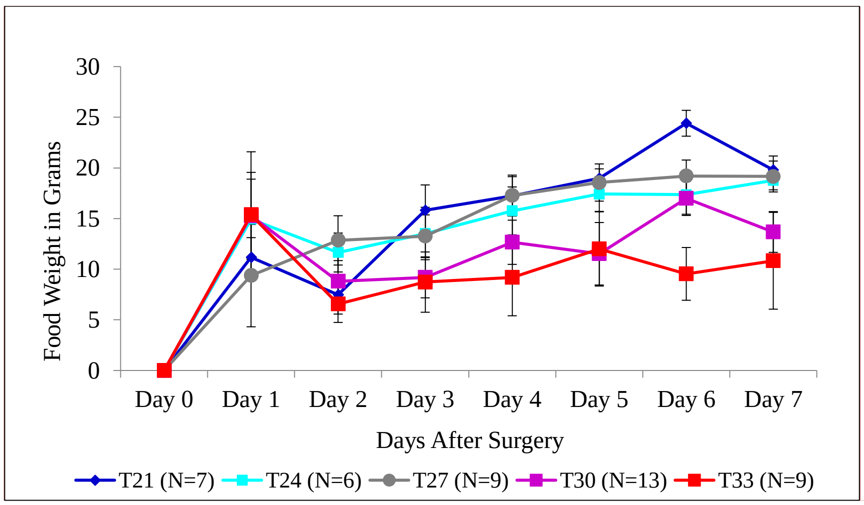

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Figure 5. **The Effect of Tamb on Food Consumption During Recovery**. "Day 0" is food consumption after surgery. Some minor differences may be observed between the 21°C and 33°C groups ~day 6.

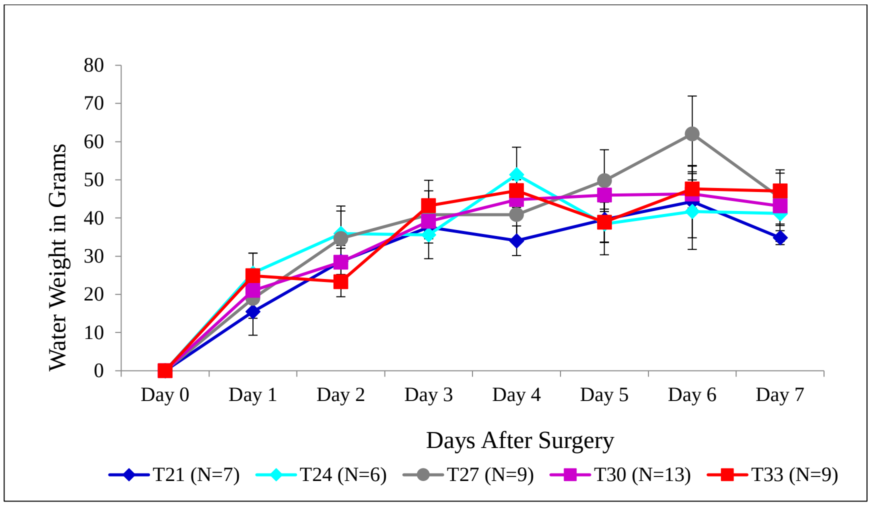

Figure 6. **The Effect of Tamb on Water Consumption During Recovery**. "Day 0" is water consumption after surgery. There does not seem to be significant difference in drinking behaviors between the groups.

VII. Discussion

Overview

The findings of this project demonstrate some of the effects Tamb has on the recovery of rats after surgical instrumentation. It was hypothesized that rats housed at their preferred Tamb of ~27°C will be less stressed and therefore recover to a normal Tc (36.5°C) more quickly, return to their pre-surgical body weight faster, and survive better, than rats recovering at lower Tamb such as 21°C and 24°C. Alternatively, housing rats at 30°C and 33°C after surgery will facilitate recovery from surgery by recovering to a normal Tc (36.5°C) more quickly, returning to pre-surgical body weight quicker, and facilitating survival better than the control group rats. Nonetheless, the warmer Tamb groups 30°C and 33°C did not enhance their survival rate.

Surprisingly, housing rats at a low Tamb (21°C) for the entirety of recovery did not hinder their survival while mild warming of rats (30°C and 33°C) did not enhance their survival (Table 2). In addition, there may be subtle differences among the Tamb groups in the recovery of Tc circadian rhythms during recovery (Figure 2). Unfortunately, there appears to be minimal differences in return to a pre-surgical weight, food consumption, and water consumption among the Tamb groups (Figures 4, 5, and 6). The hypothesis was not completely supported, but further statistical analysis and larger sample sizes are needed to make any accurate conclusions for this project. Performing surgeries at the same time of day for each rat will be considered for the future to limit variation in Tc that occurs naturally throughout the day.

<u>Tamb Effect on Survival Rate</u>

Table 2 shows the survival rates of the rats in each Tamb group. It was expected that the rats in the 21°C and 24°C groups would have had the lowest survival rates due to the combined surgical and thermal (cold) stress. However, the 21°C group rats had the highest survival rate of all groups. This may be because if they were housed at 21°C they were so stressed that they didn't even survive 24 hours after surgery. The assumption made at the outset of the analysis was that if rats did not survive 24 hours after surgery there was a surgical error and therefore, they should not be included in this study. This assumption may have to be revisited. If all animals, regardless of surgical error were included, perhaps the 21°C group survival rate may be very different and therefore the conclusions from this study would also change. For example, if the 21°C group were excluded altogether, and a graph of the survival rates of all the other groups were created, then a bell-shaped curve could exist with the peak survival rate at 27°C. This would suggest that 27°C is not only the animals preferred Tamb when allowed to choose its own

Tamb (Le and Brown 2011) but it is also the temperature at which rats survive stress the best when surgically stressed. These data, as well as previous data published in the lab suggesting 27°C is ideal for SD rats, would instead support a portion of the hypothesis that suggests that housing rats at 21°C or 24°C would hinder surgical recovery. It would also suggest that warming rats by housing them at 30°C or 33°C would not facilitate surgical recovery and therefore would not support the second portion of the hypothesis. These are purely presumptuous conclusions based on a hypothetical deletion of an entire experimental group however. Clearly, more analysis of the subtleties of the data are needed.

Tamb Effect on Circadian Rhythm and Core Temperature

Measurement of circadian rhythm of Tc, motor activity, blood pressure etc. is useful in determining if an animal is stressed. The data suggests that a circadian rhythm may have been established by day 3 of recovery just by observing the consistent fluctuations in Tc between daytime and night (Figure 2). Without further statistical analysis it is unknown when a circadian rhythm was established among each group and if there are any differences. This will be a focal point of a future project in the lab.

The analysis of Tc within the first day of recovery seems to be a critical time period to investigate within the recovery period. Tc drastically increases towards a normal Tc within a day (Figures 3). These data suggest that Tamb may not influence the return to a normal Tc since the 24°C and 33°C group returned the most quickly.There is not a trend on whether a slight warming and cooling of the rats' environments affected their return to a normal Tc after surgery. It was expected that the 33°C group would recover normal Tc quickest because less heat production is needed and heat loss to the environment is reduced when an animal is held at a higher Tamb. Conversely, it was expected that the 21°C would recover the slowest because more heat production is needed to overcome environmental heat loss. These were not seen in this data.

Tamb Effects on Weight, Food Consumption and Water Consumption

As with any invasive surgery, body weight will drop post-surgery due to evaporative water loss, the reduction in feeding time the rats have during the preparation for surgery and the surgery itself, and then the recovery to a point when feeding can begin again. After surgery, the body weight of rats continued to drop among each Tamb until ~ day 3 of recovery (Figure 4). These drops in body weight may be due rats not eating as much as usual since reaching up for food is uncomfortable due to soreness secondary to abdominal surgery. However, food was supplied on the bottom of the cage so that rats would not have to reach for food. This food was also counted in the 'feeding' amounts of each rat. The dramatic drops in weight, as much as an 8%, can be due to multiple factors such as surgical stress and thermal stress; "significant weight loss in rats exposed to stressful conditions could be due to an increase in corticosterone levels" (Park et al. 2017). The group that returned to pre-surgical weight the most quickly were the 24°C or 27°C groups. Even though it was unknown whether Tamb would influence feeding and water consumption behaviors, this finding partially supports the prediction that the control group would more quickly return to pre-surgical weight than the colder Tamb groups.

Rats' feeding behaviors did not seem to differ between groups except for the 21°C and 33°C group data on day 6 of recovery (Figure 5). It was expected that rats housed at cooler temperatures would need more caloric intake to support heat generation from metabolic sources, like brown adipose tissues, compared to those housed at warmer temperature. The increased caloric intake in the 21°C group, although mild, would be consistent with that conclusion. The reduction in food/caloric intake in the 33°C on the same day would be consistent with an animal that does not need to generate body heat via metabolic mechanisms, and therefore would not need the caloric intake to support that. However, these conclusions are preliminary. More animals are needed in the study to tease out differences in between groups, if any.

The water consumption of rats among each Tamb group do not appear to differ but further statistical analysis would need to be done. It was expected that warm stressed rats would drink more to match evaporative water loss to a warmer environment. This was not seen in the data as the 27°C Tamb group had the highest water consumption by day 6 of recovery.

Project Limitations

The main limitation to this project was shortened length of time to complete the statistical analysis due to COVID-19. A more in-depth statistical analysis will need to be completed in the future. Also, due to COVID-19 and the closing of the Bioscience building, data from each of the rats pertaining to the survival data could not be accessed. Additionally, the sample size of each Tamb group was too small to make any accurate conclusions.

Project Pitfalls

During the project, there were several times when there were malfunctions in the computer data acquisition of Tc. For some rats that were a part of this project, there were large gaps of missing data in their Tc, therefore, we could not include these rats in the Tc analysis. This is partially why there was a different number of sample sizes among the Tc data versus the body weight, water consumption, and food consumption data. Additionally, the mismatch in sample size between these analyses can also be contributed to human error. For example, the wrong recording of a rat's body weight during recovery resulted in excluding that rat from the body weight data. Overall, when computer software malfunctions and human error occurred, the animal was not included in the project.

Future project ideas

Various adjustments to this project can be done in the future to clarify aspects of the vague animal care guidelines regarding recovery from invasive surgery. One idea would be to measure the daily body weight, food consumption, and water consumption of rats for one week before surgical instrumentation. Since rats cannot be returned to the vivarium once they are removed according to IACUC rules, these measurements would need to be taken in the JMU vivarium daily. Then, following surgical instrumentation, the same measurements would be taken for the duration of recovery. This would be a strong addition to this project because it would help us better understand the effect Tamb has on these measurements.

Secondly, the measurement of stress of levels of rats during surgical recovery is of vast importance in determining whether a rat is fully recovered and ready for experimentation. However, it can be challenging in determining their stress level primarily by a rat's body language and behavior patterns, therefore, the measurements of hormones are often used since they are quantifiable. Corticosterone (cortisol) is the main stress hormone in rodents and is often measured to determine the stress level of a rat. One study found that obtaining blood samples from rats and using an ELISA kit demonstrated the greatest percent increase in plasma corticosterone from baseline to acute stress conditions in rats (Bekhbat et al. 2018). There is great variation in testing kits that measure corticosterone, but the ELISA kit appears to be the most used and accurate kit. Another study that measured corticosterone in rats obtained blood samples by submerging their tails in 42 °C water to dilate their blood vessels, then drew blood from the lateral tail vein using a syringe. Then, after a few more procedures the ELISA kit was used to quantify the corticosterone levels, and the final concentration of corticosterone was calculated according to a fitted standard curve (Park et al. 2017). This method of measuring corticosterone in rats is minimally invasive and barely stressful for rats. Another way of measuring the corticosterone levels in rats is through saliva, urine and feces, but each method of measurement comes with pros and cons. “Blood and saliva yield an index of stress at one brief moment in time (point samples) and are, therefore, influenced by circadian variation, making it crucial that samples be collected at the same time each day (e.g., at the nadir of the rhythm). In contrast, urine and feces yield an index of stress reactivity over hours or possibly several days (steady-state samples) and are, therefore, less vulnerable to circadian variation” (National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals 2008). It is possible that using urine and feces to measure corticosterone could be the best way to measure stress over a few days of surgical recovery. In all, measurement of a rat’s corticosterone levels is a good indicator to determine if the rat is fully recovered, on top of other measurements such as return to a normal Tc, circadian rhythm, and return to pre-surgical weight. Although expensive, investing in an ELISA kit in the future would be a tremendous addition to this project and lab in general if funding is available.

Measuring more physiological processes through radio telemetry would also be an accurate way to measure the stress level of the rat to monitor its recovery from surgery. Since the development of stress is not necessarily associated with activation of the HPA axis, as hormonal changes are not necessarily present under all clearly stressful conditions. For example, animals that experience chronic neuropathic pain do not exhibit changes in circadian corticosteroid levels or oscillations in HPA responsivity to restrain (National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals 2008). Assessment of glucocorticoid levels has many limitations, therefore, measurement of physiological processes such as heart rate and blood pressure in addition to Tc can be a good addition to this project. "Telemetry has become the 'gold standard' for measuring blood pressure in laboratory animals, mainly because this technology reduces or eliminates discomfort caused to the animals" (Braba and Prabhakar 2009). Investing in radio telemetry probes that can measure Tc, heart rate, and blood pressure all at once would be helpful in comparing the stress levels of rats throughout surgical recovery. However, blood pressure, heart rate, and ECG have all be measured using radiotelemetry probes in the lab before (Le, et al 2011) but the surgical stress from implantation of those probes is exceptionally stressful on the animal and surgical success rates are also very low.

One key aspect of future work will be to determine when circadian cycling of Tc restarts after surgery in each Tamb group. This was beyond the scope of this study due to time constraints but was considered at the outset of this project. In fact, that could be an honor's thesis project of its own.

Conclusion

In conclusion, current housing guidelines for SD rats allow them to recover from surgery at Tamb that are well below their preferred Tamb of 27°C. These include 21°C -24°C which may lead to a cold stressed animal. Combining this with surgical recovery stress may negatively affect the rat’s survival. Although the 21°C group did not have the lowest survival rate, it may have been due to methodological design issue (discarding data from rats that did not survive 24 hours after surgery) instead of a preservation effect of housing at 21°C. The remainder of the survival rate data is in line with the hypothesis that housing rats at cool Tamb hinders survival. Although it was predicted that housing rats at warmer Tamb (30°C and 33°C) would facilitate recovery the survival rate and recovery of a normal Tc data do not support this. More animals need to be included and statistical analysis needs to be done to solidify these conclusions, however. Finally, analysis of how Tamb affects circadian cycling of Tc after surgery remains an intriguing future project as does the monitoring of other physiologic variables such as cortisol levels, and perhaps even cardiovascular parameters.

VIII. References

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