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The role of ambient temperature in the recovery from major surgical stress

Mary Katherine Garbarini
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The Role of Ambient Temperature in the Recovery from Major Surgical Stress

A Project Presented to

the Faculty of the Undergraduate

College of Science and Mathematics

James Madison University

in Partial Fulfillment of the Requirements

for the Degree of Bachelor of Science

by Mary Katherine Garbarini

May 2014

Accepted by the faculty of the Department of Biology, James Madison University, in partial fulfillment of the requirements for the Degree of Bachelor of Science.

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Introduction

Surgical trauma is a significant physiologic stressor on the body. A stress response is thought to be necessary for wounded animals to survive in the wild, however, in a controlled environment; the animal’s physiologic response to surgical injury may be a cause of postoperative morbidity (Hall, 1985; Kehlet, 2000). The hormonal and metabolic response to surgery has been a cause of investigation for many years (Desborough, 2000).

The endocrine response to surgery has been described as increased secretion of the pituitary hormones and sympathetic nervous system activation (Desborough and Hall, 1993). The changes in pituitary secretion stimulate the release of cortisol from the adrenal cortex, arginine vasopressin from the posterior pituitary, and increased glucagon release and decreased insulin secretion in the pancreas (Desborough, 2000). The overall effect of the endocrine stress response is increased catabolism to provide energy, and the retention of salt and water to maintain fluid volume and cardiovascular homeostasis (Desborough, 2000). The likely evolutionary explanation for this stress response is that it developed to allow injured animals to sustain themselves through stored body fuels and retaining salt and water, until healing and recovery had taken place (Desborough, 2000). Recovery is affected not only by this stress response, but also by the common side effects of decreased gastrointestinal motility and pain (Kato et al., 1997).

Another potential stressor during recovery is a decrease in body core temperature (Tc) due to the use of anesthesia results of surgical intervention (Diaz & Becker, 2010). The use of anesthesia eliminates accurate control of Tc through the impairment of hypothalamic thermoregulatory centers (Diaz & Becker, 2010). An animal may experience a cold stress
because of surgical intervention due to the proclivity of anesthetics not only to affect hypothalamic thermoregulation, but also to create heat loss through vasodilation (Diaz and Becker, 2010).

The added effects of the hypothermic response caused by anesthesia, and other physiologic stressors likely hinder subsequent surgical recovery. After surgery is complete, the animal attempts to raise Tc back to normal levels as the anesthetic-induced vasodilation decreases. However, this return to normal thermoregulation is impaired by housing the animal in a cold environment (Díaz and Becker, 2010). Therefore, the use of external heating sources is required until the effect of anesthesia dissipates (Guide for the Care and Use of Laboratory Animals, 2012).

Current guidelines suggest that animals be allowed to recover from surgical stress on a heating pad for approximately one hour to support the recovering animal because autonomic thermoregulation is still not possible due to the effects of the anesthesia (Guide for the Care and Use of Laboratory Animals, 2012). Rodents are then returned to the normal animal holding areas where ambient temperature (Tamb) should be maintained within the range of 20°C-26°C (Guide for the Care and Use of Laboratory Animals, 2012). The Tamb of most vivaria is approximately room temperature (22°C) to make the environment more comfortable for human workers. However, previous data suggest that a rodent’s preferred ambient temperature is actually closer to 27°C (Brown and Le, 2011).

It is possible that housing rats at room temperature, below their preferred temperature, leads to a long-term cold stress that impedes surgical recovery, especially when the animal is already significantly stressed due to the metabolic and endocrine effects of surgical injury. Many
studies have examined the relationship between a chronic cold stress, behavior, and physiology, in rats (Fukuhara et al, 1996; Pardon et al, 2003; Weisenfeld and Hallin, 1981). However, the long-term consequences of cold stress due to post-surgical environmental conditions remain unexplored.

This study will investigate the effect of ambient temperature on surgical recovery. Indications of thermal stress during recovery include changes in food and water consumption, inadequate weight gain, and alterations in other homeostatic systems. Therefore, this study will specifically explore food and water intake, and body weight as measures of surgical recovery while animals are housed at ambient temperatures of 21°C, 24°C and 27°C for one week following surgical intervention.

We hypothesize that surgical recovery outcomes improve if rats are maintained at their preferred Tamb (27°C), instead of room temperature (22°C), even though 22°C is within current animal care guidelines. To this end, rats were housed at various Tambs to determine whether there is a significant difference in surgical outcomes based on the ambient recovery temperature. Increased weight gain, and food and water consumption at their preferred temperature of 27°C. The clinical signs of body weight change, and food and water consumption have often been used to estimate rodent stress (Martini et al, 2000; Roughan and Flecknell, 2001; Shavit et al, 2005). These data will facilitate a better understanding of how current animal care guidelines may enable a significant thermal stress following surgical intervention in laboratory animals. These stressors potentially affect an animal’s physiologic function, and thereby affect subsequent viability for use in research.
Methods

To quantify the effect of ambient temperature on major surgical recovery, eleven male Sprague-Dawley rats, weighing approximately 200-400g were instrumented with an abdominally placed radiotelemetry thermoprobe device (Model TA10TA-F40, Data Sciences International, St. Paul, MN) using aseptic techniques. The thermoprobe enabled body core temperature and motor activity to be recorded without handling the animal. These data are part of a separate project and will be analyzed later, when a more reliable and efficient method of data storage is established.

Rats were housed for one week following surgical instrumentation in Nalgene polycarbonate cages (11.5 in. x 7.5 in. x 5 in.) with wood chip bedding, fed with Rodent Laboratory chow (Harland Talked, LM-485, Madison, WI). Rodents were provided food and water ad libitum under standard laboratory conditions (~23°C 12:12 h L:D cycle, lights on at 0700h), except for ambient temperature which was varied by experimental group. All procedures were approved by the James Madison University Institutional Care and Use Committee and all procedures performed adhered the NIH Guide for the Care and Use of Laboratory Animals, 8th edition.

Before surgery, each rat was anesthetized with ketamine (75mg/kg) and xylazine (10mg/kg) via intraperitoneal injection before surgery. Supplemental dosages were administered as needed. Therefore, there was some variance in the amount and duration of anesthesia among subjects. The rat’s abdomen was shaved, and then swabbed with providone-iodine. A midline incision of approximately 2-3 cm was made in the abdominal skin and linea alba for sterile insertion of the probe into the abdomen. Each rat was implemented with a cranial cannula, which targeted select brainstem nuclei as part of a separate study. All surgical procedures were equally invasive. The abdominal muscle layers were closed with interrupted sutures and the skin was
closed with surgical staples. Post-surgery, ibuprofen was provided as an analgesic (0.2mg/ml in water supply). Cage warmers, which circulated warm air at ~2L/min into the rat’s cage, were used to maintain ambient temperature (Tamb) at 21°C, 24°C, or 27°C during the allotted recovery period.

For one week after surgery, food and water consumption, and body weight was measured daily between 11:00am and 2:30pm. To correct differences in body weights among subjects in each group, a water intake to body mass ratio was calculated. These measured values were averaged for each group, and then analyzed with the ANOVA statistical function of Excel to determine whether there was a significant difference between treatment groups.
Results

I. Food Consumption Results

Figure 1. Food Consumption of Rodents Housed at 21°C, 24°C, and 27°C During Surgical Recovery Period of One Week. The averaged food consumption of rats held at 21°C (n=2), 24°C (n=6), and 27°C (n=3), with standard deviations.

Figure 1 demonstrates that food consumption increased throughout the week of recovery in each treatment group, especially after day 3. However, on days 4, 5, and 6, the rats held at 27°C actually consumed less food than the other groups. On days 4 and 6, the rats held at 21°C consumed more than either the 24°C or the 27°C groups. On day 5, the 24°C group consumed more than either the 21°C or 27°C group. Overall, the food consumption results are variable and there is no clear trend of any group increasing or decreasing more rapidly than another group.
Table 1. Food Consumption Single Factor ANOVA Results. Table depicting the results of an Excel ANOVA analysis comparing each treatment group.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>P-value</th>
<th>F crit</th>
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<tbody>
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<td>Between Groups</td>
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<td>2</td>
<td>10.07486</td>
<td>0.192197</td>
<td>0.826816</td>
<td>3.554557</td>
<td></td>
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<tr>
<td>Within Groups</td>
<td>943.5498861</td>
<td>18</td>
<td>52.41944</td>
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<td></td>
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<tr>
<td>Total</td>
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<td>20</td>
<td></td>
<td></td>
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</tr>
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To quantify statistically whether there was a significant difference in the average food consumptions of each group, ANOVA statistical analysis was performed using Excel. As seen in Table 1, the ANOVA resulted in an F crit of 3.55, which is greater than the F of 0.19, indicating that there is no significant difference between the mean food consumptions of each group. This was supported by the calculated p-value of 0.82, indicating that there was no significant effect of Tamb on the food consumption of recovering rats.

II. Water Consumption Results

![Water Consumption Chart](image)

Figure 2. Water Consumption of Rodents Housed at 21°C, 24°C, and 27°C During Surgical Recovery Period of One Week. The averaged water consumption of rats held at 21°C (n=2), 24°C (n=6), and 27°C (n=3), with standard deviations.
As seen in Figure 2, the rats held at 21°C had decreasing water consumption until day 4. Water consumption increased over days 4, 5, and 6. In the 24°C group, water consumption increased until day 3, after which water consumption plateaued on days 4, 5, and 6. The 27°C showed water consumption increasing throughout the week, especially on days 4, 5, 6. Water consumption in the 27°C group was greater than that of the 21°C and 24°C groups on days 4, 5, and 6. Water consumption gradually increased throughout recovery in the 27°C group.

Table 2. Water Consumption Single Factor ANOVA Results. Table depicting the results of an Excel ANOVA analysis comparing each treatment group.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
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</thead>
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<td>50.81121</td>
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<td>0.627983</td>
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<tr>
<td>Within Groups</td>
<td>1915.490377</td>
<td>18</td>
<td>106.4161</td>
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<td>20</td>
<td></td>
<td></td>
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To determine whether there was a significant difference between the average water consumptions of each group, ANOVA statistical analysis was performed. As indicated by Table 2, the F crit value of 3.55 was greater than the F of 0.477, demonstrating that there was no significant difference between the water consumptions of rats held at 21°C, 24°C, or 27°C. This conclusion was supported by the p-value of 0.62, indicating that there was no significant effect of Tamb on water consumption.
III. Water Intake to Body Mass Ratio

Figure 3. Water Intake to Body Mass Ratio of Rodents Housed at 21°C, 24°C, and 27°C During Surgical Recovery Period of One Week. These values were calculated from the ratio of the average water intake over the average weight of rats held at 21°C (n=2), 24°C (n=6), and 27°C (n=3), with standard deviations.

The water intake to body mass ratios calculated from the average water intake divided by the average body mass accurately reflected the same trends observed in the water consumption results (Figure 3). These trends, such as increased consumption of water in the 27°C and plateauing of water consumption in the 24°C during the last 4 days of recovery, are seen more clearly on the water intake to body mass ratio graphs.

Table 3. Water Intake to Body Mass Ratio Single Factor ANOVA Results. Table depicting the results of an Excel ANOVA analysis comparing each treatment group.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
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<td>Between Groups</td>
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<td>0.123655</td>
<td>3.68232</td>
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<td>Within Groups</td>
<td>0.011932</td>
<td>15</td>
<td>0.000795</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.015766</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
To determine whether there was a significant difference between the average water intake to body mass ratio of each group, ANOVA statistical analysis was performed. As indicated by Table 3, the F crit value of 3.68 was greater than the F of 2.41, demonstrating that there was no significant difference between the water intake to body mass ratios of rats held at 21°C, 24°C, or 27°C. This conclusion was supported by the p-value of 0.12, indicating that there was no significant effect of Tamb on water intake due to body size.

IV. Weight Change Results

![Recovery Weight Changes](image)

Figure 4. Recovery Weight Changes Shown as a Percent of the Control of Rodents Housed at 21°C, 24°C, and 27°C During Surgical Recovery Period of One Week. The average weights of rats held at 21°C (n=2), 24°C (n=6), and 27°C (n=3).

As shown in Figure 4, each treatment group showed a similar pattern of recovery. The 21°C, 24°C, and 27°C groups all showed weight loss through the first 3 days post-surgery. All of the treatment groups began to recover on days 4 – 6. A difference in percentage of weight gained during recovery is visible at day 3. The 24°C group gained back a higher percentage of weight on each day of recovery, as compared to the 27°C and 21°C groups.
Table 4. Recovery Weight Changes Single Factor ANOVA Results. Table depicting the results of an Excel ANOVA analysis comparing each treatment group.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>3166.740761</td>
<td>2</td>
<td>1583.37</td>
<td>11.81114</td>
<td>0.000529</td>
<td>3.554557</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2413.032764</td>
<td>18</td>
<td>134.0574</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5579.773525</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To determine whether there was significant difference between the recovery weight changes of each group, ANOVA statistical analysis was performed. The results are shown in Table 4. The F value was greater than the F crit value, and the p-value of 0.0005 indicated that there was an initial significant difference among groups.

Figure 5. Regression analysis of Food Consumption Versus Body Weight Change in Rats Housed at Various Ambient Temperatures during a Week of Surgical Recovery.

Figure 5 describes the relationship between food consumption and weight change. In the 24°C group and 27°C group, a strong positive correlation was observed, $R^2=0.88$, and $R^2=0.91$, respectively. Rats housed at 21°C did not have a strong-positive correlation between food intake and body weight, as did the rats housed at 24°C and 27°C.
Figure 6. Regression analysis of Water Consumption Versus Body Weight Change in Rats Housed at Various Ambient Temperatures during a Week of Surgical Recovery.

Figure 6 demonstrates that there was a positive correlation between water consumption and weight change in rats maintained at 24°C and 27°C. This analysis resulted in an $R^2$ value of 0.79 for the 24°C group and 0.80 for the 27°C group. Rats housed at 21°C did not have a positive correlation between food intake and body weight as did the rats housed at 24°C and 27°C ($R^2=0.59$).
Discussion

Food consumption increased in each treatment group, throughout the week of recovery (Figure 1). It was predicted that rodents held at 27°C would consume the most food during recovery; however, on days 4, 5, and 6, the rats held at 27°C actually consumed less food than the other groups. One explanation for this unexpected observation is related to another unusual finding -- on days 4 and 6, the rats held at 21°C consumed more than either the 24°C or the 27°C groups. It is possible that the rodents in the 21°C consumed more food on days 4 and 6 as a response to an ambient cold stress. These observations are unsubstantiated by a p-value of 0.82, indicating that there was no significant difference between treatment groups (Table 1). However, their increased consumption of food may have been to boost caloric intake in order to support an increased metabolic rate. Small rodents rely more on metabolic changes to regulate body core temperature (Gordon, 1993).

The generalized stress response of rodents is described by decreased food intake during recovery from the stressor (Shavit et al, 2005). However, after chronic cold stress, rodents have been observed to increase food intake (Kawanishi et al, 1997). Although metabolic rate was not measured in this study, it is presumed that the increase in caloric intake in the rats housed at 21°C would have been used to support an increased metabolism and subsequent heat generation in order to maintain thermal homeostasis. Therefore, measuring oxygen uptake and carbon dioxide production to determine metabolic rate would be a more exact evaluation of cold stress than monitoring food consumption. This could be accomplished through use the metabolic box technique (Musch et al, 1988).

In the analysis of the water consumption data, days 1, 2, and 3, water intake trends were variable among groups (Figure 2). Similar trends were observed in the analysis of all of the
measures of surgical recovery. It is possible that clear indications of recovery are only visualized after day 3, regardless of treatment group. This may be explained by impairment in the rat’s ability to eat or even move around in the first three days after surgical trauma. After surgical wound healing, the rats may have been better able to regulate food and water consumption.

The rats held at 27°C gradually increased water consumption throughout the week of recovery, at a higher rate than the 24°C and 21°C groups (Figure 2). Increased water consumption was expected in the 27°C group as an indication of health; however, it is also possible that recovering at 27°C leads to more evaporative water loss and subsequently a greater need to increase water intake, leading to a mild heat stress.

Water consumption plateaus on days 4, 5, and 6, in 24°C group. It is likely that rodents in this group increased intake to restore fluid volume lost during surgery. They then plateaued, indicating normal homeostatic regulation of fluid balance. Perhaps this trend was a result of a having a larger number of subjects in this group. This trend is expected in all groups as groups increase in subject number. However, this trend may indicate that 24°C is an ideal Tamb for optimized recovery. The similarity between these graphs indicates that the size of rats in each group was not substantially different to produce a change in water consumption.

As the water consumption data were analyzed, differences in water intake based on body mass were considered. The water intake from the smaller rats was less than the larger rats. It was not known if this was simply due to body size or if the smaller rats were actually consuming more water per body mass. The body mass to surface area ratio was higher in smaller animals allowing for more evaporative heat loss (Gordon, 1993). Dehydration is a more significant concern in smaller rats. Therefore, to correct for body weight variations in the rat subjects, the ratio between body weight and water intake was analyzed.
In reviewing the water intake to body mass ratios for each temperature, there was no significant difference in the calculated ratio values (Table 3). This indicates that there was no significant variation in water intake due to body mass and recovery temperature. Overall, the calculation of the water intake to body mass ratio allowed for more clear visualization of water consumption trends by treatment group, which further support our observations from the water consumption data.

In regards to the recovery weight change data (Figure 4), similar trends were seen in the weight loss and gain of all three treatment groups. However, when ANOVA statistical analysis of these data was performed, a significant difference was observed between groups, indicated by a p-value of 0.0005 (Table 4). This finding is problematic because it suggests that there may have been a pre-existing difference in weights between groups. To correct this problem in the future, each group should either contain rats of a narrower weight change, or each group should contain a similar spectrum of initial rodent weights.

When the same data are viewed as a percentage of weight gained after surgery, it is clear that the 24°C group gained weight more consistently than the 27°C or 21°C (Figure 4). The increased subject number in the 24°C group may explain this observation. However, this graph suggests that rats which recover at 24°C may be less stressed.

The regression analysis performed investigating the relationship between weight change and food consumption (Figure 5) illustrated that there was a strong positive correlation between weight change and food consumed in the 24°C and 27°C. A similar trend was seen in the water consumption and weight change regression analysis for both the 24°C and 27°C groups (Figure 6). The 21°C group lacked a positive correlation between weight change and food consumption or water consumption. These results are more difficult to understand.
According to Desborough (2000), water and salt may be retained to maintain fluid volume and cardiovascular homeostasis after surgical stress. The strong positive correlation between weight change and water consumption may be an indication of traditional surgical stress. However, in the 21°C group, no strong correlation was seen between food consumption and weight change, which may indicate that the colder temperature has an effect on the manifestation of surgical stress.

In the future, there are several improvements to be made to enable better data collection and analysis. The addition of control groups without surgical instrumentation held at 21°C, 24°C, and 27°C will further clarify results. To substantiate any of this investigation’s observations, comparisons and statistical analysis must be conducted with control groups. To improve this project, more rats must be added to each group. The most successful and useful data collected thus far resulted from the 24°C group, in which n=6. A greater number of animals being included in the extreme Tamb groups is essential. However, survival rates of these groups seem to be less than rats maintained at 24°C or 27°C. This emphasizes the need to explore this area of research to determine the best Tamb to house surgically stressed animals.

The first relevant finding of this preliminary project includes the fact that 21°C group consumed the most food as compared to the 24°C and 27°C groups on days 4 and 6, which could be an indication of cold stress (Figure 1). However, food consumption is likely to be a poor estimation of cold stress. In the future, metabolic rate should be used to determine the degree of cold stress. The second relevant finding was the realization that clear recovery signs are only seen after day 3 (Figure 2).

It should be noted that the 24°C group water consumption plateaued during the last few days of recovery as an indication of homeostatic regulation of fluid balance (Figure 2).
Plateauing of water intake is expected in all groups as subject number is increased and as recovery from surgical stress progresses. However, if this trend is only observed in the 24°C group, it may indicate that 24°C is an ideal Tamb for surgical recovery. This finding was supported by Figure 4, which indicated that the rats held at 24°C gained weight more consistently than those held at 21°C or 27°C. The increased weight gain in the 24°C additionally supports the possibility that recovering rats held at 24°C may have better surgical outcomes.

The strong positive correlation between weight change and water consumption may be an indication of traditional surgical stress due to the retention of water and salt (Desborough, 2000). However, in the 21°C group, no strong correlation was seen between food consumption and weight change, which may indicate that the colder temperature has an effect on the way the animal responds to surgical stress.

Rats prefer 27°C when healthy (Brown and Le, 2011). However, they seem to recover from surgical stress better when housed at 24°C. There have been no clear indications of a significant difference in weight gain, food consumption or water consumption in the 27°C group versus the other groups. The lower ambient temperature of 24°C may have a protective effect. This protective effect of temporary hypothermia has been demonstrated in other exogenous stressors including hypoxia (Gordon, 1993) and hemorrhage (Brown et al, 2005).

Finally, these data suggest that it takes at least one week for surgically stressed rats to fully recover, which can be influenced by the Tamb in which the animal is housed. Monitoring of these factors at appropriate Tamb for strain and body weight would be essential to the rat’s health and facilitate quality data collection from the animals once recovery in complete. However, no findings from this study can be substantiated without comparison to and a statistical
analysis of a control group. The project is still ongoing, and with increased subject number, the intergroup differences may clarify results.
Literature Cited


New York, N.Y.


