Effects of Supplemental Heat when Provided to Rodent Housing Units

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Background

- Laboratory rodents are typically kept in animal housing rooms set at temperatures between 20-22ºC as recommended by the *Guide for the Care and Use of Laboratory Animals*. [5]
- Rodent thermoneutral zone (TNZ) is a range of temperatures where rodents can maintain their body temperature and do not expend energy to warm or cool themselves. [1]
- The TNZ of the mouse is 29-34ºC. [2]
- Housing rodents in temperatures lower then their TNZ subjects them to cold stress, which leads to changes in their physiology, behavior, and immune function. [3]
- The aim of this study was to explore the potential for the heated OptiMice® Smart System Rack to offset physiological & behavioral effects in cold-stressed mice.

In particular, what effect (if any) does supplemental heat supplied by the rack have on breeding, behavior, nest building and anesthetic recovery in laboratory mice?

Methods

With MSU IACUC approval, these experiments were performed:

Experiment 1: Voltage-Temperature Guide

- For each voltage on the Smart System Rack with OptiMice® cages, temperatures were collected at the cage front, cage floor quadrants, and the center and edge of the heating plate to create a guide for later experiments based on the ambient room temperatures of the animal housing facility.

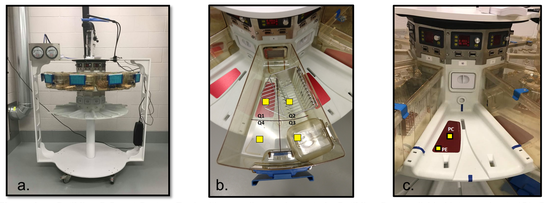


Figure 1. a. OptiMice ® Smart System Rack with recording equipment. **b.** OptiMice ® cage divided into quadrants (Q). Q1 is directly over the heat plate and rest of quadrants were assigned in a clockwise order. Yellow squares represent where cage temperatures were collected. **c.** Heat plate (red/brown rectangle) with control panel displayed. Blue tape corresponds with cage quadrant perimeter. Yellow squares represent where plate temperatures were collected at the plate center (PC) and plate edge (PE).

Experiment 2: Behavior and Nest Assessment

- SQ temperature transponders were placed in mice (n=12) 48 hours prior to introduction to the Smart System Rack.
- 24 hour recording sessions of one heated and one unheated cage were obtained at day 0, day 14, and the day of delivery starting at the dark cycle (7pm).
- SQ temperatures, cage temperatures, and nest scores were collected after the first 24 hours and then three times weekly.

Experiment 3: Anesthetic Recovery Assessment

- SQ temperature transponders were placed in mice (n=16) at least 1 week prior to study.
- Mice were anesthetized with ketamine (80 mg/kg)/xylazine (10 mg/kg) injection IP for a 15 minute “mock procedure” time.
- Mice were allowed to recover in cages on the Smart System Rack (11, 14 or 15V) or on a warm water blanket (38ºC setting); heat was only supplied to quadrant 1 (Q1).
- SQ temperatures were recorded every 5 minutes and times to anesthetic recovery were noted.

Experiment 4: Reproduction Assessment

- Mice (n=12) from behavior study were randomly assigned into 6 breeding pairs.
- Three pairs received 11V of supplemental heat, three served as a control.
- The following outcomes were evaluated: number of pups delivered and pup weights at day 3, 7, and 14 days post-delivery.

Results

Experiment 1: Voltage-Temperature Guide

Voltage	OptiMice® Smart System Rack Temperature by Voltage Guide										
	6	7	8	9	10	11	12	13	14	15	16
Q1 Temperature (°C)	23.2	24.6	25.9	27.8	29.4	30.7	33.4	31.4	32.8	35.6	37.6
PC Temperature (°C)	24.7	26.4	28.0	31.0	33.5	36.2	39.2	34.6	35.4	38.3	41.5

Table 1. Temperatures (ºC) collected at the center of the heat plate (PC) and over the heat plate in quadrant 1 (Q1) of the cage floor for each voltage. Highlighted is the voltage which created a cage floor temperature of approximately 30ºC in Q1.

Experiment 2: Temperature, Behavior, and Nest Assessment

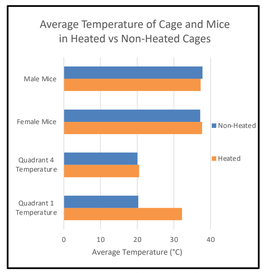


Figure 2. Average temperatures of the mice (n=12) and cage floors in heated (n=3) and non-heated (n=3) cages. Temperatures were taken initially and then 3 times weekly. Cage floor temperatures were taken in Q1, over the heat plate, and Q4 for comparison as temperatures in Q2, 3, and 4 did not greatly differ. Mouse SQ temperatures were collected via IPTT-300 SQ temperature transponders and handheld receiver.

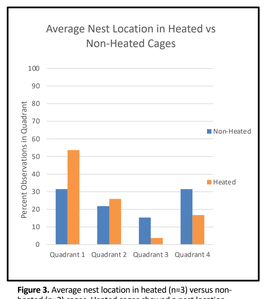


Figure 3. Average nest location in heated (n=3) versus non-heated (n=3) cages. Heated cages showed a nest location preference over the heat plate while non-heated cages did not have an obvious preference for nest location.

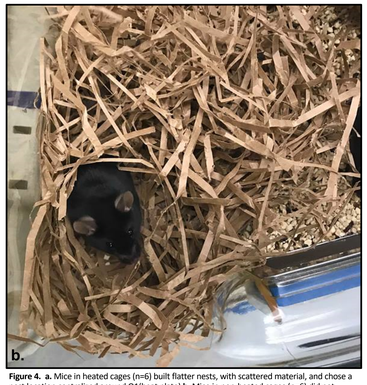
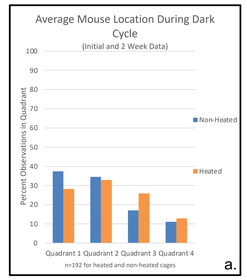
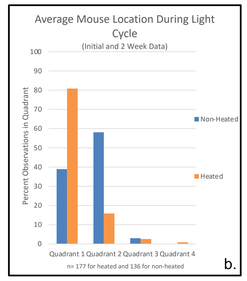
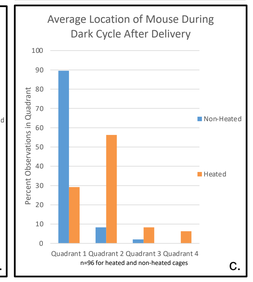


Figure 4. a. Mice in heated cages (n=6) built flatter nests, with scattered material, and chose a nest location centralized around Q1(heat plate) **b.** Mice in non-heated cages (n=6) did not exhibit preference for nest location, tended to build better quality nests with higher nest sides, and were less likely to scatter their nesting material throughout the cage.

Figure 5. a. Average location of the mice during the dark cycle (7pm-7am), also known as the active phase. Graph shows that mice (n=4) tend to travel with little preference for a specific quadrant. Observations of nest location (n=96) noted that 49% were in Q1, 25% in Q2 and 26% in Q4 for non-heated caged mice (n=2). Nest observations (n=96) for heat cage mice (n=2) noted 74.5% in Q1 and 25.5% in Q4. **b.** During the light cycle (7am-7pm), or inactive phase, mice tend to stay in their nest location. However, mice in the heated cages do show more willingness to leave their nest. Nest observations for non heated cages (n=94) noted 50% in Q1, 25% in Q2, and 25% in Q4. Nest observations for heated cages (n=94) noted 75% in Q1 and 25% in Q4. **c.** Average location of the mice during the dark cycle after delivery. Different from previous dark cycle behavior, mice in heated and non-heated cages tended to stay in their nests (n=48 for heated and non-heated) just after delivery, though heated caged mice were still more likely to travel away from their nests when compared to non-heated cage mice. Both non-heated and heated cage mice had nests that were 50% in quadrant 1 and 50% in quadrant 2.



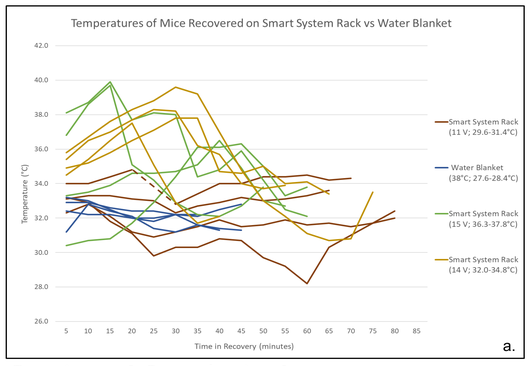


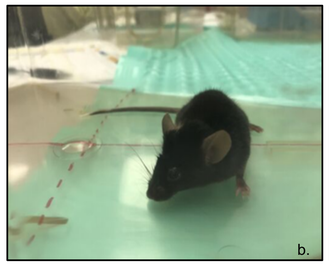


Cage Type	Average Nest Score
Non-Heated	3.82
Heated	3.15
Difference	0.67

Table 2. Nests were scored based on the Naturalistic Nest Scoring System.[4] Mice in heated cages tended to build flatter nests with lower nest scores when compared to mice in non-heated cages.

Experiment 3: Anesthetic Recovery Assessment





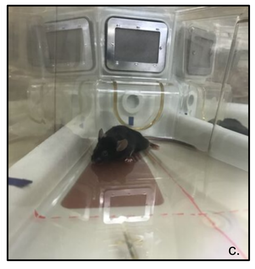


Figure 6. a. Graphic of SQ temperatures recorded every 5 minutes during an anesthetic recovery period until mice were conscious. Mice (n=12) were recovered on the Smart System Rack (voltage; temperature in Q1) or mice (n=4) were recovered on a warm-water recirculating blanket (water blanket temp; temperature in Q1). The mouse with the lowest temperature (~28ºC), in the 11V group, regained normal body temperature without intervention. Mice (n=3) with higher temperatures (~40ºC), in the 14V and 15V groups, were repositioned away from the heat element, which brought temperatures within normal range on the next reading. **b.** Example of a mouse in a static Opti cage placed on a water blanket (38ºC setting) where heat supplied to Q1 was between 29.6-31.4ºC. **c.** Example of a mouse recovered on the Smart System Rack where heat was supplied in Q1 by the heat plate. Voltage was set to either 11, 14, or 15 which translated to a temperature of 29.6-31.4ºC, 32.0-34.8ºC , or 36.3-37.8ºC inside the cage at the mouse level in Q1.

Experiment 4: Reproduction Assessment

Figure 7. a. Example of a nest containing a female nursing her pups in a heated cage. **b.** Example of using a gram scale to weigh a pup from a heated cage 7 days post-delivery.

Cage Type	Days to Delivery	Number of Pups Born	Pup Weight at 3-days (g)	Pup Weight at 7-days (g)	Pup Weight at 14 days (g)
Heated	25.3	5.3	1.57	3.29	7.08
Non-Heated	26.3	5.0	1.96	3.98	7.56
Difference	1	0.3	0.39	0.69	0.48

Table 3. Summary of the reproductive study data (averages between heated versus non-heated cages). Time to delivery (in number of days post-pairing) and average number of pups delivered to mice is represented from heated (n=3) and non-heated (n=3) breeding pairs. One breeder pair of non-heated mice were then removed from study due to dystocia. Pup weight reflects data from the remaining heated (n=3) and non-heated (n=2) breeding pairs. Difference between the highest score and lowest score is shown. Note: pilot data was not statistically analyzed.

Discussion

- Mice kept in standard animal housing rooms are subjected to ‘cold stress’ that can impact physiology, behavior, and immune function, potentially decreasing efficacy for modeling human disease. [3,5]
- When provided with a microenvironment within their TNZ, as created by setting the Optimice® Smart System Rack to ~30ºC (11V), mice were shown to have a decreased nest score and also chose to centralize their nest over the heat plate in quadrant 1 (Q1).
- The Optimice® Smart System Rack can be an asset after anesthetic procedures as mice will be allowed to recover in a heated cage that is supplied with proper air ventilation. Food and water can also be provided to the cages, allowing the researcher to decrease the amount of time spent physically monitoring the animal.
- Limitations of this study:
  - The temperature of Q1 created by the plate voltage is dependent on the ambient housing room temperature. Therefore each rack needs to be calibrated to meet temperature ranges within the conditions of any particular housing room. The data collected to create the voltage-temperature guide can serve as a starting point for other investigators interested in the rack.
  - The aerial view of the GoPro videography was beneficial to remote cage assessments; however Q2 was obstructed from view by the food hopper, which impacted accuracy of data collection from that quadrant.
- Future Studies:
  - Explore the potential of Optirat® Smart System Racks for laboratory rats.
  - Continue to try to find a voltage on the Smart System Rack that will minimize time to recovery after anesthesia.
  - Determine if there is a difference in data collected from the breeding pairs with their second litter.
  - Follow pup development for differences in feed consumption, blood cell counts, blood glucose, behavior, and breeding that are directly related to exposure to appropriate TNZ housing.

Conclusions

- The pilot studies performed using the Optimice® Smart System Rack further demonstrate that rodents prefer to be housed in an environment within their TNZ.
  - In heated cages, mice trend towards building nests over the heat plate.
  - Mice in heated cages spend more time over the heat plate during the light cycle.
  - Mice in heated cages tend to build poorer nests suggesting they are more thermally comfortable.
- These pilot studies served as a baseline for further studies investigating differences between heated and non-heated cages when looking at:
  - Blood work
  - Anesthetic recovery
  - Behavior
  - Growth

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