The Development of A Non-Invasive Behavioral Model of Thermal Heat Stress In Laboratory Mice (*Mus musculus*)

J.T. Mufford[1], M.J. Paetkau[1], N.J. Flood[1], G. Regev-Shoshani[2], C.C. Miller[2], J.S. Church[1]

[1]Thompson Rivers University, 900 McGill Road, Kamloops, British Columbia V2C 0C8, Canada

[2]Department of Medicine, Respiratory Division, University of British Columbia, Vancouver, British Columbia V5Z 3J5, Canada

Previous research investigating physiological and behavioral responses of heat stressed mice have often relied on invasive biotelemetry methods in order to collect crucial data such as core body temperature [1, 2]. Biotelemetry generally requires surgical implantation of a telemeter, which subjects the animal to surgical distress and discomfort along with the risk of infection [2]. Furthermore, the invasiveness of a surgical procedure may influence the behavior of the animal test subjects.

We wished to develop a method for studying thermal heat stress in mice that did not require invasive surgical procedures and that allows precise control of the ambient heating conditions while measuring the external surface temperature of the animal test subject. The new experimental design was configured such that both the behavior and surface temperature of mice can be continuously monitored automatically while undergoing different heating regimes in a modified 30x30cm open field (Med Associates Inc, St. Albancs, VT) (Figure 1). To induce heat stress in mice, one wall of the open field was modified to include a ceramic heat emitter (Hagen, Baeie d'Urfé, QC Canada) that was directed toward the inside of the field, providing an increase in the ambient air temperature that was held constant in our experiments at 36°C. The ceramic heat emitter was controlled with a variable voltage transformer (R. Mack & Co. Ltd., Vancouver, BC, Canada) and a power monitor (P3 International, New York, USA). A cooling fan (KDE1205PFB2-8, Thermal FX, Gardner, NV, USA) connected to a DC Power Supply (HP, Palo Alto, CA, USA) was also installed in the bottom corner of the modified wall of the open field for air circulation. A custom-built cage top was fabricated, to keep the heated air trapped in the open field box, while still allowing the use of the infrared thermography (IRT) camera (FLIR E60 infrared camera, FLIR Systems Inc., Wilsonville, USA) external to the open field. The key component of the cage top included a sheet of thin plastic stretched tightly on a wooden frame. This allowed us to simultaneously record the behavioral activity of the mice with a colour CCT camera (Panasonic WV-CP504, Newark, USA) and the surface temperature of both the mouse while in the open field, and the surface temperature of the open field with the IRT camera. The surface temperatures were recorded by streaming radiometric MPEG to a computer using FLIR Tools+ software (FLIR Systems Inc., Wilsonville, USA) (Figure 2).

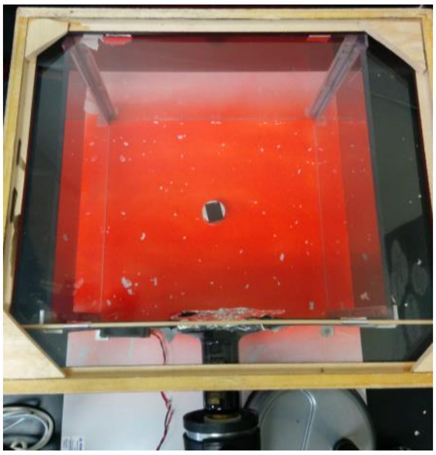

Figure 1. Open field with a custom built plastic top and a modified wall.

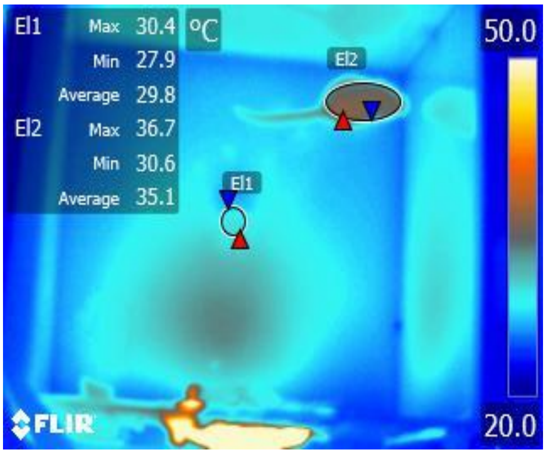

Figure 2. Snapshot of streamed IRT video recording. Surface temperature of the mouse and the open field are simultaneously being measured.

A thermocouple (LM 35 Heat Sensor, Texas Instruments, Dallas, Texas, USA) was installed at the bottom of the cage, raised 3cm from the bottom and enclosed with aluminum, for secondary temperature validation. The surface temperatures of the open field was also recorded by taking the IRT measurement of a piece of vinyl electric tape with known emissivity (Scotch Super 88, 3M, St. Paul, Mn, USA) attached to the thermocouple. The temperature recorded by both the thermocouple as well as the IRT measurement of the tape were nearly identical. The novel plastic cover also enabled other environmental variables to be investigated such as the effect of electromagnetic radiation of visible light on different mouse coat colors by using LED video lights (Gentec International, Markham Canada) placed adjacent to our open field.

The colour CCT camera recorded mice activity through Media Recorder 2.5 (Noldus, Wagenigen, The Netherlands) using a Euresys Picolo U4 H.264 capture card (Euresys, San Juan Capistrano, USA). The behavior of the mice in response to thermal loading was automatically tracked using Ethovision 10.0 software (Noldus, Wagenigen, The Netherlands) (Figure 3) to determine the total distance travelled and velocity of the mice inside the open field using the CCT camera video file. In general, the total distance travelled by mice in the heated open field was far less than that of the mice in the control open field, which was maintained at room temperature in the laboratory (22°C). Ethovision was also used to generate heat maps representing the areas in the open field where the mice had spent the most time (Figure 4). Four continuous data streams (ambient air temperature of the open field measured by a thermocouple; surface temperature of the open field measured by IRT mpeg radiometric video of the electrical tape, surface temperature of mice measured by IRT mpeg radiometric video, along with the behaviour of mice captured by avi video by the CCT camera) can now be readily statistically compared. The development of this novel non-invasive model of heat stress improves the animal welfare of the study animals, while still allowing for very detailed control of both heat and lighting factors, along with unimpeded behavioral responses of the animals.

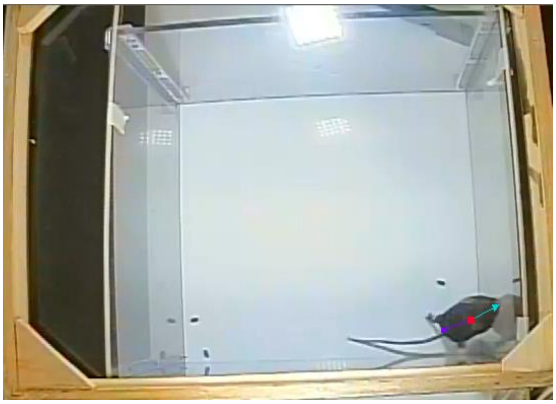

Figure 3. Automated mouse tracking in Ethovision 10.0. Mouse center, nose and tail points are tracked to determine velocity and total distance travelled.

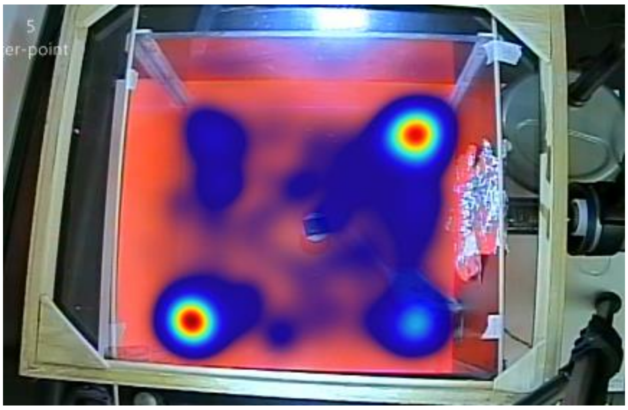

Figure 4. Heatmap representation of the areas in the open field where the mouse had spent the most time. Duration is scaled from blue, representing the least duration, to red, representing the highest duration.

Ethical Statement

This study was approved by the Thompson Rivers University Animal Care Committee and followed the Canadian Council on Animal Care Guidelines.

References

1. Leon L.R., DuBose D.A., Mason C.W. (2005). Heat stress induces a biphasic thermoregulatory response in mice. *American Journal of Physiology* **288**: 197-204.

2. Gaskill B.N., Gordon C.J., Pajor E.A., Jeffrey R.L., Davis K.D., Garner J.P. (2013). Impact of nesting material on mouse body temperature and physiology. *Physiology & Behavior* **110-111**: 87-95.