WHITE PAPER //
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Improvement project:

Enhancing animal welfare and surgical monitoring in surgical rodent models

The increasing array of surgically modified rodent models is providing researchers with novel tools to increase the productivity of their studies and to better model complex diseases for the development of novel therapies.

However, there are challenges associated with generating surgically modified rodents, including monitoring them in the pre- and post-surgery settings. Current surgical monitoring techniques are typically time consuming because laboratory personnel perform welfare checks multiple times per day and must update their records accordingly. Manual monitoring techniques also induce stress in the animals and technicians, provide only limited monitoring windows (i.e., usually during standard daytime work hours), and may fail to identify important opportunities for interventions that might improve survival outcomes, such as providing a heat source.

Actual size of the RFID Temperature microchip is 2.1mm x 13mm in a Sterile Blister Pack 12.5G cannula

Thus, there remains ample opportunity to continually advance and optimize surgical monitoring of rodents, which is of utmost importance because it achieves the following:

- Enhances animal welfare by ensuring that pain is adequately controlled.
- Improves survival rates via effective control of infections and closely tracking weight gain/loss, temperature, and activity.
- Improves consistency of surgical outcomes by ensuring catheters are implanted correctly and patent and, for infarct models, that the infarct size is correct and has low standard deviation.
- Bolsters the quality of data that is collected by identifying and addressing any potential confounding variables and reduces the likelihood of having to repeat a study.

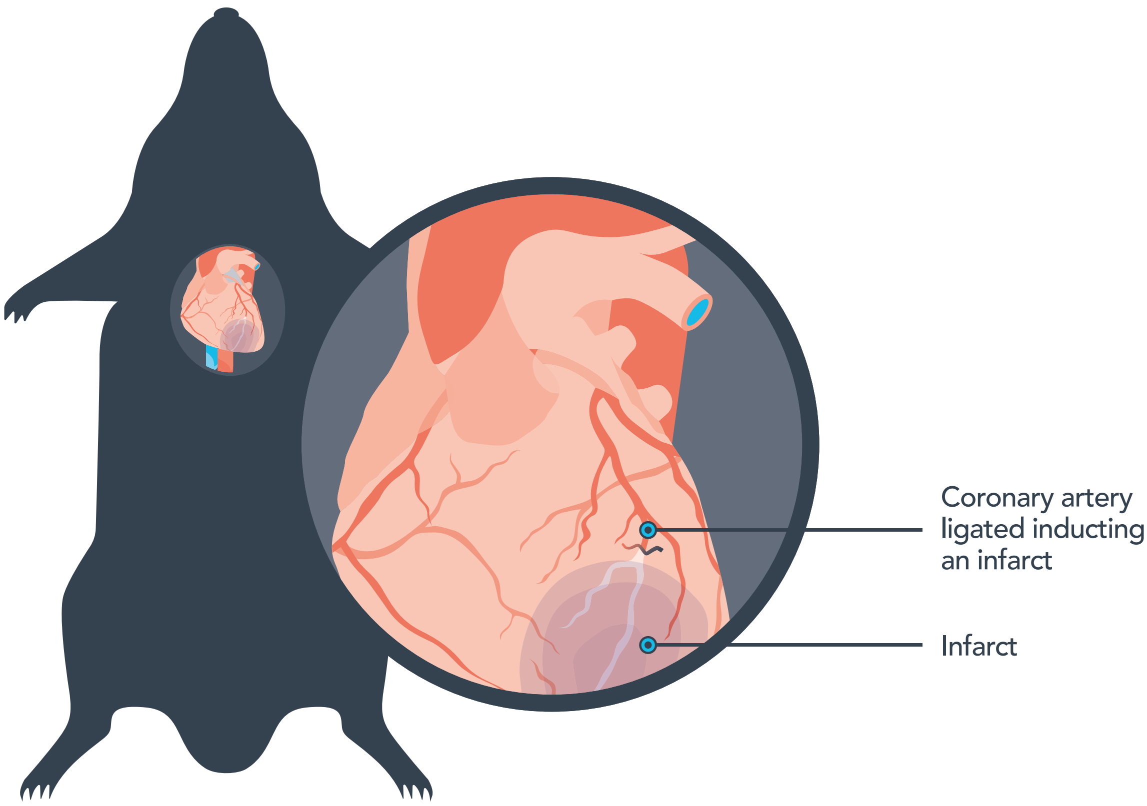
As part of Envigo's commitment to continuous improvement in rodent surgery, it undertook an improvement project to enhance the pre- and post-surgical monitoring of cardiac surgical models while improving animal welfare. This paper presents the results of a collaborative project with a technology company that offers implantable radio frequency identification (RFID) microchips to monitor rodents' home cage temperature and activity.

Rodent Cardiac Surgical Models

The myocardial infarction (MI) and transverse aortic constriction (TAC) rodent models have unique characteristics and great utility for studying the downstream effects of ischemia. Selecting the optimal model for a specific research need will depend on the context of the research question(s) and a clear understanding of the differences between the models.

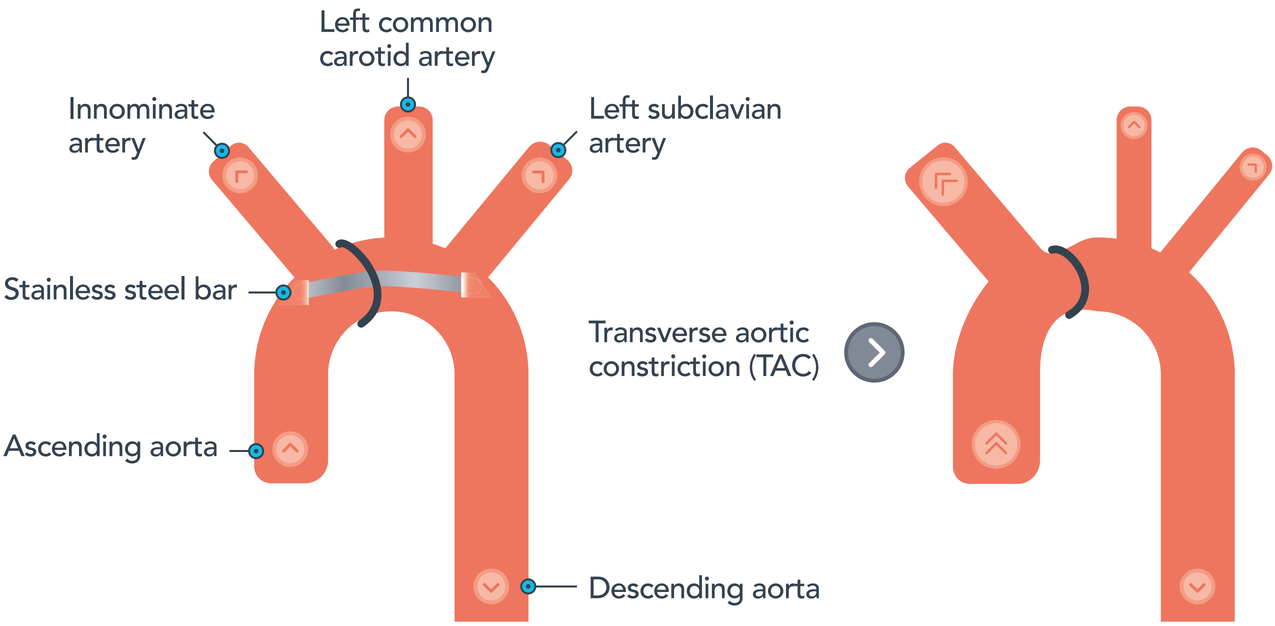
The MI model is generated by inducing an acute period of ischemia by ligating the left anterior descending artery (LAD) under a 20–40X stereomicroscope, followed by reperfusion of the heart tissue by releasing the ligation (See Figure 1). Cardiac output is usually reduced to 40% or below (confirmed by echocardiogram), depending on the size of the infarct (35–40% reduction in ejection fraction for medium infarcts and 30–35% for large infarcts). Notably, there is a proportional increase in LV volume as a function of infarct size, as first described by Fletcher (1981).

Figure 1: Schematic depicting the ligation of the LAD and subsequent infarction



The TAC model is generated by constricting the aortic arch of young animals between the innominate and left common carotid artery (See Figure 2). This increases blood flow velocity through the constriction; the blood flow into the left common carotid artery is greatly reduced, whereas blood flow into the innominate artery increases. Cardiac output (ejection fraction) is usually reduced to <60% two weeks post-surgery and <40% by 6–8 weeks post-surgery. Research has also shown evidence of LV hypertrophy in rats at 2–4 weeks post-constriction, and by 16–18 weeks, overt signs of heart failure associated with LV hypertrophy are evident, including tachypnea, edema, pleural effusions, and ascites (Litwin 1995). In mice, LV hypertrophy occurs more quickly: TAC has been shown to induce a ~50% increase in LV mass within two weeks (Patten 2009).

Figure 2: Schematic depicting the constriction of the aortic arch between the innominate and left common carotid artery and downstream changes in blood flow



Surgical experts at Envigo have been at the forefront of developing MI and TAC models that are consistent across cohorts and validated both internally and by industry partners (for additional information on these models, please refer to Envigo's paper on Surgical Cardiac Models).

Improvement project

Envigo collaborated with Unified Information Devices (UID), which supplied the RFID implanted microchips, RFID reader plate, and software.

The overarching goal was to assess temperature and activity pre- and post-surgery in mouse and rat MI and TAC models. By using a technology that allows for continuous monitoring of temperature and activity day and night, the project provided insights into heat loss during surgery and post-surgery with existing equipment and how heat affects animal recovery times.

Methods

All surgical modifications were reviewed and approved in advance by Envigo's Institutional Animal Care and Use Committee (IACUC). Veterinarians provided oversight and technical and professional support to surgical personnel. All surgical procedures were performed by trained surgeons using aseptic techniques in surgical facilities.

Rodents were assigned to either the MI (n = 3) or TAC (n = 3) group and housed in one cage per study group (the same project groups were used for both species).

The animals served as their own controls (i.e., pre-surgery baseline data). Body temperature was assessed pre-surgery, during surgery, and post-surgery over a period of five days, which was the total study time. Animal characteristics are provided in Table 1, and the study groups are shown in Table 2. Animals that were recovered with post-surgery heat were housed in ThermoCare® intensive care units (ICUs). Animals without immediate post-surgery heat were placed on heat at Day 3 post-surgery to minimize any discomfort.

Table 1: Rodent characteristics

Species	Mice	Rats
Strain	C57BL/6	Sprague Dawley
Sex	Male	Male
Age	7–8 weeks	6–8 weeks
Weight	20–24 grams	200–224 grams

Table 2: Study groups (the same groups were used for the mouse and rat studies)

Without Post-Surgery Heat	With Post-Surgery Heat
Control	Control
Transverse Aortic Constriction (TAC)	Transverse Aortic Constriction (TAC)
Myocardial Infarction (MI)	Myocardial Infarction (MI)

Intraperitoneal injection was used to implant all animals with a standard RFID microchip with the addition of a programmable microchip that provided each animal's temperature and identification. Intraperitoneal injection was used because this produces the most accurate core body temperature readings. The microchips allow for the monitoring of temperature and activity in a noninvasive, real-time, continuous manner for single or grouped animals. Activity and temperature data are recorded 24/7 and as often as every 0.6 seconds.

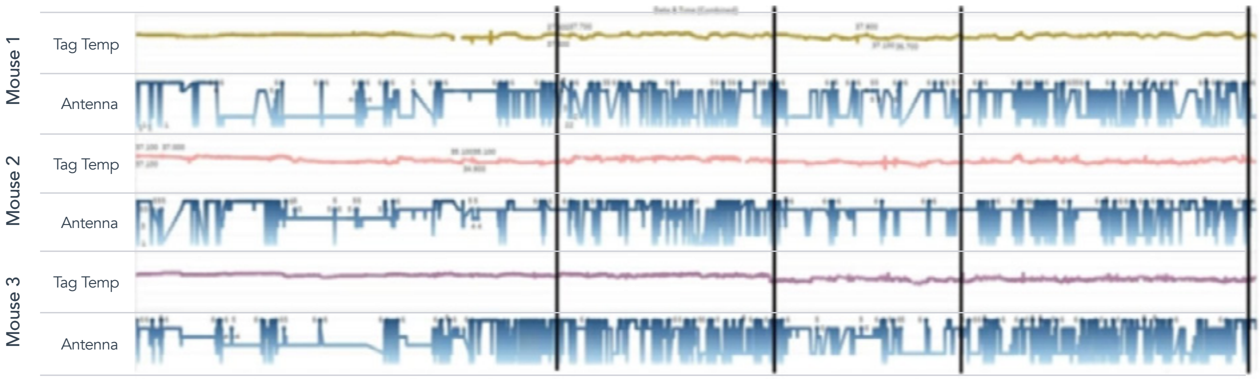
Cages were placed on a plate that contained the RFID reader mechanism and six antennas (zones). The reader plate gathered information on the activity of specific animals based on their zone (animals had unique identification codes) and their temperatures. An activity was scored when an animal moved from one zone to another. The data was sent to the software, which collected and output it. The data could be viewed in real time and remotely.

Results

Over the course of the five-day study, over two million data points were amassed for the 12 groups (i.e., six groups total for the mouse and rat studies).

Figure 3 shows the control mice data (temperature and activity) for each animal in that group. The top plot (the yellow, red, and purple lines) shows the temperature, which was observed to be very steady over time. The lower graph for each animal (blue line) represents activity. The left-most portion of the plot confirms that the mice were not very active during the day (as expected), but once the lights were shut off in the evening, their activity increased substantially (to the right of the first black bar).

Figure 3: Control mice temperature and activity data



Surgery was then conducted on the "control" animals to generate the TAC and MI models. Post-surgery, the animals were placed back into their cages with or without heat and their temperature and activity were monitored.

Mouse TAC model data

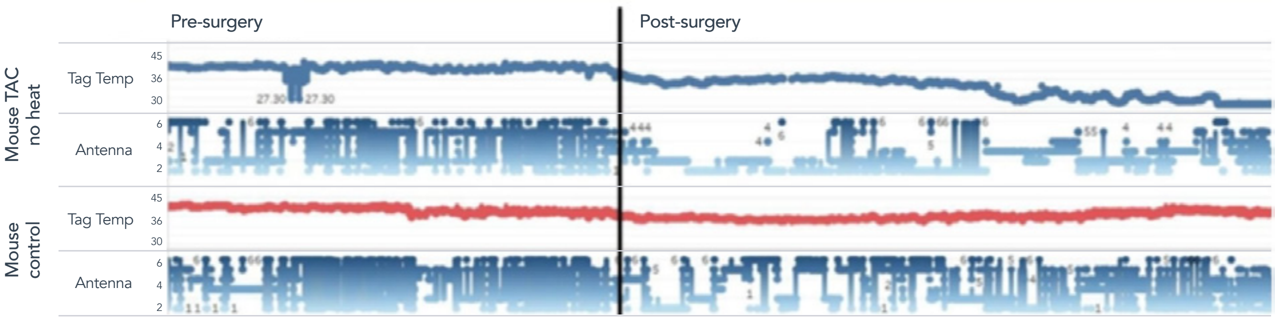
For comparative purposes, Figure 4 shows the average pre-surgery and post-surgery plots for the mouse TAC model group that was not recovered with heat.

The left portion of the graphic (to the left of the black line) shows the pre-surgery data, with an average temperature of 37.5°C, which is a close match to historical values.

However, the animals' temperatures began to drop almost immediately after removal from the surgical heating pad. A significant decrease in activity was also observed over the first two days post-surgery. As shown in Figure 4, during the pre-surgery period, the animals had an average of 110,256 recorded activity movements over 5 days, and this dropped to approximately 34,200 in the post-surgery setting, despite receiving analgesic.

At about Day 2.5 post-surgery, animals were removed from the study and provided with heat to aid their recovery and minimize discomfort.

Figure 4: Mouse TAC model recovered without heat



Group	Average temperature (°C)	Average activity
Mouse control	37.5	110256
Mouse TAC no heat	32.5	34230

Surgery was then conducted on the "control" animals to generate the TAC and MI models. Post-surgery, the animals were placed back into their cages with or without heat and their temperature and activity were monitored.

Mouse MI model data

The mice recovered without heat showed a very similar pattern of substantial drops in temperature and activity post-surgery. Once the animals were provided with heat, beginning at Day 2.5, they made a full recovery.

The activity of the surgical mice that were recovered with heat rebounded much quicker compared to those without heat. Indeed, their average temperatures and activities were similar to the control mice. The animals recovered with heat were more active, fed and drank more, and had higher feces and urine output, which reflects their increased food and water intake.

Table 3 provides a summary of the temperature and activity data gathered from the mouse groups recovered with or without heat.

Table 3: Mouse average temperatures and activity

Group	Average temperature (°C)	Average activity (movements)
Mouse control	37.5	110,256
Without heat		
Mouse TAC	32.5	34,230
Mouse MI	33.1	33,051
With heat		
Mouse TAC	36.9	101,235
Mouse MI	37.1	110,235

Rat model data

For the rat models, the observed differences were not as great as those seen with the mice, likely because of rats' larger body size and better ability to thermoregulate post-surgery.

While there was no dramatic difference in average body temperature, the rats' post-surgery activity was reduced (although this could have been due to the opioid analgesic that was provided). Slight temperature and activity increases were observed for the heat-recovered MI and TAC rat groups compared to the no-heat MI and TAC groups.

Table 4: Rat average temperatures and activity

Group	Average temperature (°C)	Average activity (movements)
Rat control	37.8	106,523
Without heat		
Rat TAC	36.8	80,523
Rat MI	36.7	81,562
With heat		
Rat TAC	37.1	87,520
Rat MI	36.9	85,426

Conclusions

For the mouse study, core temperatures began to drop immediately after anesthesia induction, and post-surgery heat significantly improved recovery time and led to increased feeding and drinking, so that weight increased quicker compared to the group without heat. Mice recovered without heat had significantly lower body temperatures and activity scores when compared to the heat-recovered animals. All groups had increased activity at Day 3 post-surgery, but animals recovered with heat returned to control levels on Day 5 post-surgery, which was more rapid than the animals without heat.

In rats, post-surgery heat slightly improved recovery time. While heat is certainly important for post-surgery recovery in rats, it was less so in rats as compared to mice because of the rats' larger body size. Thus, only slight differences in temperature and activity were observed in the rat groups recovered with or without heat, and temperature and activity returned to control levels at Day 3 post-surgery for both groups.

Summary

The MI and TAC surgical rodent models have unique characteristics and are invaluable tools for the study of the downstream effects of ischemia. However, monitoring of these and other surgical models in the pre- and post-surgery settings can be challenging using current surgical monitoring techniques. Novel technologies such as the implantable RFID microchips described in this paper allow for the monitoring of temperature and activity in a noninvasive, real-time, continuous manner for single or grouped animals which enhances animal welfare and allows for more effective surgical monitoring.

As of July, 2019 Envigo surgically modified rats can have RFID microchips with temperature monitoring implanted, and surgically modified mice can be implanted with RFID microchips and the addition of temperature monitoring can be requested. Please inquire when ordering.

References

1. Fletcher PJ, Pfeffer JM, Pfeffer MA, Braunwald E. Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction. Effects on systolic function. Circ Res. 1981 Sep;49(3):618-26.
2. Litwin SE, Katz SE, Weinberg EO, Lorell BH, Aurigemma GP, Douglas PS. Serial echocardiographic-Doppler assessment of left ventricular geometry and function in rats with pressure-overload hypertrophy. Chronic angiotensin-converting enzyme inhibition attenuates the transition to heart failure. Circulation. 1995 May 15;91(10):2642-54.
3. Patten RD, Hall-Porter MR. Small animal models of heart failure: Development of novel therapies, past and present. Circ Heart Fail. 2009 Mar; 2(2): 138-144. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. Card Fail Rev. 2017 Apr;3(1):7-11.

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