Evaluation of Shipping Stress in Surgically Altered Rodents During Commercial Air Transport

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Planes, Trains and Sometimes Automobiles

- When establishing our Global Surgical program, we looked for solutions that would keep projects moving forward.
- A need arose on the west coast for telemeterized animals.
- The strain and model was not available on the west coast.
- There was hesitation to shipping a surgical model over the road.
- Why can't we fly? ....causes too much stress on the animals.
- How could we measure physiological indicators of stress during shipping?
- Continue our partnership with Charles River (CRL)
Background

- Understanding shipping stress of surgically altered rodents is relevant to animal welfare as well as the science and research that these models support.

- The changes in normal physiological parameters associated with shipment related stress can affect scientific validity and consequently alter study results.

- This study focused on heart rate, which is one of the physiologic parameters previously documented and associated with stress.

- Based upon this assumption, increased heart rates would be correlated with increased stress.

- Many stress evaluation studies include cortisol levels.
  - Cortisol levels were not included in this study because the effects of stress and the associated levels have previously been researched extensively and documented in the literature.
  - Previous publications are used to guide the assessment of the data for our conclusions with respect to cortisol levels.
Background

- Pfizer outsources the production of many rodent surgical models.
  - At this time, the only approved method for shipment is by ground courier due to concerns about the impact of shipping stress on the animals.
  - Transit time can sometimes take up to four days from origin to destination.

- There is limited published information on the duration of physiological stress indicators during the time before, during, and after transportation.
  - Data has never been collected during shipping in rodents.
  - Discovered a novel device that would allow us to focus on physiological data during travel
  - Evaluate stress in rodents following surgery, in relation to time of shipment and acclimation
  - Assist in establishing guidelines for humane shipping post-operatively.

- We focused this investigation on the departure from supplier to landing at destination.
  - However, data was collected for 14 days.
  - Future presentations
Star Oddi Device

- Key Features
  - Small size - only 3.3g
  - Long battery life – typically over 3 months
    - Depends on data collection
  - Automatic stress-free measurements
  - Leadless, minimally invasive
    - The DST micro-HRT simultaneously measures long term heart rate and temperature in the study animal.
    - The logger has no external wires, which makes it especially simple to implant.
    - Made of unique ceramic housing and epoxy and is hermetically sealed.
• Data collection
  – ECG derived heart rate, including data verification
    • The heart rate is derived from a leadless single channel ECG.
    • The logger takes a burst measurement of ECG at the set time interval and calculates the mean heart rate for each recording.
    • For validation purposes, individual ECG bursts can be saved. In addition each burst is graded with a QI (quality index).
    • Heart rate is recorded every 2 minutes based on a 1 second ECG measurement.
Materials and Methods

- All procedures involving animals were in accordance with regulations, and established guidelines. They were reviewed and approved by Pfizer and CRL’s Institutional Animal Care and Use Committee.
- Star Oddi DST micro-HRT data logger devices were utilized to evaluate heart rate and temperature in rodents from the time of surgery to delivery and acclimation.
- Surgical procedures were performed at CRL Raleigh.
- Twelve male, 8-10 week, variable weight, CRL CD Sprague Dawley rats were used.
  - Group A (n=6) control group data logger implants only.
  - Group B (n=6) Surgery group jugular catheter and data logger implantation.
Surgical Description

• Anesthetized with ketamine (75 mg/kg) and xylazine (6.0 mg/kg) administered intraperitoneally and provided buprenorphine (0.02 mg/kg) subcutaneously.

• The jugular vein was isolated and ligatures were placed using non-absorbable suture material.
  – A phlebotomy was made in the jugular vein, a polyurethane catheter was inserted, and secured with ligatures.
  – The data logger was subcutaneously tunneled to the left chest (close to apex of the heart).
  – The extravascular portion of the catheter was tunneled subcutaneously to the dorsal scapular region.
  – The catheter was locked with heparinized dextrose solution and the skin incision was closed using a subcuticular suture.
Post Surgical Activities

Significant events as well as behavioral observations within the housing room were recorded.

• Immediately following surgery, assessments were completed daily as per CRL guidelines.
  – Monitored for pain and healing
  – The behavioral observations recorded evaluated movement, posture, body condition, respirations, and other parameters.

• Day 3
  – Body weight assessment and Physical exam was performed.
  – Animals were packaged for shipment.
• Animals undergo surgery and recover smoothly
• Animals maintain normal rhythm
  – Minor effect on Surgery group

Body Temp
Control
Heart Rate

Body Temp
Surgery
Heart Rate
Comparison Surgery to Packaging

- Increased heart rate of surgical group noted
- Body temperatures were similar with no significant differences.
### Day 4 Day of Travel

Approximately 29 hours of travel time  
Packaged 17 hours prior to departure  
46 hours of box time

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:41</td>
<td>Depart CRL</td>
</tr>
<tr>
<td>4:41</td>
<td>Processed in at airport (2 hour 30 minute layover)</td>
</tr>
<tr>
<td>7:17</td>
<td>Loaded onto plane</td>
</tr>
<tr>
<td>7:30</td>
<td>Departed for Los Angeles</td>
</tr>
<tr>
<td>9:40 (12:40)</td>
<td>Arrive Los Angeles (5 hours 10 minutes flight)</td>
</tr>
<tr>
<td>10:07 (13:07)</td>
<td>Checked into Los Angeles (4 hour 36 min layover)</td>
</tr>
<tr>
<td>14:43 (17:43)</td>
<td>Loaded onto plane for San Diego</td>
</tr>
<tr>
<td>15:58 (18:58)</td>
<td>Departed for San Diego</td>
</tr>
<tr>
<td>16:12 (19:12)</td>
<td>Arrive San Diego (19 minute flight)</td>
</tr>
<tr>
<td>16:39 (19:39)</td>
<td>Checked into San Diego</td>
</tr>
<tr>
<td>17:15 (20:15)</td>
<td>Arrive at warehouse</td>
</tr>
<tr>
<td>17:16 (20:16)</td>
<td>Checked into warehouse (Held over night 15 hours)</td>
</tr>
<tr>
<td>08:10 (11:10) (PST) (EST)</td>
<td>Delivered CRL SAN</td>
</tr>
</tbody>
</table>
Shipping Departure Raleigh to Los Angeles

- HR begins to increase and reaches peak at 1 hour into flight.
- Body temperature decreases throughout flight
Shipping Departure Raleigh to Los Angeles

- Significant increase in HR during flight
- Significant decrease in body temperature during flight

Blue = Surgery
Red = Control

Heart Rate

Body Temp
Surgical group body temperature decreased during the initial shipping period

Surgical group showed a 56 bpm increase compared to the control group
Flight Temperatures in Transport Box

- **RDU/LAX**
- **LAX/SAN**

Temperature range from 10.0 to 30.0°C.
Packing for Shipment to Un-Packing at Delivery

- Packaging to unpacking which is 10:00am on 12/18 through 10:00am on 12/20
- Increased HR and decreased temperature during flight through unpacking
- Surgery group temperatures are slow to normalize after flight
Receipt to 72 Hours Post Delivery

- Establishing normal rhythm within 24 hours
- Resting heart rate of surgery group remains higher through 72 hours
Surgery to the End of Study (14 days)

- Surgery
- Flight
- Peak HR
- Resting HR
- Normalization
Summary

- **Behavior**
  - All animals appeared normal through the study
  - Normal rhythm observed at 24 hours post delivery

- **Body weight**
  - Animals met expected weight gain

- **Heart Rate**
  - Statistically significant increase in HR during flight to LAX
    - Surgical group difference of 55.9 BPM
  - Surgical group maintained a higher peak HR through day 8 post surgery
  - Surgical group resting HR normalized at day 13 post surgery

- **Temperature**
  - Significant decrease seen during air shipment
  - Synchronized with increased HR

- While Surgical group animals did experience significantly higher heart rates, they did appear to recover quickly after the flight

- No long-term adverse effects were seen with air shipment
References

  – Contains approximately 90 references on shipping stress

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