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

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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.
• 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.
Star Oddi Device

- 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.
Surgery to Packaging

- Animals undergo surgery and recover smoothly
- Animals maintain normal rhythm
  - Minor effect on Surgery group
Comparison Surgery to Packaging

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

![Heart Rate Graph](image)

- Blue = Surgery
- Red = Control

![Body Temperature Graph](image)
### 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</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)</td>
<td>Delivered CRL SAN</td>
</tr>
</tbody>
</table>
Travel Day

4:41 Airport
7:17 Loaded on PLN #1
12:40 Arrive LAX
17:43 Loaded on Plane #2
19:12 Arrive SAN
19:39 Checked SAN
20:15 Arrived warehouse

11:10 Delivered CRL SAN

Mean HR in Surgically Altered Rodent Model
Mean HR in Subcru implanted Rodent Model
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
Surgery Versus Control Group Differences

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

10.0 12.0 14.0 16.0 18.0 20.0 22.0 24.0 26.0 28.0 30.0
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**

![Graph showing HR intervals during different periods](image)
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
  – Contains approximately 90 references on shipping stress
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