2013 OSU ASLAP Summer Student

Extern: Briony Smith, ASLAP Foundation Summer Fellowship

- Currently a student at The Ohio State University, Columbus OH
- Projected graduation 2015
- Involved with ASLAP (President), SCAVMA, Ohio Veterinary Medical Association, Veterinary Business Management Association, Omega Tau Sigma, OSU Surgery Club, OSU Equine Club (SCAAEP), OSU Radiology Club, Buckeyes for Greyhounds

Mentor: Dr Judy Hickman-Davis, DVM, PhD, DACLAM, Director of Training and Director, Quality Assurance Laboratory, University Laboratory Animal Resources; Associate Professor Clinical Veterinary Preventative Medicine.

Dr Hickman-Davis is the Director of the Laboratory Animal Medicine Training Program and the Quality Assurance Lab and provides clinical and didactic support for the Laboratory Animal Residency Program. She is a member of ASLAP, ACLAD and AALAS, serves on the BSL3 Advisory Committee, Radiation Safety Committee and as an alternate for the IACUC and the Institutional Biosafety Committee.

Research Project: “Micro Isolator Filtered Cage Lids for Verification of Sanitation Standards”.
MICRO ISOLATOR
FILTERED CAGE LIDS FOR
VERIFICATION OF SANITATION
STANDARDS

B Smith, D Harrison, C Hofer, J Petty, M Nicolaus, V Bergdoll, JM Hickman-Davis
University of Laboratory Animal Resources, The Ohio State University, Columbus, Ohio

SO WHY DO WE CARE?

• It takes time and money to wash lids
  • If they aren’t dirty, then why do it!
• It is a should, not a must
• To determine a possible source of contamination and transmission for disease

AGENDA

1. Guide standards vs ULAR standards
2. Reference papers
3. Part I of experiment
   • Lid Sanitation Levels
4. Part II of experiment
   • Glow Powder contamination
5. Summary

The most wonderful time of the year……
(well every 3 years)
1. STANDARDS

THE GUIDE

“In general, enclosures and accessories such as tops, should be sanitized at least once every 2 weeks”

ULAR REQUIREMENTS

A. Any piece of equipment in contact with the animal is replaced every 2 weeks
ULAR REQUIREMENTS

B. Equipment not in contact with the mice are sanitized every 6 months

Investigation of Appropriate Sanitization Frequency for Rodent Caging Accessories: Evidence Supporting Less-frequent Cleaning

- Used ATP tests and RODAC plates
- Four groups:
  - Mouse and Rat ventilated, static wire bar with or without filter tops
  - Costs & establish a sanitation frequency

2. REFERENCE PAPERS

ATP Results

Mouse Ventilated

Mouse Static

Rat Ventilated

Rat Static

***ATP between 14d and 90d-no significant△****
**RODAC RESULTS**

- **Mouse Ventilated**
- **Mouse Static**
- **Rat Ventilated**
- **Rat Static**

***Colonies between 14d and 120d=no significant change***

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**Effects of Cage Density, Sanitation Frequency, and Bedding Type on Animal Wellbeing and Health and Cage Environment in Mice and Rats**

- Body weight was not affected by cage density or sanitation frequency
- Only sporadic effects of cage density and sanitation and bedding type on ATP cage concentrations containing rats
- Even with monthly cage change (versus weekly as recommended by The Guide), ATP concentrations were not affected

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**Cage Change Intervals for Opossums in individually Ventilated Cages**

- Looked at temperature, relative humidity and ammonia levels
- Although not outlined for this species in The Guide, used same parameters as rats
  - Ammonia<25ppm
  - Temperature= 18 to 26°C
  - Humidity=30% to 70%
- If individually housed: cage changing frequency extended from 7 →→→14d

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‘All filter tops and wire bars remain in place on cages for at least 90 days with no significant change in the amount of organic material or gram (-) bacterial contamination’
Evaluation of Housing and Sanitation Practices as Compared to Guide Recommendations Using Laboratory Animal Performance Indices

- Harlan only changes rat cages every 5 weeks, but did not see a significant difference in ammonia concentration level.
- They believe that results indicate no important effects on cage conditions, behavior or reproduction.

3. PART I OF STUDY: LID SANITATION LEVELS

- The sentinel mice in Rodent Health Status rooms 1-4 were used.
- 144 cage lids were changed out to sterile lids on 5/8/2013.
- At two week intervals, 12 lids were ATP-swabbed and RODAC tested.
- These lids were replaced with sterile lids.

LID TESTING MATERIALS

- Sanitation levels of the mouse IVC cage lids within the facility were tested using Replicate Organism Detection and Counting (RODAC) plates and ATP monitoring system (SystemSURE Plus™ Luminometer and UltraSnap swabs).
- Testing will continue up to 180 days.
**RODAC PLATES**

- Replicate Organism Detection And Counting
- Raised agar surface to allow surface sampling
- Plates are incubated at 35°C for ~48 hours
- After incubation, colony counts can be recorded

**ATP TESTING**

- Provides a method for real-time monitoring of cleaning process
- ATP testing is used for its sensitivity, speed and convenience.
- Based on the measurement of adenosine triphosphate (ATP)
  - ATP is present in all animal, bacterial, yeast and mold cells
  - NOT IN VIRUSES

**HOW RODAC PLATES WORK:**

- Plates contain Trypticase Soy Agar with Lecithin and Polysorbate 80
- Casein and soy peptones provide the nutrients for organism growth
- Polysorbate 80 neutralizes phenols, hexachlorophene and formalin
- Lecithin inactivates quaternary ammonium compounds

**HOW TO DO AN ATP TEST**

- To test:
  - Premoistened swab for area to be tested.
  - Swab area 4in. X 4in.
  - Snap the end with reagent to release
    - The enzyme luciferase catalyzes the consumption of ATP, which produces light
  - Place within luminometer, wait 15 seconds for results
BASED ON THE PREVIOUS EXPERIMENT:

- Pass standards were defined as:
  - 0-15 colonies for RODAC
  - 0-30 RLU for ATP
- Lid testing included standard barrier and sterile barrier areas
  - Cage lid=barrier to spread disease
  - Dirty lid=fomite for transmission of disease to new animals from handling/cage changes

PASS RATES FROM PREVIOUS EXPERIMENT:

- **Barrier lids**
  - ~70% at 14 days
  - ~50% at 30, 90 and 120 days
  - Failure at 150 and 180 days
- **Sterile lids**
  - ~80% at 120 and 150 days
  - No difference between 14 and 180 days

PASS RATES FOR OUR EXPERIMENT:

- **ATP**
  - Pass <10 RLU’s
  - Caution=11-29 RLU’s
  - Fail >30 RLU’s
- **RODAC**
  - Pass= 0-15 colonies
  - Fail >15 colonies

***Manufacturer standards

***ULAR standards

TEST RESULTS

<table>
<thead>
<tr>
<th>Date</th>
<th>ATP Pass</th>
<th>ATP caution</th>
<th>ATP Fail</th>
<th>RODAC Pass</th>
<th>RODAC Fail</th>
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</thead>
<tbody>
<tr>
<td>5/22/2013</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>5</td>
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<td>6/5/2013</td>
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<td>5</td>
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<td>8</td>
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<td>7/17/2013</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
OVERALL RESULTS: ATP

- ATP Fail
- ATP Caution
- ATP Pass

OVERALL RESULTS: RODAC PLATES

- RODAC Fail
- RODAC Pass

ROOM HEALTH STATUS RESULTS

<table>
<thead>
<tr>
<th>Barrier</th>
<th>ATP Pass</th>
<th>ATP Caution</th>
<th>ATP Fail</th>
<th>RODAC Pass</th>
<th>RODAC Fail</th>
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<tbody>
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<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Week 4</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
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<td>0</td>
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<td>1</td>
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<td>4</td>
<td>2</td>
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<td>Week 10</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
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</table>
Frequency of lid washing

- Currently, ~8% of cage lids are sanitized every 14 days
  - ~8% X 12 two week intervals = 96%
  - After 6 months, at this rate, all cage lids should have been washed
  - In order to wash 12,187 cage lids every six months, 182 lids would need to be washed every day during the week

To verify this, cage lids were manually counted in clean cage wash

<table>
<thead>
<tr>
<th>Date</th>
<th># of lids washed</th>
</tr>
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<tr>
<td>5/28/2013</td>
<td>767/3=256</td>
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<tr>
<td>6/25/2013</td>
<td>191</td>
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</table>

SOME BACKGROUND INFORMATION

- In one hour, ~2,800 lids can be washed
  - This breaks down to 47 lids/minute
- For 1 hour of run time, it costs ~$10 for the tunnel washer to operate
  - This breaks down to $0.17/minute
- Staff: $11.50/hr = $0.19/minute
  - But there are 2 people (clean and dirty cage wash), so it costs $0.38/minute

SO HOW MUCH DOES IT COST TO WASH EXTRA LIDS???

- To wash 102 lids, it would take 2.18 minutes
  - 2.18 minutes X $0.38/minute (staff cost) = $0.83
  - 2.18 minutes X $0.17/minute (tunnel wash) = $0.37
  - So altogether it costs $1.20 to wash 102 lids per day
- In a 6 month period, washing 5 days/week = $144.00
WASHING LIDS EVERY 2 WEEKS:

- 12,187 lids x 12 washes in 6 months = 146,244 to be washed
- If the lids needed to be washed every 2 weeks, 1,224 lids would need to be washed each day
- To wash 1,224 lids, it would take 26.13 minutes
- 26.13 minutes X $0.38/minute (staff cost) = $9.93
- 26.13 minutes X $0.17/minute (tunnel wash) = $4.44
- So altogether it costs $14.37 to wash 1,224 lids per day
- In a 6 month period, washing 5 days/week = $1,724.40

$1,724.40 - $144.00 = $1,580.40 in six months
In twelve months, this would add up to $3,160.80!!!

...but this doesn’t even include the extra time it will take the technicians to change all these lids (and the cost associated)

4. PART II OF STUDY:
GLOW POWDER CONTAMINATION

PROTOCOLAIMS

1. To evaluate the ability of IVC filter tops to prevent the spread of particulate matter through the interior of the ventilated rack and adjacent cages on the rack over time.
PROTOCOL AIMS

2. To evaluate the potential spread of particulate matter embedded in an IVC filter top between and extending over several changes where filter tops and not changed with the cages and wires

FILTER PAPER SPECIFICATIONS

- The filter is produced with either straight or crimped polyester fibers which give the various product styles different filtration and other general performance properties.
- Crimped fibers yield softness, conformability, and greater porosity
- Straight fibers yield stiffness, tighter structure, and finer arrestance.

FILTER PAPER SPECIFICATIONS

<table>
<thead>
<tr>
<th>Particle Micron Size</th>
<th>0.3-0.5</th>
<th>0.5-0.7</th>
<th>0.7-1.0</th>
<th>1.0-2.0</th>
<th>2.0-3.0</th>
<th>3.0-5.0</th>
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</thead>
<tbody>
<tr>
<td>% Efficiency</td>
<td>14</td>
<td>56</td>
<td>143</td>
<td>295</td>
<td>521</td>
<td>630</td>
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</tbody>
</table>

Percentage Efficiency based on 8-10µ size particles=91%
CONTAMINANTS THAT REMAIN IN MICROENVIRONMENT

- Contained/partially contained within microenvironment
  - Helicobacter: 0.5-0.9μm wide by 2-4μm long
  - Pasteurella pneumotropica: not usually transmitted by fomites, therefore transmission due to new animal
    - 0.5 X 1.2 μm
  - Pseudomonas aeruginosa: 0.5 – 1.0 x 1.5- 5.0 μμ

CONTAMINANTS THAT PASS THROUGH TO MACROENVIRONMENT

a) Viruses: 5-300nm
b) Bacteria:
  - Coccus 0.5-1.0μm in diameter
  - Bacillus 0.5-1.0μm wide X 1.0-4.0μm long
  - Spirochetes range from 1μm to over 100μm in length

KRYPTON POWDER

- Dosed in 2 teaspoons/cage
- Glows bright green in the dark and fluoresces under UV light
- Contamination powder is milled at a 325-to-2500 mesh, which approximates a 44-μm final size

EARLY REMOVAL CRITERIA

- Chemical Hazards of glow powder:
  - Reported as non-toxic by the supplier
  - Potential for eye and respiratory irritation
  - ERC:
    - Respiratory distress that lasts more than 24 hours after being placed into a clean environment
    - Dermatitis that does not resolve with treatment after removal from powder
    - Other ERC: >20% loss of body weight OR have a BCS <1.5
Mice placed 4/cage (with the exception of one cage that contained 5 mice). Glow powder was added to 3/6 cages. Mice were placed in racks at D1, D5 and D10.

**ACTIVITY TIMELINE: DAY 0:**

 Remaining cages placed on other side of rack in the bottom corner

**BEFORE...**

**IMMEDIATELY AFTER ADDITION OF MICE**
ACTIVITY TIMELINE CON’D

• Cages were removed from rack and placed in BSC 3 times per week for 30 minutes, left unopened

Day 2: First time in hood

ACTIVITY TIMELINE CON’D

• Leakage from glow powder was evaluated periodically using UV light
• Cages were changed biweekly
  • Included cage bottom, bedding, nestlet, wire rack and food
  • The filter/lid was NOT changed
• This process was repeated until there was no visible glow powder under UV light on the mice, cages or filter tops

DAY 4
DAY 14

Mice from the glow powder cages were removed and placed into clean cages to determine how long it would take before you could no longer find glow powder on them.

Pictures!!

AND the lixits inside and out were contaminated as well as the plenum.

DAY 14-CHANGE OUT PROCEDURES

Mice from the clean cage, ie. no glow powder, were added to a clean cage.

The ‘dirty’ lids with glow powder were placed on top of these cages.

The purpose was to check and see if the ‘dirty’ lid would contaminate the new cage.

Trace residue on the lid=???
DAY 14-CAGE CHANGE OUT

Minimal contamination!

DAY 16…AND WE FOUND??

DAY 16: CLEAN MICE WITH THE GLOW POWDER LID

DAY 16: GLOW POWDER MICE AFTER A CLEAN CAGE

NO GLOW!!!
DAY 18
No change...

DAY 21/23/25......
Still nothing had changed!
So then it was time to repeat the study......

DAY 0.....AGAIN. BUT WITH EXTRA GLOW!!!
4 Teaspoons

DAY 0
IMMEDIATELY AFTER ADDITION OF MICE

DAY 0

DAY 0

Day 2
The lid sanitation level portion of the study will continue for the full 6 months to monitor changes. Previous Study: 90d minimum. Based on our data? If lids need to be washed more frequently, this will take time and money! Technician handling/spor klenz.
Based on the glow powder portion of the experiment, the filters work!

However, there are other parts of the cage to worry about:

Port, lid itself

The mice may be euthanized to determine if ingested glow powder deposited in any tissues.

REFERENCES

• http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3314521
• http://www.blacklightworld.com/Contamination/EnviromentPowder.htm
• http://www.bd.com/ds/technicalCenter/promotionalFlyers/sx_index.ai.pdf
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• http://www.bvsanfrancisco.com/Products/veevey/custompages/veepages/111768.pdf
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catalog/PCS/001/002/003/004/2000000idN25ITH78E/rispJOCSD
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• http://www.essentialprotein.com/counts/efficiency/comparepeated_test-96a.html
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MORE REFERENCES...

• http://www.hpa.org.uk/pdfs/bacteria/detail.jsp?collection=not/deflated/NCTC%3
29848/Advisory/061210a.pdf
• http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3508182
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• http://www.sciencedirect.com/science/article/pii/S0378109703003276
• http://www.sigmaaldrich.com/edui/pdf/Fluka/Brochure/1/mibi_focus_2_4.Par.0001.File.tmp/mibi_focus_2_4.pdf
2013 OSU Cass Summer Fellowship

Extern: Carolyn Doerning, OSU Cass Summer Fellowship

- Currently a student at The Ohio State University, Columbus OH
- Projected graduation 2015
- Involved with ASLAP 2011 to present, OVMA, SCAVMA, 2015 Student government, AAFP Student chapter, Behavior Club, ECC Elective, Equine ICU Elective, Food Animal Club, Theriogeneology Club, Hoofbeats (CVM choir)

Mentor: Valerie Bergdall, DVM, DACLAM, Director, University Laboratory Animal Resources (ULAR) Professor Clinical Veterinary Preventative Medicine (VPM).
Dr. Bergdall is the Director of the University Laboratory Animal Resource Department (ULAR) and also serves as the Institutional Attending Veterinarian overseeing all animals at The Ohio State University. Dr. Bergdall has served on the ACLAM Training Program Oversight Committee, Career Pathways Committee, Standard Setting Study Task Force, Minimal Competency Task Force, and Recertification Oversight committees. She is a member of AALAS and ASLAP and serves as an AAALAC ad hoc site visitor. She currently serves on the NCRR/NIH scientific review group and is a co-investigator on an NIH grant investigating wound healing. Dr. Bergdall provides fiscal, clinical and didactic support for the Laboratory Animal Residency Program.

Research Project: “Effects of Rodent Analgesia on Post-Operative Pain and the Inflammatory Healing Response”
Introduction

An integral component of veterinary medical care is prevention or alleviation of pain.

Analgesics used to relieve signs of pain can impede the inflammatory process potentially resulting in obscured data.

OUTLINE

• Introduction

• Part 1 - Does peri-operative analgesia affect the pain response of mice undergoing a wounding procedure?

• Part 2 - Does peri-operative analgesia affect the inflammatory healing response to an excisional wound?

• Conclusions and discussion
Project Aims

1. To evaluate the response of various peri-operative analgesics in the surgical model

2. To investigate the effect of these agents on the inflammatory response of wound healing

Analgesic agents

- **Bupivacaine** [5]
  - Long-acting local injectable anesthetic
  - Acts locally, temporarily blocking nerve impulse conduction
  - Used to reduce the perception of pain
  - Most effectively used in conjunction with general anesthesia

- **Buprenorphine hydrochloride** [5]
  - Partial agonist with high affinity for the mu-opioid receptor
  - Primarily used for postoperative analgesia
  - Analgesic affects may last 8-12 hours and may be used preemptively
  - More potent than morphine

Collaborating group

- Dr. Sashwati Roy - Biology of diabetic wound healing study
  - Investigates mechanisms underlying impairment of diabetic wound healing
  - Primarily use C57Bl/6 and BKS Obese mice
  - Creation of wounds left to heal by secondary intention
  - Analgesics have not previously been used
Why do we need live animal models?

- To analyze local and systemic inflammatory response of wound healing
- To assess a clinical pain response to an analgesic/anesthetic therapy
- To study the dynamics of wound healing and associated analgesic responses

Which model do we choose?

- **C57BL/6 mice**
  - Strain is well genetically characterized
  - Previous wound healing studies by the Dr. Roy’s lab have used this strain as their model of choice
- **BKS.Cg-m +/- Lepr^db/J mice**
  - Spontaneous mutation of the leptin receptor gene
  - Ideal model for investigating diabetic-impaired wound healing

Chosen animal model

- **C57BL/6 male mice, 8 weeks old**
  - Our study only looks at the acute analgesic effects on behavior
  - The diabetes-impaired healing process would be an unneeded variable

Part 1

- Does peri-operative analgesia affect the pain response of mice undergoing a wounding procedure?
Surgical training

- Survival surgery training was received from both ULAR and Amitava Das of Dr. Roy’s lab

- Covered anesthesia, sterile prep, wounding procedure and suturing techniques
Acclimation phase and overview

- Upon arrival, mice are individually housed and acclimated
- Baseline data is collected
- Wounding procedure is performed on 4 mice at a time (total of 32 animals)
- Behavioral assessments are performed during first 24h post-op

Surgical prep

- All mice are clipped and Nair is applied at least 48h before wounding procedure

Wounding procedure - Materials

- **Prep**
  - Nair, clippers
  - Silicone, 8mm punch biopsy
  - Isoflurane, eye lube, alcohol, povidone

- **Surgery**
  - 6mm punch biopsy
  - Rodent surgery pack
  - Insulin syringes
  - Bupivacaine, Buprenorphine, saline
  - Surgical glue
  - 5.0 Polypropylene suture
  - Tegaderm, cloth tape

- Creation of silicone stents
  - Stents prevent wound contracture and allow wound to heal from second intention
Wounding procedure - Materials

- 4 mice underwent the wounding procedure each day, for a total of 32 animals over 8 days
- Each mouse was assigned to 1 of 4 treatment groups:
  - Bupivacaine: 5mg/kg at 2.5 mg/ml concentration
  - Buprenorphine: 0.1 mg/kg at 0.03 mg/ml concentration
  - Buprenorphine + Bupivacaine
  - Saline control: 0.1 to 0.15 ml

Wounding procedure

- Mice were anesthetized with isoflurane
- Eye lube was applied
- A scrub of alcohol and povidone was applied

Wounding procedure - Methods

- 2 symmetrical 6mm punch biopsy outlines were created
- Analgesic or saline was administered as a line block
- Surgeon/behavioral tester was blinded to treatment groups
Wounding procedure

- Full thickness punch biopsies were created in place of the outlines

Part 1

Wounding procedure

- Surgical glue applied to stents to help hold in place during suturing
- Stents affixed to outside of punch biopsy site
- Stents sutured in place using 5.0 prolene

Part 1

Wounding procedure

- Tegaderm applied on top of each wound
- Cloth bandage wrapped over stents and around midsection
- Mouse recovered and placed into cage for the first behavioral assessment
Wounding procedure

Does peri-operative analgesia affect the pain response of mice undergoing a wounding procedure?

- Various behavioral tests performed during first 24h post-op to assess pain level of each mouse
  - Mouse Grimace Scale
    - First 10min post-op
  - Nest Complexity Score
    - 8h post-op
  - Hind Paw Withdrawal using Von Frey Fibers
    - 4h and 6h post-op
  - Open Field Test
    - 8h and 24h post-op

Mouse Grimace Scale - Background

- Behavioral responses alone may only reflect nociceptive input, while facial expression may better reflect the affective component of pain [4]
- Nociception is a neurobiological process encoding noxious stimuli into neural impulses [5]
- Pain is the cognitive and emotive interpretation of the stimuli as hurtful or unpleasant [5]
Mouse Grimace Scale - Background

- “Coding of facial expressions of pain in the laboratory mouse” [3]
  - The Mouse Grimace Scale is a coding system consisting of five facial features (action units) that have been determined as reliable indices of pain, defined as follows:

  **Orbital tightening**
  - Narrowing of the orbital area, a tightly closed eyelid, or an “eye squeeze” [3]

  ![Orbital tightening](image)

  **Nose bulge**
  - The skin and muscles around the nose will be contracted, creating a rounded extension of skin visible on the bridge of the nose. [3]

  ![Nose bulge](image)

  **Cheek bulge**
  - Will appear to be convex from its neutral position. Considered to be the area directly below the eye to the beginning of the whiskers. Distance from eye to whisker pad may appear shortened. [3]

  ![Cheek bulge](image)
Ear position

- Ears may be pulled back from baseline or laid flat against the head. Tend to rotate outwards and/or back away from face when in pain. [3]

Mouse Grimace Scale - Materials

- Recording equipment
  - Night Owl Security Cameras and software
  - Sony Camcorder
  - Canon PowerShot Camera
- Clean cage
- Computer to analyze videos

Mouse Grimace Scale - Methods

- Mouse placed in clean, clear cage following recovery
- Recorded for 10 minutes uninterrupted
- Videos are transferred to a computer for analysis
- Screen shots are taken every 2-3 minutes when AU are present
- Photos will be coded based on presence and intensity of specific AU
  - 0 = AU not present
  - 1 = AU moderately visible
  - 2 = AU severe
Mouse Grimace Scale - Results

- Images from initial recordings were difficult to discern
- Quality of recording equipment not adequate to observe AU

Part 1

Nest Complexity Scoring - Background

- “Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring”
- Laboratory mice have high motivation for nest building
- Nest building is a simple, non-invasive indicator for estimating neurological dysfunction or pain
- Healthy mice build complex nests regardless of strain, sex, or housing conditions

Part 1
Nest Complexity Scoring - Background

- “Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring” [2]
  - Nest building performance decreases in correlation with the invasiveness of the surgical procedure
  - Scoring should be performed at end of light phase
  - Mice rebuild or repair an old nest at the end of the dark phase

Nest Complexity Scoring - Materials

- Clean cage
- 1 Fresh nestlet
- Camera
- Blinded observer(s) to score photos

Nest Complexity Scoring - Methods

- Photos were taken 8h post-op and NCS was determined using the established 0-5 scoring system

Nest complexity scoring [2]:

- Score 0 = nestlet not manipulated
- Score 1 = nestlet slightly manipulated, more than 80% of nestlet intact
- Score 2 = nestlet noticeably manipulated, less than 80% of nestlet intact
- Score 3 = noticeable nest site; less than 80% of nestlet intact, hollow in bedding, mice start building walls
- Score 4 = flat nest, hollow in bedding, walls mainly higher than mice
- Score 5 = complex, bowl-shaped nest, walls higher than mice
Nest Complexity Scoring - Results

- Photos were randomized and sent to 4 blinded observers to score
- Average, median, standard deviation, and standard error of mean were calculated from each set of scores

<table>
<thead>
<tr>
<th>Date</th>
<th>Mouse</th>
<th>Time point</th>
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Part 1

Nest Complexity Scoring - Initial impressions

- Majority of mice showed no nestlet activity at 8h post-op time point
- May be worth investigating effects of anesthesia alone on nest building activity

Von Frey Testing

- Von Frey filaments were borrowed from Lesley Fisher of the Spinal Cord Research Group
- Baseline data was collected before surgical wounding procedure
Von Frey Testing

- A filament is placed at a perpendicular angle to the mouse's hind paw and pressed against the paw
- Withdrawal to filament is recorded as positive or negative
- If no response, filament size is increased to the next highest diameter
- This process is continued until a positive withdrawal is observed
- The same procedure is then performed on the opposite hind paw

Part 1
Von Frey Testing

- Withdrawal response can convey:
 ▫ Hyperalgesia - exaggerated response to painful stimulus ([5]
 ▫ Allodynia - pain response to a non-noxious stimulus ([5]
 ▫ Hypersensitivity - reduced threshold to noxious stimuli
- This can become apparent when compared across different treatment groups or when comparing baseline to post-operative results
Von Frey Testing - Initial impressions

- Can be difficult to perform
- Stress levels of mice may interfere
- May be too subjective
- Can occasionally distinguish hypersensitivity

Open Field Activity Test

- Materials:
  - Camera/tripod
  - Plexiglas open field box
  - Cardboard box
- Methods:
  - Performed at 8h and 24h PO
  - Mouse placed in center of open field and recorded for 5 minutes
  - Videos transferred to computer and various actions counted

Open Field Activity Test - Background

- “Animal models of anxiety in mice” [1]
- Most standardized measure of general motor function
  - Allows observation of behavioral patterns
  - Most important is tendency to stay on the periphery of the field, or thigmotaxis
  - Thigmotaxis is often a reliable indicator of anxious behavior
Open Field Activity Test - Initial impressions

- Easy assessment, quantifiable data
- Activity levels of most mice were uniform
- Occasional mouse would display less activity at 4:00pm vs. 8:00am time points

Complications

- Few complications were noted post-operatively
- One animal developed a severe air pocket
- Several animals developed urine scalding or penile prolapses
- Almost all animals had some level of debris stuck in their bandage
- 1 of 32 died under GA

Part 2

- Does peri-operative analgesia affect the inflammatory response to an excisional wound?

Materials and Methods Overview

- Bandages were changed and wounds photographed every other day until end of study
- Mice were euthanized on day 3, 5, and 7 post-op
- Both wounds were harvested and prepared for analysis at a later time point
- Tissue samples were flash frozen in liquid N and placed in -80C freezer, or preserved in formalin
Materials and Methods

Part 2

Tissue preparation methods

- Wound progression photographed
- Sutures and stents removed
- Square was cut around each wound

Wound #1

- Dissect a 1 mm ring of tissue around the wound
- Each strip of skin is placed in an Eppendorf tube and placed in liquid nitrogen
- Will eventually be analyzed for RNA/Protein expression
Wound #2 - 1st half

- Cut square of wound tissue in half
- Place wound in OCT and into liquid N to flash freeze
- Will be sectioned and stained in the future

Wound #2 - 2nd half

- Place onto cassette and preserve in 10% formalin for submission to comparative pathology lab
- Will be sectioned and stained in the future
Upcoming conclusions

• Was there an effect on pain response in the groups receiving analgesic versus the control?
• Does the use of anesthesia alone impact behavioral tests?
• Were the tests used to assess pain behavior adequate and reliable?
• Can analgesia be used without confounding data?

Thank you to the many people who helped with this project!

• Dr. Matthew Hogan
• Dr. Valerie Bergdall
• Dr. Sashwati Roy
• Amitava Das
• Briony Smith
• ... and more

Questions?

References