Group housing during hindlimb unloading to simulate weightlessness

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Rodent hindlimb unloading (HU) as a ground-based analog for weightlessness

- Analog for musculoskeletal disuse and cephalad fluid shift observed in spaceflight (Morey-Holton, Bikle and Globus 1984)
- Tail traction along a pulley system to elevate hindlimbs; single housing
- Variations
  - Partial unloading (Spatz et al 2017)
  - Single or group housing; tail piercing at intervertebral disc space (Ferreira, Crissy and Brown 2011)

Images courtesy of Dr. Ralph Muller
Rationale for group housing during HU

- Rodents are social animals

- Long-term social isolation → altered behavior and organ-system responses
  
  *Single vs group housed mice*
  
  - Altered synaptic plasticity; ↑ voluntary ethanol intake (Talani et al 2014)
  - Immune system changes (Ref)
  - Smaller; ↓ soft-lean tissue mass, ↓ BMC and BMD (Nagy et al 2002)
  - ↑ variance in % body fat, BMC and BMD (Nagy et al 2002)

- Better simulation of housing conditions in ISS (AEM)
Key topics

1. Group (paired housing) HU design
2. Group vs single housing HU: effects on spaceflight-relevant tissues
3. Behavioral analysis: preliminary results
Group vs single housing HU

Hypotheses

- Long-duration HU in group housing → deficits in musculoskeletal structure; comparable to single housing HU
- Group housing will provide opportunities for olfactory, visual, auditory and physical stimulation and interaction → improved animal well-being; minimize effects of stress-induced social isolation
### Experiment design

<table>
<thead>
<tr>
<th>Experiment Parameters</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Group housing HU</td>
<td>Single housing HU</td>
</tr>
<tr>
<td></td>
<td>Group housing control</td>
<td>Single housing control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group housing HU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group housing control</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Strain</td>
<td>C57BL/6J</td>
<td>C57BL/6NJ</td>
</tr>
<tr>
<td>Age at start of HU</td>
<td>16-17 weeks</td>
<td>16 weeks</td>
</tr>
<tr>
<td>Duration of HU</td>
<td>30 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Measurements</td>
<td>Soleus, adrenal and spleen weights; body weights;</td>
<td>Soleus, adrenal and spleen weights; body weights;</td>
</tr>
<tr>
<td></td>
<td>food consumption</td>
<td>food consumption; behavior</td>
</tr>
</tbody>
</table>

IACUC approval obtained prior to any animal experiments.
No differences in body weights in single vs group housing HU

**Study 1**  
Group housing, C57BL/6J

**Study 2**  
Single vs Group housing, C57BL/6NJ

Stats: t-test at p<0.05

Stats: One-way ANOVA at p<0.05
Increased food consumption in single vs group housing HU animals; strain differences in response to HU

**Study 1**  
Group housing, C57BL/6J

**Study 2**  
Single vs Group housing, C57BL/6NJ

*Significant by one-way ANOVA followed by Tukey-Kramer post-hoc test at p<0.05; Means and SD shown*
Group housing HU leads to expected responses in postural and type II fiber-enriched muscles; response not different from response to single housing HU

**Study 1**  
Group housing, C57BL/6J

**Soleus**

```
*  
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**Tibialis anterior**

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*  
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**Study 2**  
Single vs Group housing, C57BL/6NJ

**Soleus**

```
*  
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*p<0.05 by t-test; Means and SD shown; N=8/group

*One-way ANOVA and Tukey-Kramer post-hoc test at p<0.05; Means and SD shown
No differences in spleen weights in single vs group housing HU

**Study 1**
Group housing, C57BL/6J

* *p<0.05 by t-test; N=8/group

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>30D HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, mg</td>
<td>80</td>
<td>70</td>
</tr>
</tbody>
</table>

**Study 2**
Single vs Group housing, C57BL/6NJ

* *One-way ANOVA and Tukey-Kramer post-hoc test at p<0.05;
NS: Not significant;

<table>
<thead>
<tr>
<th></th>
<th>Control, Single</th>
<th>30D HU, Single</th>
<th>Control, Group</th>
<th>30D HU, Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, mg</td>
<td>90 ± 5</td>
<td>85 ± 4</td>
<td>100 ± 3</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

* NS: Not significant;
Group housing HU leads to a modest increase in adrenal weights; not different from response to single housing HU

**Study 1**
Group housing, C57BL/6J

- Control
- 30D HU

* p<0.05 by t-test; N=8/group

**Study 2**
Single vs Group housing, C57BL/6NJ

- Control, Single
- 30D HU, Single
- Control, Group
- 30D HU, Group

N=8 N=8 N=12 N=12

*Wilcoxon test at p<0.05;
NS: Not significant;
a: Not significant vs 30D HU Single
Group and single housing HU: preliminary results from behavioral analysis

Methods
• 24hr video acquisition (5 frame/sec) started on unloading day 28
• 15min samples analyzed within 3 hrs of lights on/off (12hr light:dark cycle)

Qualitative observations
• Group-housed HU mice did not exhibit physical contact, although occasionally observed to ambulate toward cagemate
• Singly-housed HU mice look similar to group-housed in terms of behavioral repertoire
• Singly-housed control mice appeared fairly inactive
Summary

• Group (paired housing HU) design
  - Portable, low footprint for storage
  - Compatible with automated vivarium cleaning equipment
  - Source materials readily available
  - Building requires standard tools

• Group housing HU (vs controls)
  ↓ soleus mass, no change mass of type II fiber-enriched muscle (TA)
  ↓ spleen mass; consistent with findings from 13-day spaceflight mission (Gridley et al 2013)
    Slight increase in adrenal weights

• Single vs group housing HU differences: only in food consumption

• Group housing HU well tolerated
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