Untwisting Questions about Nesting Materials and Laboratory Mice

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Why Do We Care?

- While laboratory mice greatly benefit from nesting material[1,2,3,4], there are several barriers to implementation in USA-based research institutions:
  1. Institutions are apprehensive to provide necessary amounts of nesting material for mice to maintain core body temperature due to difficulty visualizing animals during daily welfare checks.
  2. There is a high cost of labor required to manually add nesting material to cages.
- As a result, USA-based institutions often provide mice with inadequate amounts of nesting material, or do not provide any nesting material at all, therefore compromising the welfare of the mice.

Background:

- Mice maintain their core body temperature regardless of access to nesting material.[1]
- Consistent with lower thermoregulatory burden, mice with nests reproduce more successfully than mice without nests while consuming the same amount of food.
- Mice adjust the shape of their nests in response to thermal stress.[1,3]
- The eye and inner ear of a mouse are representative of core body temperature (35°C– 37°C); tail temperature changes via vasoconstriction and vasodilation in response to thermal stress.[3,4]

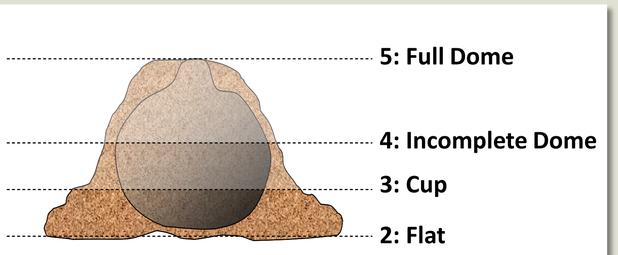
What is the Ideal Nesting Material?

- The ideal nesting material should be highly insulative and easily manipulated by mice. A nesting material with this combination will encourage the construction of a flatter nest without compromising an animal's ability to maintain core body temperature.

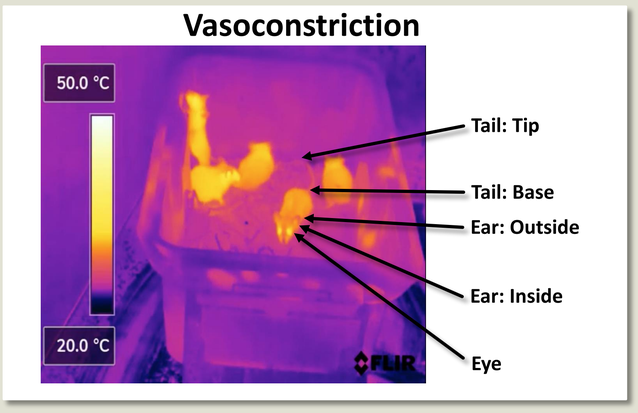
Predictions:

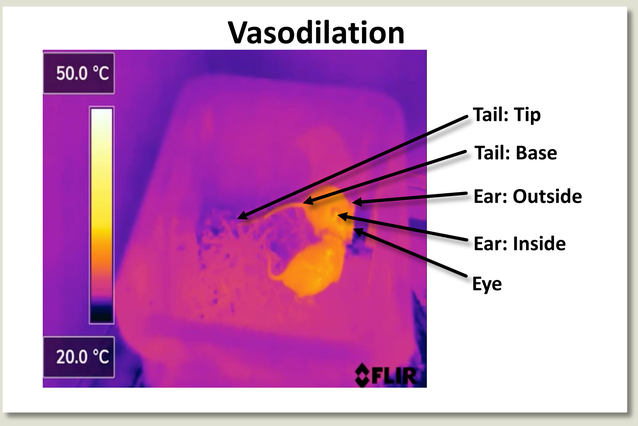
- Here, we tested the impact of three nesting materials (EnviroDri, Loose Alpha Twist, and Tight Alpha Twist) on the well-being of laboratory mice.
- We anticipated that mice would have difficulty interacting with both the AlphaTwist materials, thereby leading to poorer nest construction, and ultimately an inability to maintain thermoneutrality.

What Do We Mean by Nest Score?

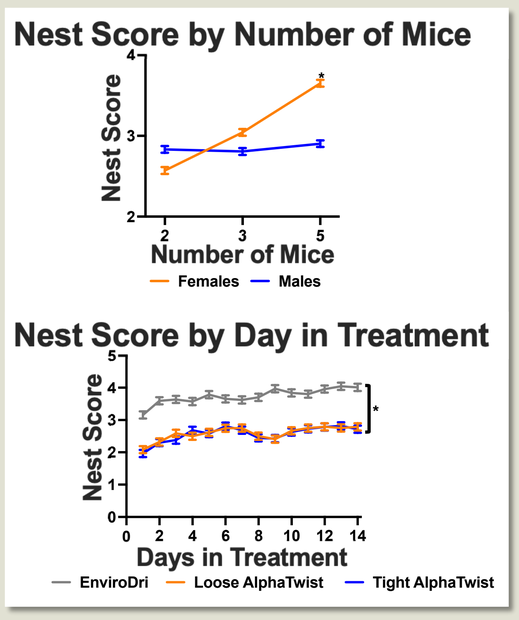


Thermal Regions of Interest:



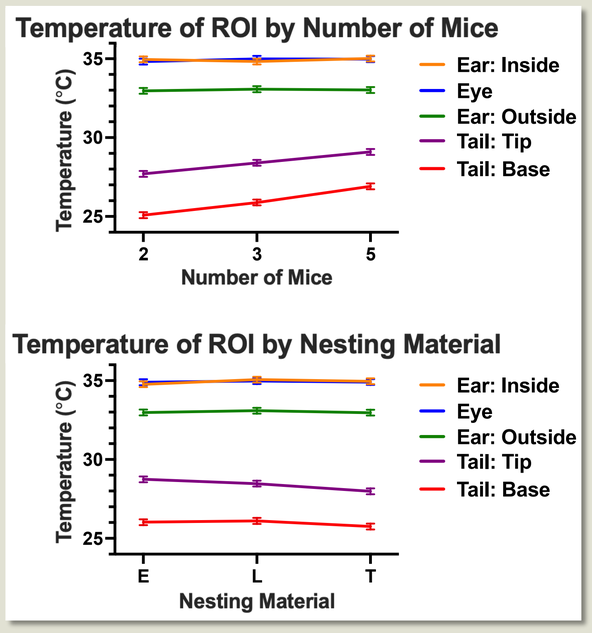


Does Nest Score Differ Between Nesting Material?



- Female mice build higher scoring nests than male mice ($p < 0.0001$).
- Nest score increases as the number of mice in the cage increases ($p < 0.0001$).
- Nest score is higher in EnviroDri ($p < 0.0001$) than in AlphaTwist.

Does Thermoregulation Differ Between Nesting Material?



- Mice are able to thermoregulate more effectively in larger group sizes ($p < 0.0001$).
- There is no difference in the ability of mice to thermoregulate in any of the materials studied ($p = 0.4061$).

Conclusions:

- Mice build higher scoring nests with EnviroDri than with either of the AlphaTwist nesting materials.
- All three nesting materials support the thermal needs of laboratory mice.
- Loose AlphaTwist provides necessary warmth while encouraging the construction of flatter nests.
- These data further support the importance of group housing laboratory mice.

Future Directions:

- We will assess fecal corticosterone levels to ensure the flatter nests do not increase animal stress levels.

References:

1) Gaskill, B.N., Gordon, C. J., Pajor, E. A., Lucas, J. R., Davis, J. K., Garner, J. P., (2013). *Physiol Behav.* 110-111: 87-95.
2) Gaskill, B.N., Karas, A. Z., Garner, J. P., Pritchett-Corning, K.R., (2013). *J. of Vis. Exp.* (82): e51012.
3) Gordon, C.J., (2017). *Physiology and Behavior.* (179): 55-66.
4) Meyer, C. W., Ootsuka, Y., Romanovsky, A. A., (2017). *Front. Physiol.* 8: 520.

Methods:

- **Subjects:** *N* = 40 C57Bl/6 mice (20 males; 20 females) that were slated for euthanasia.
- **Housing:** We housed mice in same-sex groups of 2, 3, or 5 animals in standard IVC cages (Innovive) on a standard 12hr/12hr light/dark cycle with food and water available *ad libitum*.
- **Study design:** We used a cross-over factorial design in which each cage was its own control. Mice experienced each nesting material type for two weeks; we randomized the order of exposure to nesting material type to account for potential influences of previous experience on nest score. To account for potential interactions of cage location, number of mice per cage, and sex, we randomized cage location on racks and blocked analyses by cage.
- **Cage Change and Nesting Materials:** We changed cages weekly, during which time we recorded thermal video to assess temperature, collected fresh fecal samples to measure corticosterone, and scored body condition of the mice. We provided the mice with 8 grams of nesting material (EnviroDri or AlphaTwist). When switching within treatment, we transferred a small amount of the original nest with the mice into a clean cage. When transitioning between treatment types, we did not include the original nest when transferring mice.
- **Nest scoring:** Trained observers scored nests daily at approximately 9 am daily. Observers gave each quadrant of the nest a score, which were averaged to produce nest score. We then averaged nest scores from four observers to produce an overall nest score, which was used for the purposes of analyses.
- **Thermal Image Recording and Visualization:** Using FLIR Thermal Studio, we obtained temperatures of the eye, inner ear, outer ear, base of tail, and tip of tail for each individual mouse when they exited their nest during cage change.
- **Statistical analysis:** All statistical analyses were blocked by cage, nested within number of mice and sex, and number of mice and sex were crossed with treatment. For thermal imaging, ROI was included as a crossed term to conduct a compositional analysis. All analyses were conducted using JMP16 Pro for Windows using GLM.

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