

Pain Assessment in Mice

What if you see ...?

Analgesics must be administered as outlined in the IACUC-approved protocol. When “as needed” analgesics are specified, animals must be monitored for signs of pain, and treated accordingly.

Listed below are easily identifiable indicators of pain in mice. This is neither a comprehensive nor specific list, and as such, other observations should be taken into account when assessing pain status in mice. Please contact ULAM veterinary staff for additional assistance with identification and/or treatment of pain.

Appearance

Interpretation of **facial expression** can be used to qualify pain. Below are images of mice who show varying levels of pain.

Orbital Tightening

There will be a narrowing of the orbital area, with a tightly closed eyelid or an eye squeeze (denoted by a wrinkle around the eye).

Nose Bulge

A rounded extension of skin is visible on the bridge of the nose.

Cheek Bulge

There will be a convex appearance of the cheek muscle (between the eye and whiskers) from its baseline position.

Ear Position

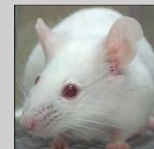
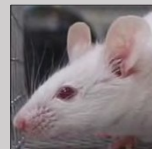
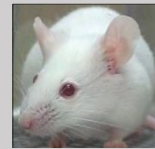
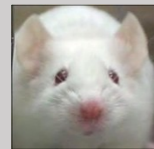
Ears pulled apart and back from their baseline position or featuring vertical ridges that form owing to tips of ears being drawn back.

Whisker Change

Movement of whiskers from their baseline position either backward, against the face or forward, as if standing on end; may also clump together.

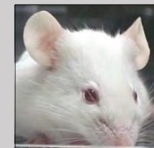
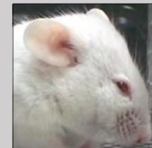
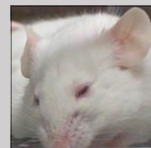
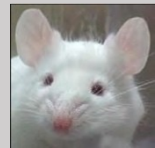
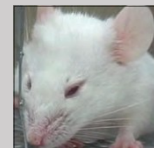
Not present

“0”



Pronounced

“2”



Rough and/or dirty hair coat and piloerection are indicators of lack of grooming, pain and/or stress.

Piloerection

The involuntary erection or bristling of hairs.



Behavior

When mice are experiencing pain, they may demonstrate the following **abnormal behaviors**:

Appetite

Reduced food and/or water intake

Activity and Posture

Reluctance to move

Decreased interaction with the environment and/or cage mates

Reduced grooming activity

Decreased cage organization

Hunched posture

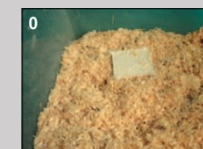
Increased aggressiveness and/or self-mutilation

Nest Building

Static cage side assessment of nesting behavior via nest score:

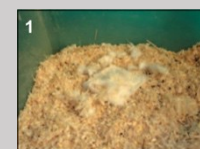
Score 0

Nestlet not manipulated, possibly dragged around cage.



Score 1

Nestlet slightly manipulated, more than 80% intact, possibly a few shreds picked out.



Score 2

Nestlet noticeably manipulated, less than 80% intact, shreds spread around or in one area.



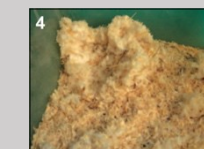
Score 3

Nestlet noticeably manipulated, less than 80% intact, shreds placed mostly in nest site, hollow building +/- walls.



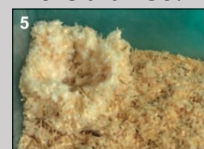
Score 4

Flat nest, hollow in bedding, walls mainly higher than mice and encasing the nest less than 50%.



Score 5

Complex nest, more than 50% shreds picked out, bowl-shaped nest, walls higher than mice and encasing the nest by more than 50%.



Dynamic cage side tests for pain-related depression of nesting:

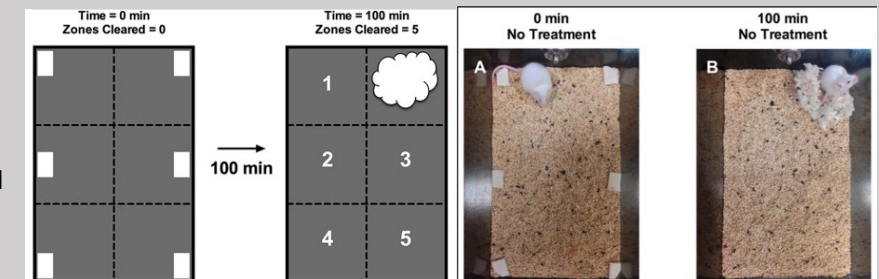
Zone Clearance Test

1. Remove the existing nest.

2. Cut a nestlet square into 6 pieces and place at 6 zones as pictured below.

3. Measure how many of these zones were cleared after 100 minutes.

Possible scores range from 0 to 5.



Time to Integrate into Nest Test (TINT)

1. Add a quarter of a nestlet to the mouse cage.

2. Observe the nesting behavior immediately thereafter.

Positive TINT = the square is missing from its original location after 10 minutes.

Negative TINT = square remains in the original location after 10 minutes and so, additional observations are needed to determine if the mice require veterinary attention.

For information on how to carry out this test, please watch the following video:

<https://www.jove.com/video/51012/nest-building-as-an-indicator-of-health-and-welfare-in-laboratory-mice>

References

1. The Mouse Grimace Scale images and accompanying descriptions are borrowed verbatim from: Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Ingrao, J., Klassen-Ross, T., LaCroix-Fralish, M.L., Matsumiya, L., Sorge, R.E., Sotocinal, S.G., Tabaka, J.M., Wong, D., van den Maagdenberg, A.M.J.M., Ferrari, M.D., Craig, K.D., and Mogil, J.S. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods*, in press.
2. Piloerection image and description from: Assessing the Health and Welfare of Laboratory Animals. Basic Assessment of Animal Health and Welfare Tutorial: www.ahwla.org.uk/site/tutorials/HW/HW17-Fur2.html
3. Behavioral Indicators from: Guidelines for the Assessment and Management of Pain in Rodents and Rabbits: http://www.aclam.org/Content/files/files/Public/Active/position_pain-rodent-rabbit.pdf
4. Nest Building image and description from: Jirkof P, Fleischmann T, Cesarovic N, Rettich A, Vogel J, Arras M. Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring. *Lab Anim.* 2013 Jul;47(3): 153-61.
5. Time to Integrate into Nest Test description taken from: Gaskill, B.N., Karas, A.Z., Garner, J.P., Pritchett-Corning, K.R. Nest Building as an Indicator of Health and Welfare in Laboratory Mice. *J Vis. Exp.* (82), e51012, doi:10.3791/51012 (2013).
6. Zone Clearance Test description taken from: Negus SS, Bradley N, Altarifi AA, Carroll FL, Leitl MD, Miller LL. Effects of ketoprofen, morphine, and kappa opioids on pain-related depression of nesting in mice. *Pain.* 2015 Jun; 156(6): 1153-60.