Video Article

Nest Building as an Indicator of Health and Welfare in Laboratory Mice

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Abstract

The minimization and alleviation of suffering has moral and scientific implications. In order to mitigate this negative experience one must be able to identify when an animal is actually in distress. Pain, illness, or distress cannot be managed if unrecognized. Evaluation of pain or illness typically involves the measurement of physiologic and behavioral indicators which are either invasive or not suitable for large scale assessment. The observation of nesting behavior shows promise as the basis of a species appropriate cage-side assessment tool for recognizing distress in mice. Here we demonstrate the utility of nest building behavior in laboratory mice as an ethologically relevant indicator of welfare. The methods presented can be successfully used to identify thermal stressors, aggressive cages, sickness, and pain. Observation of nest building behavior in mouse colonies provides a refinement to health and well-being assessment on a day to day basis.

Video Link

The video component of this article can be found at https://www.jove.com/video/51012/

Introduction

Measurement of animal welfare is a difficult and often subjective assessment. When animals are used for scientific purposes, there is an obligation to provide the best care possible and minimize discomfort[1]. Species-specific objective methods are needed to measure overall well-being for scientific and husbandry purposes. Current methods of welfare assessment in laboratory mice are either time consuming, require expensive equipment, or must be analyzed later to determine if an alteration has occurred[2-3]. These techniques are useful in a scientific setting, but difficult to incorporate in day to day husbandry. A simple, mouse-oriented method that can quickly and easily identify mice in pain or distress would be a step toward the immediate identification and alleviation of those stressors.

Nests are used by wild mice to shelter from predators, retreat from harsh environmental conditions, and protect young[4]. Behaviors associated with nest building are therefore highly linked to the survival of wild mice[5]. Laboratory mice, even though removed from wild conditions for many generations, are highly motivated to build a nest when given proper materials[6,7]. Any changes to these highly motivated behaviors, or the nest itself, should indicate a substantial alteration in the environment or the animals themselves.

The goal of this method is to exploit highly motivated nesting behaviors in the laboratory mouse to quickly identify pain, illness, or other stressors that may affect or reduce the animal's overall welfare. This binary cage-side assessment technique is advantageous over other existing methods because little training is needed, testing materials are found in the average mouse colony, and the test can be completed with little to no manipulation of the animals. Additionally, the provision of fresh and or different nesting material on a frequent basis may provide a positive interaction between mice and caretakers since nesting material has been found to be rewarding to mice[8].

Protocol

Both of the following protocols are most accurate when animals are housed on solid bottom caging with bedding material.

1. Nest Scoring[6]

1. Weigh out 8-10 g of nesting material.
    1. Crinkled paper nesting material (such as Enviro-Dri) is recommended as the substrate for nest scoring since most mice build better nests with this substrate[6] but other materials can be used[9].

2. Identify the cage(s) to be scored.
    1. Animals should be housed in solid-bottomed cages for optimal performance on this test.

3. Place paper nesting material into the cage.
    1. Nesting material can be provided at any time convenient to the researcher or animal care staff

4. Return the next day at 7-9 hr after lights on for scoring.
   Note: This is the best time to score nests since mice should be inactive[10], nests score highest[11], and animal rooms are quiet. If animals are active they may trample the nest, altering the score. Variation in nest building will occur depending on the amount of time the mice are given to build, making it important to score the same day and time after new material is provided.
5. Use the nest scoring protocol by Hess *et al.*[6] to score each nest (**Figure 1**).
    1. First determine whether the material has been manipulated by the mice. This includes being processed or moved around the cage.
        1. If the material is untouched the whole nest receives a score of 0;

    2. Next determine if the majority of the nesting material has been gathered to a central nest site.
        1. No nest site: If it is <u>not</u> clear where the nest site is located the cage receives a score of 1 (*i.e.* the material is spread throughout the cage);
        2. Nest site: If a centralized nest site <u>is</u> present, consider the nest as if it were a square. Each of the 4 sides of the square can be given a separate score from 2-5. The 4 scores are then averaged for the cage nest score.
            1. 2 = the nest is flat with no shallow walls;
            2. 3 = a slightly cupped shape where the wall of the nest is less than ½ the height of a dome that would cover a mouse;
            3. 4 = a wall that is ½ the height of a dome;
            4. 5 = walls that are taller than ½ the height of a dome, which may or may not fully enclose the nest.

Note: Alternatively, scoring can occur 3-4 days after the provision of new material, when peak scores are often reached (unpublished data).

2. Time to Integrate into Nest Test[12]

Note: This test will be most accurate if the animal has a long fiber paper nesting material and has built a nest with a score of at least 2 (see above).

6. Take a 2 in square of cotton nesting material (such as a Nestlet) and cut it into quarters, resulting in 4-1 in testing squares.
7. Test animals within the first 3 hr of vivarium lights turning on.
    1. This window of testing takes advantage of increased nest building behaviors prior to daytime inactivity[11].

8. Open the cage lid and place 1 testing square on the opposite side of the cage from the main nest site.
    1. Place the test square where it can be seen from outside the cage.
    2. The test material should be placed in the same location each time the test is conducted.

9. Return to the cage after 10 min.

Note: If the test square is missing from its original location, the cage is TINT positive. If the test square remains in the original location, the cage is TINT negative and additional observations may be needed to determine if the mice require veterinary attention.

Representative Results

Temperature:

Nests built by groups of 3 C57BL/6 mice were scored in cages held at three different ambient temperatures (20, 25, or 30 °C; **Figure 2**[13]) after 24 hr. As the temperature increases, nest score decreases. The nest walls lower and nests become more open, allowing for more heat loss.

Aggression:

Groups of male mice with a high level of aggressive interactions which result in wounding can be identified by nest shape. Over a 5 week experiment, nest score was found to negatively correlate with the average number of wounded C57BL/6 male mice in a cage ($F_{1,26}$ = 13.5; P=0.0011; **Figure 3**). The specific substrain of C57BL/6 mice did not significantly explain wounding data ($F_{2,26}$ = 2.69; P = 0.086). Cages with higher scoring nests had fewer mice wounded than those in lower scoring nests regardless of substrain.

Sickness:

Mice experiencing a general malaise have been found to engage in little to no nest building behavior when forced to build a nest[14].

Pain:

Nest conformation has been observed to be altered after a painful surgical procedure[11,15]. The number of animals with a negative TINT increase by approximately 60% after a painful surgery[12] but mice returned to positive baseline TINT levels 3 days postoperatively (**Figure 4**).

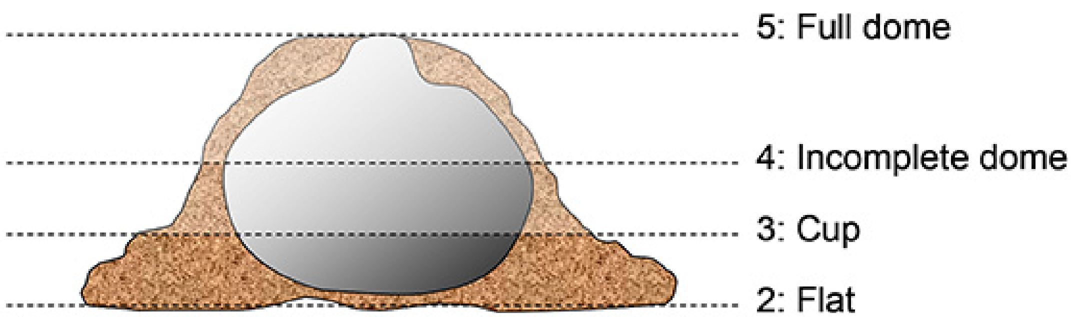

Figure 1. Nest scoring diagram. A nest, with internal cavity, that depicts different scoring wall heights[6]. The figure is reproduced with permission from the Journal of the American Association of Laboratory Animal Science. Click here to view larger image.

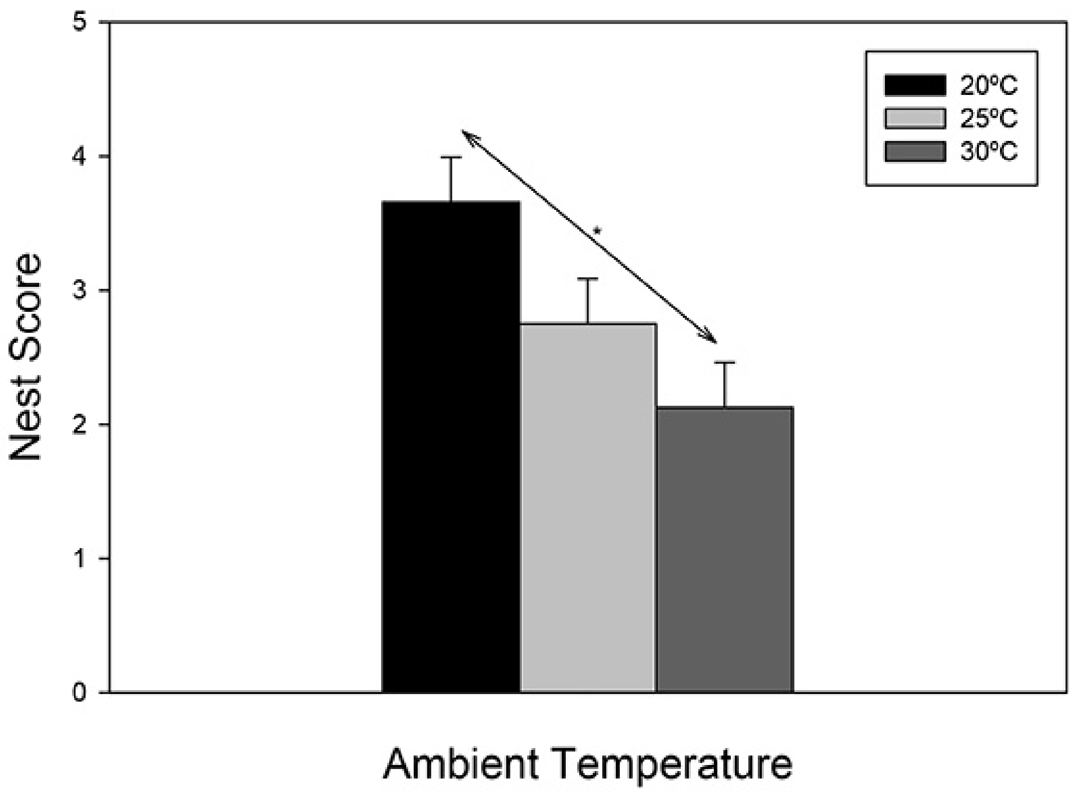

Figure 2. Affect of temperature on nest score. Average nest score of mice confined to an ambient temperature of 20, 25, or 30 °C[13]. The diagonal line indicates a significant linear contrast. The figure is reproduced with permission from Elsevier. Click here to view larger image.

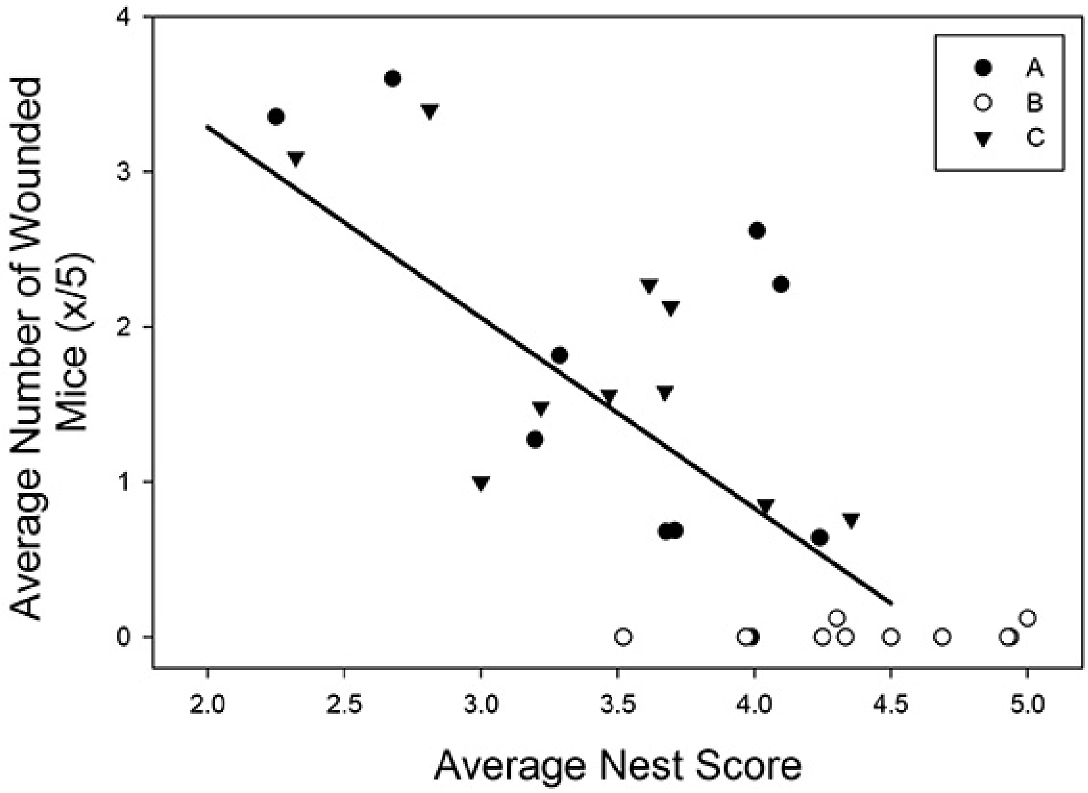

Figure 3. The effect of aggression on nest scores. This graph depicts the correlation between the average nest score of a cage with 5 male mice and the number of those males that were wounded. This was assessed in 3 different substrains of C57BL6 mice. Click here to view larger image.

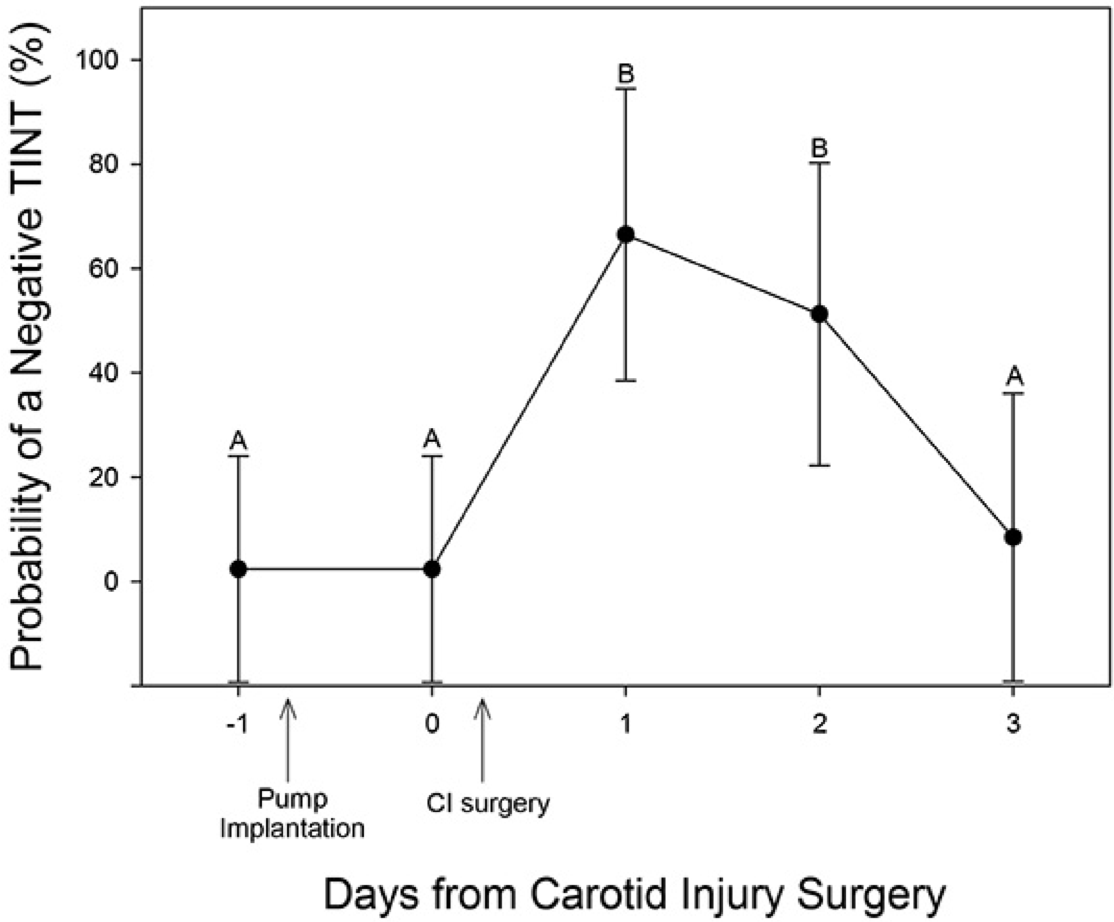

Figure 4. The effect of pain on TINT. The LSM and SE of a negative TINT are depicted before and after two surgeries[12]. The figure is reproduced with permission from the Journal of the American Association of Laboratory Animal Science. Click here to view larger image.

Discussion

Based on these results, nest building is altered depending on the degree of thermal stress, the amount of aggression present within the cage, when animals experience a general malaise, and gathering behavior is not seen in postsurgical and potentially painful mice. Since any and all of these factors will reduce a mouse's welfare, we believe that nest building can be utilized to identify cages or animals where interventions need to be made to improve their overall well-being. We believe that nest scoring may be a method best utilized for scientific purposes because the scale is more refined and can detect smaller differences than the binary TINT. However, we believe that the TINT is a more practical tool that is best applied during daily husbandry or postsurgical identification of painful animals.

Unlike social predatory species, where displays of illness or injury may be beneficial, displaying signals of weakness in prey species may be the difference between survival or death. Rabbits, for example, have been observed to respond to pain or distress by remaining motionless, especially in the presence of a human, making behavioral indicators of distress seemingly useless[16]. Ongoing pain behaviors in mice are also difficult to identify and use to quantify pain[17]. This is especially difficult in nocturnal laboratory species where, at a glance, distressed and inactive mice can easily be confused with sleeping ones.

Resources, such as food or materials which allow for nest building, are strongly tied to the survival and fitness of wild mice[5,18,19]. In studies, nesting material is a resource laboratory mice are strongly motivated to obtain[7,8]. Enviro-Dri has been shown to be an ethologically relevant enrichment for mice which allows them to alleviate thermal stress by building more insulating nests[7,10,13]. The long fiber length, which requires little to no processing, allows even poor nest builders to build better nests[6,7]. This material is also suitable for nude mice[20], which are often susceptible to eye lesions. While Enviro-Dri is superior for nest building, other material (*i.e.* Nestlets) functions better for the TINT. The presence or absence of the test square from the designated area is less subjective than attempting to determine if a portion of Enviro-Dri strips was removed.

Both nest scoring and TINT can easily be utilized in singly housed animals however issues may arise if 1 animal out of the group may be experiencing pain or distress. The data presented in this manuscript illustrate that thermal stress and cage aggression can be identified in group housed mice. Further work looking into social cohesion and interactions between healthy and unhealthy animals is currently being investigated. Therefore we caution utilizing these methods in group housed animals to detect illness or pain in a subset of the group.

Variation in building and gathering material between types of laboratory mice is well documented[7,21-23] and should be considered before using this technique for all types of mice. Specifically, C3H mice do not gather or build as complex nests as other inbred strains[21,22]. Therefore, normal nest conformation and TINT outcomes in unmanipulated mice should be observed prior to utilizing this technique in any strain or stock. It is possible that different cage types may influence building outcome. For instance, increased convective heat loss experienced in individually ventilated cages may increase overall nest scores due to additional thermal stress.

The utility of the examination of nesting behavior has only begun to be explored. The literature indicates that nests change due to the hormonal status of the dam[24,25] and therefore could also be a tool for identifying periparturient females without disturbing them. Although it has not yet been directly tested, researchers studying models of human disease might also use evaluation of nesting behavior as a humane end point or to identify disease onset, ultimately alleviating overall suffering. Alteration in this essential and complex behavioral sequence might also be a more valid, mouse-oriented, task for models of mental health disorders or even drug testing.

Disclosures

Brianna N. Gaskill and Kathleen R. Pritchett-Corning are employees of Charles River Laboratories who breeds the mouse strain used in this article.

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