UNIVERSITY OF PADUA

Department of Comparative Biomedicine and Food Science

First Cycle Degree (B. Sc.)
in Animal Care

Nesting analysis in a research facility for mice

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Index

Abstract

The observation of nesting behaviour shows promise as an non-invasive and minimally aversive home cage assessment tool for recognizing distress in laboratory mice, being particularly relevant for breeding cohorts. In this study, we highlight the remarkable variability of nest-building behaviour in laboratory mice breeding pairs, which may also serve as a relevant ethological indicator of welfare and mothering abilities.

To this aim, we conducted an observational study in which 60 breeding cages were analysed for nest building patterns along with specific biological and husbandry observations. These included genetic background, breeding parameters such as litter size, health assessments for parents and pups, and housing and husbandry conditions such as type of bedding, cage enrichment, room temperature and humidity. The investigation took place within a large Specific Pathogen free (SPF) animal unit at Queen Mary University of London. A non-invasive and minimally aversive approach was used, utilizing a comprehensive checklist to systematically annotate different clinical, husbandry and nest building patterns in the breeding cages. Daily observations were recorded using semi-qualitative techniques, and data was organized in an Excel file for further descriptive statistical analysis.

This study provides an informative observational approach on the variable nesting settings within an animal unit, supported by an effective use of a systematic nest building assessment and to associate the identification of potential stressors and adverse conditions experienced by breeding mice in a laboratory animal settings. Specifically, our data showed the impact of temperature, aggression behaviour and clinical signs of distress on the nesting patterns analysed. This support the benefits of assessing nest building behavior in mouse colonies as a good supportive indicator of health and well-being assessment.

Introduction

During the internship in the Biological Service Unit (BSU) at the Charterhouse Campus of the Queen Mary University London, it has been developed a research project to study mice nesting behaviour, with the support of my tutor Jordi Lopez Tremoleda (the designated veterinarian of the facility). The project involved gathering descriptive observational data on the factors influencing nesting behaviour across a large cohort of breeding mice housed in individually ventilated cages (IVC) using non-invasive methods to minimize stress on the mice, particularly the mothers. The dataset designed encompassed many of the factors that may influence, even minimally, the welfare of mice, and consequently, their nesting behaviour. These factors were used to design a checklist protocol that was used for the observational analysis, along with some extra information data from the mouse strains which was digitally recorded by the Automated Reporting Management Information System (ARMIS).

A scoring sheet was designed to record the nesting behavior observed in 60 randomly selected cages. Collected data was transcribed into an Excel file and binary values of 1 and 0 were assigned to support further analysis. A descriptive statistical analysis was carried out and results were presented in the form of pie charts for relative frequencies and histograms for absolute frequencies.

The practice of conducting behavioural observations in a laboratory research setting remains critical to support best welfare monitoring and husbandry care. While the use of a controlled environment promotes standardized behaviors, potential drawbacks associated with housing conditions include biases, unreliability, and a lack of representativeness. The artificial setting may hinder the replication of certain behaviours and fail to capture the full range of natural behaviours, influenced by human contact. Factors affecting semi-qualitative behavioural assessments (SQBA) include age, gender, human presence, emotional state, and individual experiences, which may also impact quantitative analysis (Reading et al., 2013).

1.1 Nest building behaviour

Nest building is an innate behavior in many animals. Mice, both male and female, build similarly-sized nests for reproductive purposes, heat conservation, behavioral enrichment, to filter out harsh light and also to predict the possible decline of cognition. All these behaviours associated with nest building are therefore highly linked to the survival of wild mice. Laboratory mice, even though removed from wild conditions for many generations, are highly motivated to build a nest when given proper materials (Gaskill et al., 2013), being a form of environmental enrichment. Changes in these behaviours generally indicate a change in health or welfare. In most cases, a lack of nest building is attributed to several factors that negatively impact well-being.

Nesting behavior in rodents is considered as a positive behavior, albeit with a few drawbacks associated with the potential build-up of ammonia and the risk of entanglement associated with certain types of nesting material, such as Paper Wool. Ammonia is an irritant that can affect the upper airways, and exposure to elevated levels has been associated with pathologic lesions in rodents (Merley et al., 2022). Furthermore, instances have occurred within the facility wherein some mice became entrapped in the nesting material, resulting in accidental strangulation. One such incident was observed in our study with one of the two sentinel mice (cage 7H, rack 5, room 16) belonging to the CD1 strain (NON-GA). This particular strain exhibits a tendency to crunch various items within the cage, including food, enrichment and nesting material, and their immune system is comparatively weaker than that of certain other strains.

Nest building scores have increasingly been used for assessing animal welfare and for measuring pain of distress in laboratory mice and can provide valuable support in the management of animal facilities. Nest building scores are easy to assess in the cage, with little discomfort to the animal.

The following list has been adapted from [https://mousebehavior.org/nesting-behavior/], where more detailed information is available.
Nesting behaviour typically follows a specific sequence:

- Digging: This initiates the nesting process by removing substrate material through fast alternating movements of the forepaws, accumulating it into a pile under the animal's abdomen.
- Push-dig: Helps in nest construction by pushing and kicking forward bedding material with fast forepaw movements, often accompanied by forward locomotion.
- Shoveling: At the beginning of the sequence, the animal burrows into the bedding material without scraping with the forepaws, unlike digging.
- Carrying: The animal transports material, such as bedding, in its mouth to the nesting site.
- Fraying: Breaking down large bedding pieces into smaller fibers for nest construction, using sideways forepaw movements and gnawing actions.
- Sorting: Organizing material within the nest, enhancing nest quality by placing specific items in particular locations.
- Pulling in: Occurring towards the end of the sequence, the animal draws nesting material into the nest, either by reaching out or grasping material with its mouth without leaving the nest.
- Fluffing: Enlarging the nest from within by hollowing out the nest's interior, causing the walls to expand visibly.

Manipulating nesting material and seeking cover offer burrowing rodents the opportunity to express these natural behaviours. Furthermore, building and retreating into a shelter provide opportunities for rodents to actively engage with their environment. Animals housed in groups may benefit in nest building, as even if not every animal in the cage actively participates in constructing the nest, others within the group may engage in this behaviour. Several studies suggest that positive social contact and a complex and familiar environment may also support recovery from injury, from invasive procedures such as surgery or from spontaneously occurring diseases (Jirkof, 2015).

Methodology

2.1 Collection of nesting parameters

The nest building observational data was collected from 60 different cages randomly collected across 7 different housing rooms over a two-month period within one of the animal units at the QMUL. Specifically, data was collected from 10 cages in rooms LG15, LG16, LG19, LG20, and LG25, and of 5 cages in rooms LG13 and LG18. Data was collected in two different days in rooms LG15, LG16, and LG20 because they contained a higher number of breeders.

The nest building data was grouped per room and rack numbers, room temperature and humidity; the hygienic conditions of the cage and the health status of mice; and the housing system, which may consist of a pair (a female and a male, or in certain circumstances, two females paired together) or a trio (two females and a male).

For this study, only breeding cages were used as nesting is critical to support good reproduction and supporting mothering care to the newborn pups. Thus the primary factors to monitor include: determining if the female(s) were/was pregnant, identifying the presence of newborns, and recording their number and whether they are alive or dead on ARMIS. Identifying the status of the litter is a crucial aspect from which genetic and health conditions of the parents can be inferred.

Additionally, mice were provided with food and water ad libitum, and various enrichments such as tunnels, chew sticks, or swings were consistently present within the cages. Finally, data was collected on nest building, categorized by assembly: Largely untouched nests (1), Partially untouched nests (2), Shredded nests without identifiable nest sites (3), Identifiable flat nests (4), and Perfect nests (5); position: corner or middle; and the materials provided and utilized, such as Sizzlenest®, Paper Wool®, Paper Shavings®, and Enrich-n'Nest®.

The factors listed earlier regarding the different rooms are documented in the BSU daily tick sheet, which must be completed at the end of each working day with the required values, while the factors concerning the behaviour of the mice and their health status are digitally available on an additional recording data tool (ARMIS). The position of each cage within the room was not taken into consideration, thus refraining from specifying the rack number or its location within the rack, as it does not hold relevance to the observation of nesting behaviour. Furthermore, the specific genetic nomenclature of the genetically altered strains was not reported due to their large number. Therefore, all genotype data was consolidated into two categories: genetically modified (GA) and non-genetically modified (NON-GA), with the latter including the C57BL/6 and CD1 strains, while all others belong to the GA group.

After categorizing all the influencing factors, all the collected data was placed in an excel sheet for the descriptive statistical analysis. Results were shown in the forms of pie charts and histograms to improve visualization of the obtained results, thereby facilitating comparison among them and discerning which factors have a greater positive or negative influence on nesting behaviour.

2.2 Score sheet

A main objective of the study was to develop a scoring sheet to assess nesting behaviour and its progression, which is consistently influenced by the various listed factors. Each parameter is assigned a score of 1 or 0, corresponding to "yes" and "no," respectively.
Here is the description of each parameter:

- Room temperature: 1 within the range 19-23°C, 0 out of the range 19-23°C
- Room humidity: 1 within the range 45%-65%, 0 out of the range 45%-65%
- Cleanliness: 1 clean, 0 dirty
- Health conditions: 1 bad, 0 good
- (Housing) 1 female: 1 yes, 0 no
- (Housing) pair 1 female + 1 male: 1 yes, 0 no
- (Housing) pair 2 females: 1 yes, 0 no
- (Housing) trio 2 females + 1 male: 1 yes, 0 no
- Pregnant female: 1 yes, 0 no
- (Enrichment) tunnel: 1 yes, 0 no
- (Enrichment) chew stick: 1 yes, 0 no
- (Enrichment) swing: 1 yes, 0 no
- (Nest building) largely untouched nest: 1 yes, 0 no
- (Nest building) partially untouched nest: 1 yes, 0 no

- (Nest building) shredded without identifiable nest site: 1 yes, 0 no
- (Nest building) identifiable flat nest: 1 yes, 0 no
- (Nest building) perfect nest: 1 yes, 0 no
- (Nesting material) Sizzle Nest: 1 yes, 0 no
- (Nesting material) Paper Wool: 1 yes, 0 no
- (Nesting material) Paper Shavings: 1 yes, 0 no
- (Nesting material) Enrich-n' Nest: 1 yes, 0 no
- Nesting location: 1 corner of the cage, 0 middle of the cage
- Genotype: 1 GA, 0 NON-GA

Attention was focused on the nest building, and since nesting behaviour should be exhibited by each individual mouse housed in each cage, the entire group of individuals housed was considered together. When any clinical or behaviour anomaly was observed in a single animal, such as signs of fighting or over-grooming, or the presence of a pregnant female, a note was made of it and only after that, the focus shifted to the individual animal.

Nest building was assessed according to this scoring criterion:

- Largely untouched nest (1): when the nesting material is not manipulated at all.
- Partially untouched nest (2): when some of the nesting material is manipulated, but over 50% of the material is partially untouched.
- Shredded without identifiable nest site (3): when over 50% of the nesting material is shredded into a platform, without a noticeable nest site.
- Identifiable flat nest (4): when almost all the nesting material is used to form a cup-like structure, so the nest is identifiable but still flat, without a covering.
- Perfect nest (5): when all the nesting material is used to make a full dome.

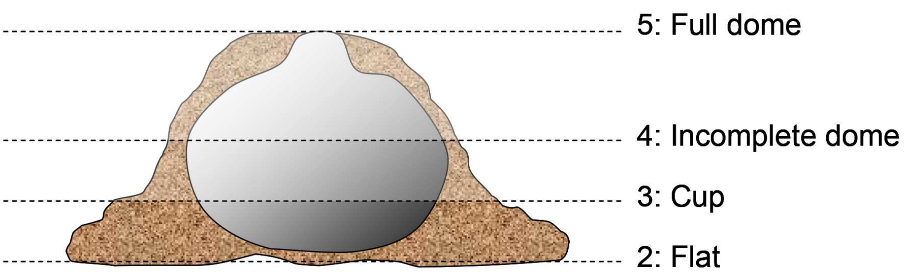

Figure 1. Nest conformation

2.3 Descriptive statistical analysis of nesting behavior

For descriptive statistical analysis, a n=60 cage observation was used to support an analytical power; taking into consideration the time available and access to breeding cages in the unit. For the values of temperature, humidity and nest building, the absolute frequencies were calculated to create histograms, while for all other values, percentages were computed and expressed as relative frequency to create pie charts.

The temperature and humidity values refer to each room, so the room names are included in the respective histograms rather than the numbers of individual cages, as indicated for the other factors. For the temperature, rooms are shown rather than the cages mainly for the purpose of graph readability, which is clearer with fewer numbers, given that there are 7 rooms and 60 cages, and also because the temperature is the same for cages belonging to the same room on the same day. In rooms LG15, LG16, and LG20, the temperature was measured on different days, resulting in two different measurements for each, so I named them LG15 (bis), LG16 (bis), and LG20 (bis). The same applied to humidity.

Results and discussion

All results are represented in the following graphs along with all factors that directly or indirectly influence nesting behaviour, either positively or negatively.

3.1 Room Temperature

The room temperature should be between 19-23°C, and all rooms fall within the optimal temperature range except for room LG25, which measures 18.30°C. Under the standard laboratory conditions, mice are subjected to conditions inducing chronic cold stress. Nevertheless, the provision of nesting material emerges as a viable strategy for facilitating thermoregulation.

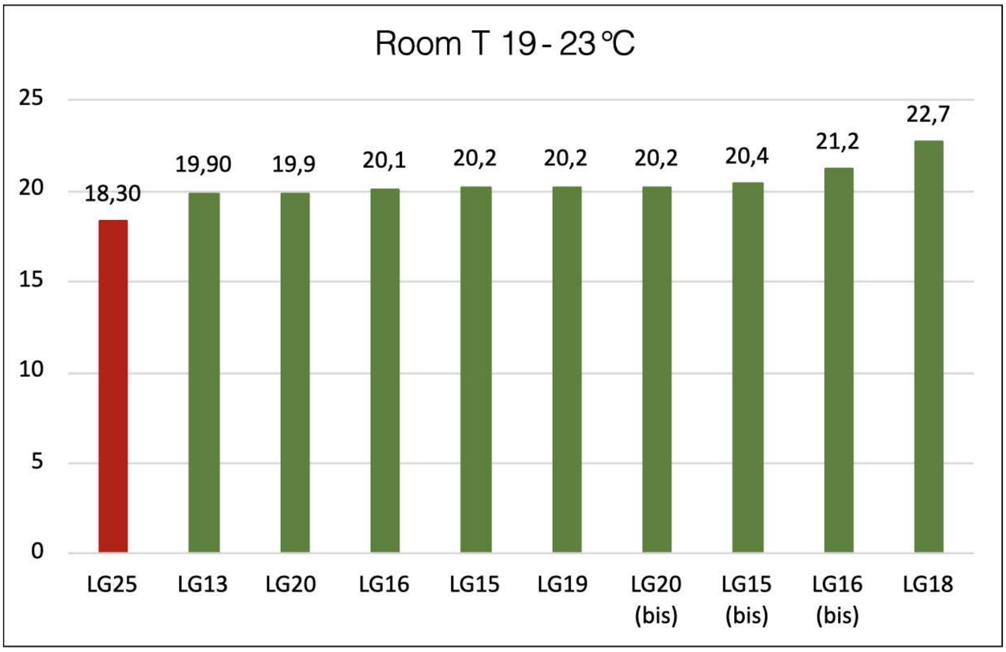

Figure 2. Histogram of room temperature

Body core temperature in mice can fluctuate between 36 °C and 37 °C, with lower values typically observed during the light cycle, which is traditionally associated with periods of rest, and higher values during the dark cycle, synonymous with periods of physical activity. Also, the homeothermic zone seems to be more a temperature point than a zone and varies about 4 ◦C across the day. Temperatures below this homeothermic point lead to increased energy expenditures, whereas temperatures above lead to a rise in body temperature. Thus, mice can adapt to different ambient temperatures, given that sufficient bedding and nesting

material is available. Moreover, they are able to adjust their body core temperature depending on activity and environmental conditions and are even able to survive ambient temperatures from −10 ◦C to 32 ◦C. Interestingly, this characteristic seems to be dependent on sex, strain, age or an interaction of these variables (Kolbe et al., 2022).
At birth, mouse pups do not have the capacity to thermoregulate and must rely on conductive and radiant heat from parents and other siblings in the nest and heat retained by the nest itself for survival (Gaskill et al., 2013).

3.2 Room Humidity

In rooms LG15, LG16 and LG19 the humidity is in the range, respectively, 45%, 52% and 47%. In rooms LG15 (bis), LG16 (bis), LG18, LG20, LG20 (bis) and LG25 the humidity is out of the range. There are 20 cages located in rooms with humidity within the specified range, while 40 cages are outside the range; thus, 33.3% of the cages fall within the "safe" range, and 66.6% fall within the "unsafe" range. Similar to temperature measurements, rooms LG15, LG16, and LG20 recorded two different humidity values on different days, but they consistently fell within the specified range. I have labeled these as LG15 (bis), LG16 (bis), and LG20 (bis).

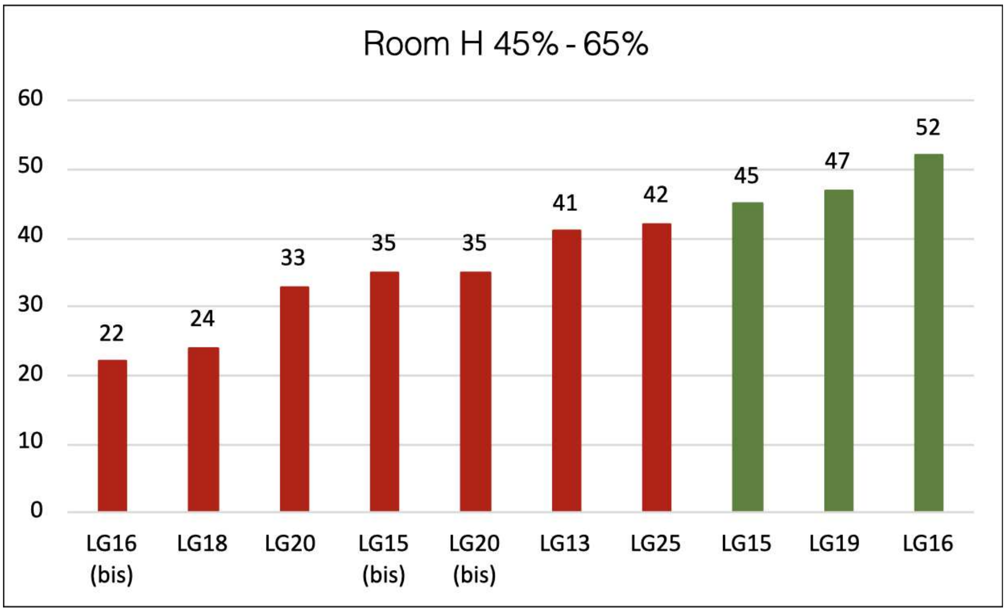

Figure 3. Histogram of room humidity

High humidity can lead to the perception of the environment as warmer and stickier, while low humidity can result in dryness of the mucous membranes. Moreover, humidity can influence the growth of mold, condensation, and other indoor environmental issues.

3.3 Health conditions

88.3% of the cages contain mice in good health, while 11.7% of the cages house mice experiencing health issues due to genetic and stress-related factors. Stress for mice living within a laboratory setting is inevitable and can only be minimized, but not eliminated. Some of the most common health issues genetic-related include diabetes, tumors, malocclusion; while health issues stress-related are over-grooming, depression and anxiety such as social withdrawal, reduced self-care and the gravest is infanticide, which is the act of parental cannibalism towards newborns. Generally, chronic stress causes various health problems in the body, affecting and weakening the nervous, endocrine, and immune systems.

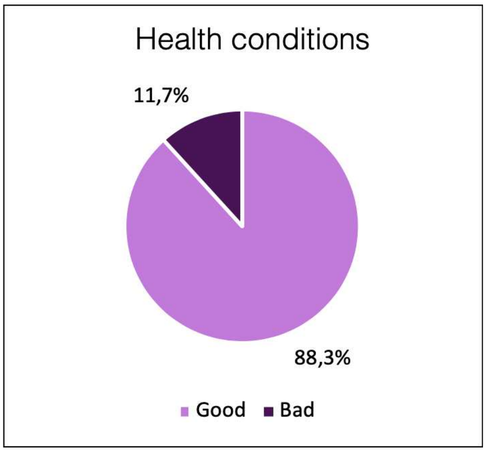

Figure 4. Pie chart of health conditions

The health conditions of the mice influence nest construction. Suboptimal health conditions can manifest through reduced motor activity, leading to a lack of response to stimuli. Mice may remain in visible areas of the cage without seeking refuge in the nest from low temperatures or predators (including humans), displaying signs of apathy. Lack of motor activity consequently indicates absence of nesting behaviour.

In one of the cages in room LG15 inhabited by a pair consisting of a male and a pregnant female, the female was engaging in self over-grooming, resulting in hair loss. Affiliative behaviors, such as grooming others, function to strengthen social bonds (Gaskill, 2014) but the over-grooming is a clear sign of stress. In a cage in room LG20, the female died due to birth complications (stocked pups) and then the males cannibalized all the pups.

My observations also include two cases of agonistic behavior occurring in two cages in room LG16, both consisting of a male and female and both of the same genetic strain ("129x1/svj"). In one of the two cages, it was the third consecutive litter that was eaten by one of the two parents, probably by the mother. It was not possible to determine whether it was cannibalism of dead pups or infanticide of live pups. The technicians of the unit informed me that this strain of mice tends to exhibit both cannibalism and infanticidal behaviours. To minimize these occurrences, the male was removed from the cages, leaving the territory entirely for the mother and her pups. Aggression is a natural behavior related to territories and the resources they contain. Resources could include food, a water source, or access to breeding females (Gaskill, 2014).

3.4 Genotype

The 88.3% of observed mice are genetically altered (GA), while the remaining 11.7% are non-genetically altered (NON-GA), which includes different strains such as C57BL/6 and CD1. Mice behaviours depend on the type of strain.

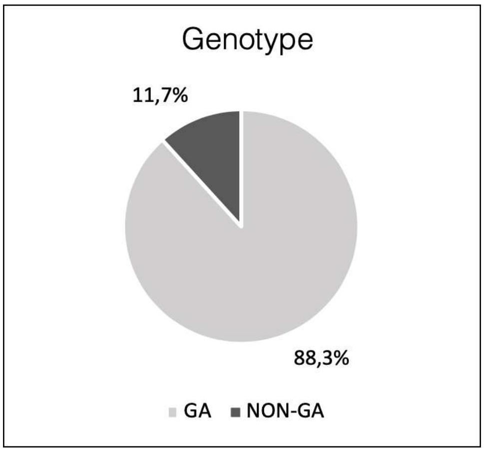

Figure 5. Pie chart of genotype

3.5 Cleanliness

The majority of the cages exhibit excellent hygiene conditions, precisely 88.30%, while the remaining 11.7% show suboptimal hygiene conditions for mice and their well-being.

Mice also avoided urinating and defecating close to their nest. However, this statement is not always accurate, as during my observations, I revised that in some cages, feces were not separated from the nesting material, which should instead be kept clean. This occurrence likely occurs frequently due to the limited space of the cage, which restricts mice and their natural behaviours. "Providing rodents with opportunities to segregate clean and dirty areas is likely an important welfare consideration. Establishing separate resting and soiling sites allows animals to engage in this naturally motivated behaviour and provides them with the opportunity to exert some control over their environment (Makowska & Weary, 2020).

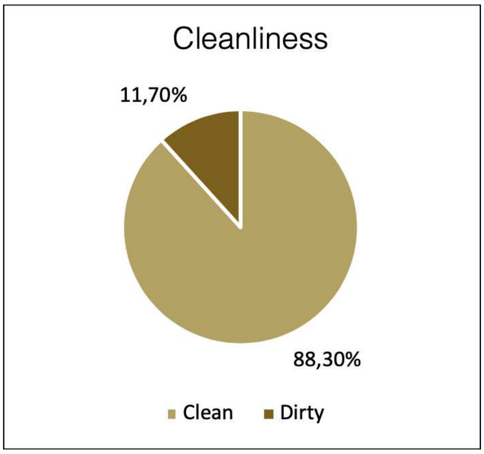

Figure 6. Pie chart of cleanliness

3.6 Enrichment

All cages were equipped with at least one item serving as enrichment, which is a positive finding. The provision of environmental enrichment to numerous species of laboratory animals is generally considered routine husbandry (Bayne, 2018).

Various types of enrichment include tunnels, swings and chew sticks. Tunnels are the most popular type of enrichment, they are found in 98,3% of the cages. They offer that secure, sheltered hiding place without adverse affecting the visibility of careers carrying out cage checks. These tunnels can also be useful as handling device, minimizing stress and offering a simple way to separate an individual in multi-animal environment. They are tinted in a choice of red or amber, providing a sense of sanctuary by reducing the level of perceived light. Suspending your rodent tunnel not only maximises available floor space, it also allows your animals to climb, providing ample opportunity to play and explore, relieving boredom and reducing aggressive behaviour. A suspended tunnel is similar to a swing. Both serve the triple function of providing shelter, climbing and handling.

Chew sticks, also called Aspen Bricks, are durable; withstanding natural chewing and gnawing behaviours. Additionally, the larger brick sizes encourage exercise, climbing and hiding, which aid in helping to reduce aggressive and apathetic behaviours and promote natural instincts (website: www.datesand.com).

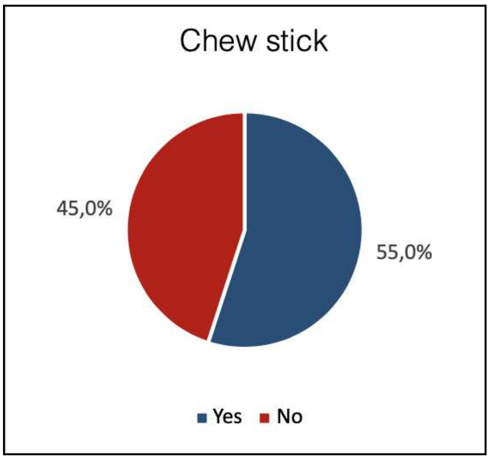

Figure 7. Pie chart of chew stick

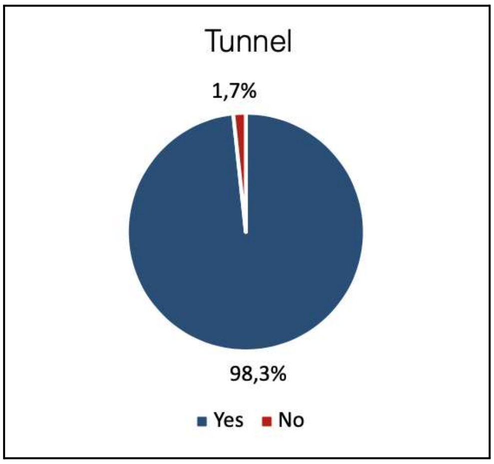

Figure 8. Pie chart of tunnel

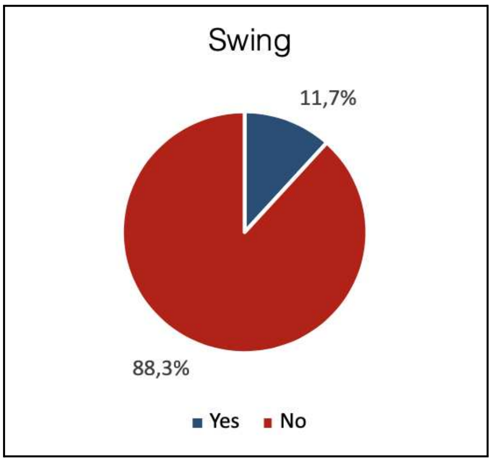

Figure 9. Pie chart of swing

The primary objective of environmental enrichment is to enhance animal well‑being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species‑typical behaviors and promote psychological well‑being through physical exercise, manipulative activities and cognitive challenges according to species‑specific characteristics (Please refer to the following website: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6388067/#ame212015-bib-0005).

Enrichment strategies vary based on specific goals. In some cases, the objective of enrichment is to increase the expression of certain behaviors while in other cases, reduction of specific behaviors, such as bar-mouthing, jumping, circling, and compulsive activities like barbering, or over-grooming. Additionally, efforts may target the alleviation of elevated stress hormone levels, fearful and anxiety-like behavior, and impaired thermoregulation. For example, mitigating the expression of stereotypic behaviors may involve providing resources such as shelters. Notably, "morphological changes to the brain are perhaps among the most well‑known effect of enrichment on rodents. In all instances, the provision of enrichment should not negatively impact the health and safety of the animal." (Bayne, 2018). In extreme cases, the elimination of enrichment resources fostering competition may be considered to mitigate aggression between individual mice.

3.7 Nest building

The graph indicates that 45.0%, nearly half of the cages, contain a Perfect nest. Level 1 indicates a percentage of 0%, indicating that no nest was identified as a Largely untouched nest, which is a positive consideration.

Reproductive female mice are highly motivated to nest building and this behavior is often elicited by odors or calls from mice pups. The nest is often defended as part of the territory by the male and female mouse (Latham & Mason, 2004).

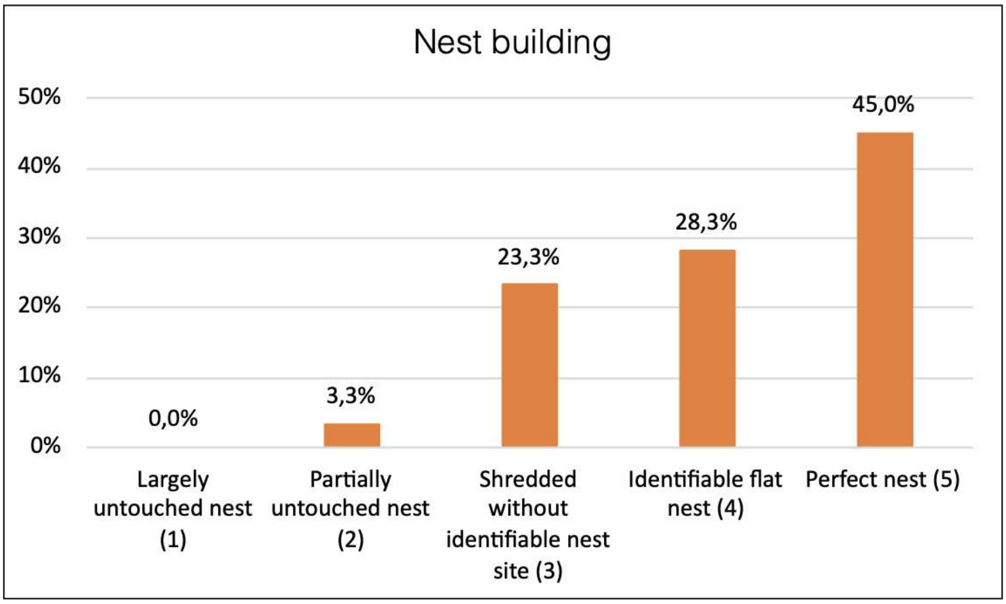

Figure 10. Histogram of nest building

I have linked nest building with the type of material employed for its construction, its location, housing conditions, and the presence of a pregnant female to better discern which of these factors predominantly affects nesting behavior.

3.7.1 Nesting material

Different materials can be manipulated and combined to create a flexible structure that decreases cold stress and provides both structural and occupational enrichment for mice. Nesting material and its transfer at cage change has been found to reduce aggressive interactions (Gaskill, 2014).

The material found in 36 cages, and therefore the most common for nest construction, is Paper Wool®, which also represents the most prevalent material for constructing a Perfect nest, accounting for 56%. The second most commonly used material, which results in an Identifiable flat nest at 50% and a Perfect nest at 42%, is a combination of the two most used materials: Paper Wool® and Sizzlenest®. The other two types of material, Paper Shavings® and Enrich-n'-Nest®, were included in 2 and 1 cages, respectively, for testing purposes.

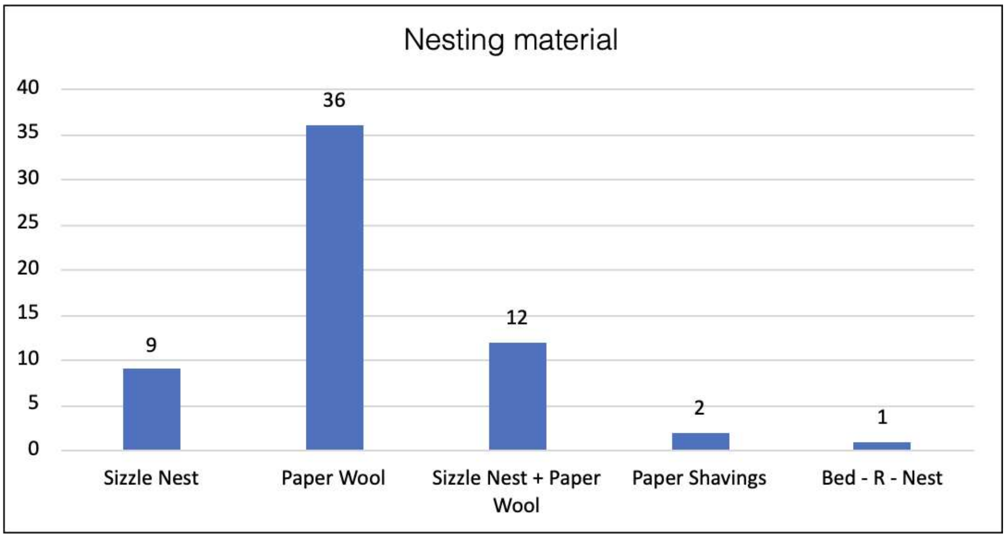

Figure 11. Histogram of nesting material

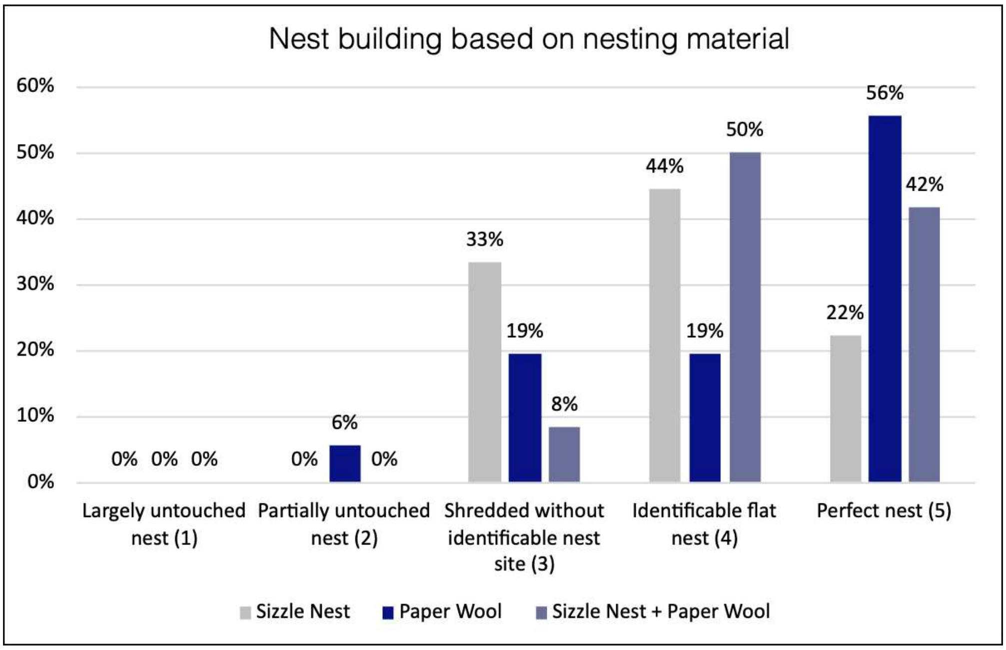

Figure 12. Histogram of nest building based on nesting material

All types of nesting materials are available in irradiated, disposable, and autoclavable forms, except for Enrich-n'Nest®, which is not disposable.

About Paper Wool®, the light and fluffy texture traps warm air and body heat, ensuring your animals have a cosy place to sleep. The naturally absorbent Paper Wool® boasts a high level of cleanliness and disposability, meaning cage changes are never a chore. The long strands make it excellent enrichment material, allowing your animals plenty of opportunities for burrowing. However, the material's natural absorbency can be problematic due to urine accumulation and subsequent ammonia release and the long strands may lead to accidental strangulation.

About Sizzlenest®, it is light enough to drag to the right location, capable of trapping pockets of nice, warm air and is soft enough to provide comfort whilst being just strong enough to hold its shape. That’s why our Sizzlenest® is made from unbleached kraft paper which is cut and folded to create the ultimate nesting substrate (website: www.datesand.com). When utilized in breeding cages, it is recommended to blend it with a softer nesting material like Paper Wool®, ensuring a suitable nest even for delicate newborns with heightened tactile sensitivity.

Composed of pure white paper rolls, Enrich-n’Nest® encourages scavenging and unfurling; however, these rolls are too short to construct an ideal nest. The same limitation applies to Paper Shavings®. Both materials offer a high level of warmth and comfort, trapping warm air and body heat, but their insufficiently long filaments hinder vertical nest development, causing the material to flatten at the cage bottom. Conversely, the carefully shredded short flakes of these materials minimize the risk of tangling.

The main result was that strands of nesting are the best, in comparison to compressed cotton materials as they don’t have much structural integrity. So nesting such as Sizzlenest® or Paper Wool® are the best. Generally, the long strands encourage natural burrowing activity and help rodents build their preferred dome-shaped nest.

Figure 13. Different types of nesting material

3.7.2 Nesting location

75,0% of the cages, and thus more than half, have the nest constructed by the mice located in the corner, while only 25,0% are located in the middle of the cage. Dark and hidden corners are often the favorite places for mice to build their shelters, this is because they consider corners relatively safe places, protected from predators and bright light.

51,1% of the cages contain Perfect nests, while 31,1% contain Identifiable flat nests, both located in the corner near the food and water. As previously mentioned in the explanation of cage hygiene, mice typically keep the clean area separate from the dirty area, hence the nesting and excrements, respectively. The more central the nest, the closer it is to the dirty area. The histogram graph demonstrates that in the case of nest building levels 2 (Partially untouched) and 3 (Shredded without identifiable flat nest site), the highest percentage values, 6.7% and 46.7%, respectively, refer to a nest located centrally in the cage, which consequently presents lower probabilities of becoming a Perfect nest.

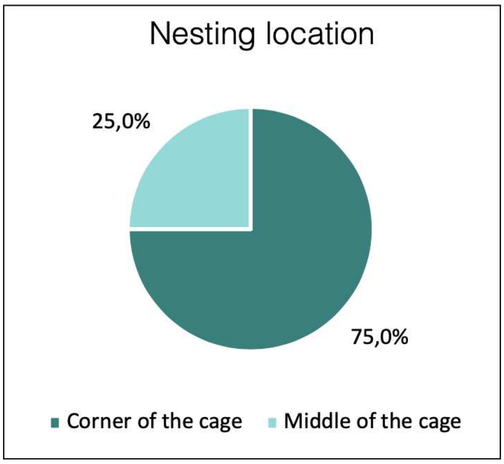

Figure 14. Pie chart of nesting location

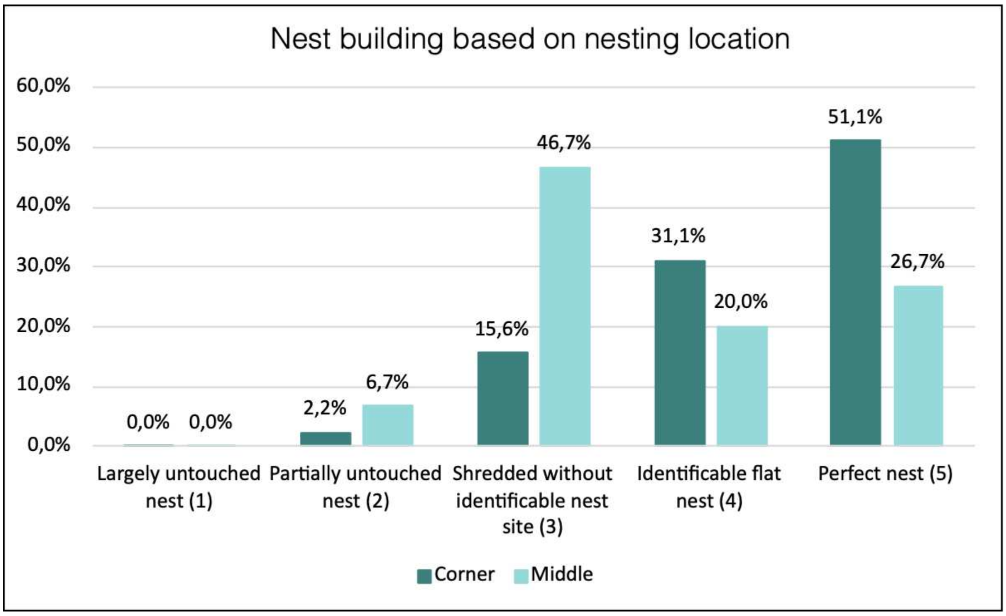

Figure 15. Histogram of nest building based on nesting location

3.7.3 Housing

Mice generally live in loose kin groups called 'demes' which include a male, 1-2 breeding females (which are usually related), subadults, and pups. Kinship and familiarity have also been found to reduce aggressive social interactions. One male owns a territory but other adults, including females, will defend it fiercely (Gaskill, 2014), especially if there are newborns.

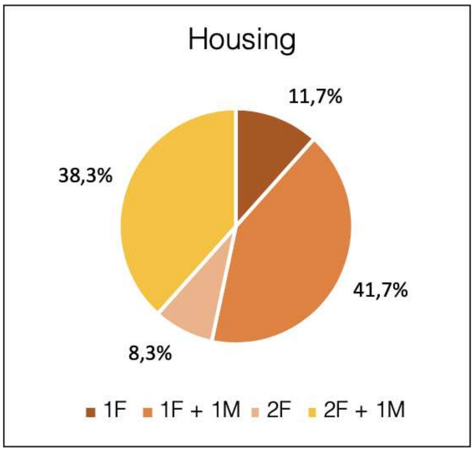

Figure 16. Pie chart of housing

Based on my observations, 62.5% of the cages containing a pair consisting of a male and a female mouse exhibit a Perfect nest, suggesting that pairs tend to work better for nest construction. Conversely, the poorest performance is observed in cages containing two females and one male (trio), represented by 25.0%. Additionally, a single female appears to perform well without the pressure of other mice, accounting for 57.1% at the Perfect nest level.

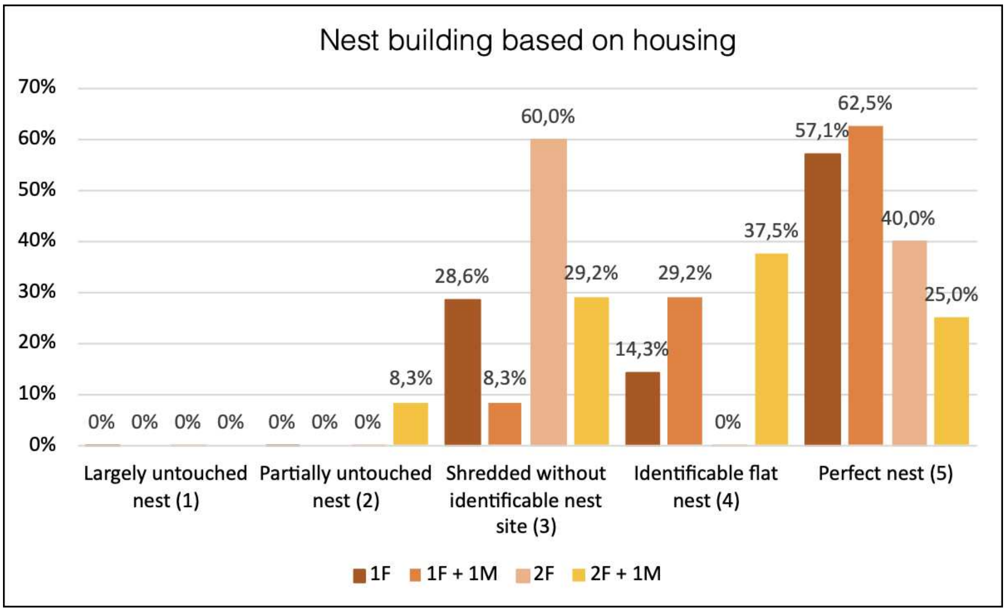

Figure 17. Histogram of nest building based on housing

3.7.4 Pregnancy

The pie chart indicates that the majority of observed cages host at least one pregnant female, representing 53.3%, while the remaining 46.7% consists of non-pregnant females. The presence of a pregnant female in a cage appears to positively contribute to nest construction. Indeed, as shown by the histogram, over 53.6% of cages with a pregnant female have a Perfect nest, compared to 37.5% of cages without a pregnant female. Similar percentages, around 28.1%, are observed for level 4 (Identifiable flat nest), which still represents a good level of nest building. Another noteworthy finding is that cages with a Partially untouched nest, level 2, are exclusively those without pregnant females, representing 6.3% of these cases.

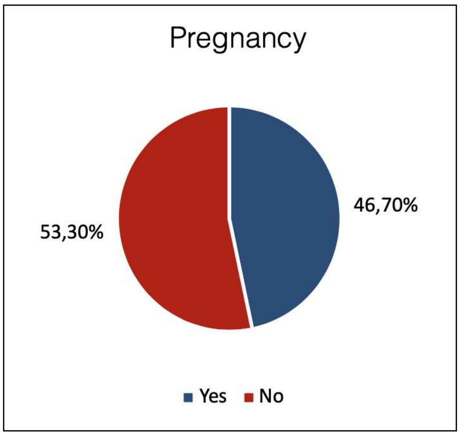

Figure 18. Pie chart of pregnancy

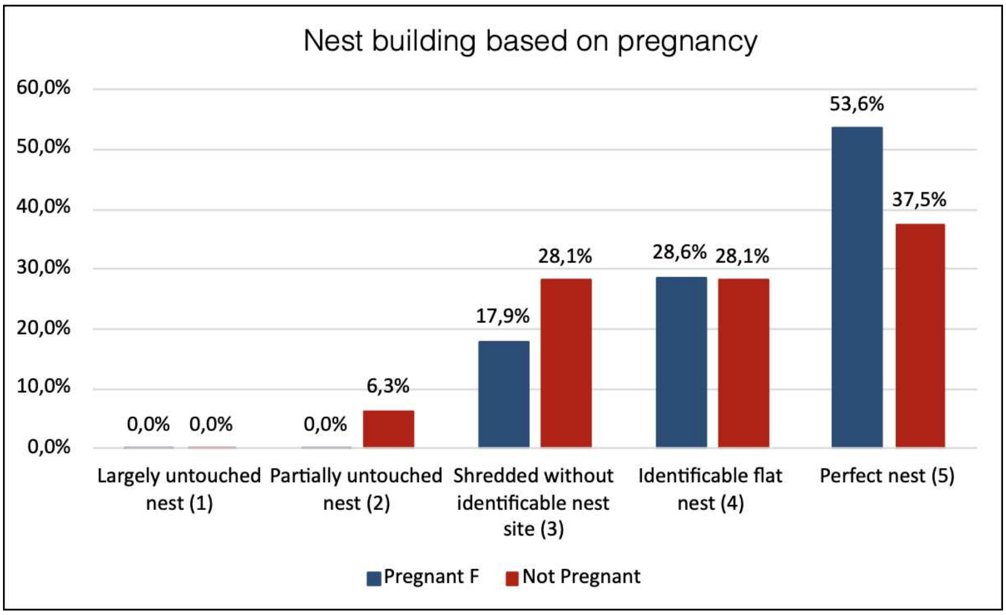

Figure 19. Histogram of nest building based on pregnancy

Maternal caregiving plays a key role in childhood development. More pronounced maternal responses start appearing in preparation for birth, as expectant dams demonstrate increased levels of aggression toward intruders and display nest building behavior to protect and prepare the environment for the litter. After birth, the dam engages in licking and nursing the pups, which is necessary for pup development. Levels of maternal progesterone are correlated with nest building. Females and breeders with pups in the home cage built more complex nests than males, and these behavioral differences may be due to associated changes in progesterone concentrations. Several types of postnatal manipulations have been proposed to affect the rodent dam-pup relationship and generate a stressful condition early in life, including models that involve handling of neonatal pups, maternal separation, and dam-pup exposure to reduced nesting material. Regarding the different models of maternal separation, they can be distinguished according to the duration period the pups remain away from the home cage and dam. A short period of time, ranging from 10 to 60 min, is classified as brief maternal separation, but if the duration exceeds the 60 to 480 min range, it is classified as prolonged maternal separation may engender an inadequate environment for neurodevelopment. The limited bedding protocol, where mothers are provided with reduced nesting and bedding material, results in changes in maternal behavior and fragmented maternal care. Furthermore, the introduction of a new environment, without the necessary material to build an adequate nest, can cause the dam to manifest potentially harmful behaviors, which can have a profound impact on the development of the pups (Orso et al., 2019).

Overall, any changes to these highly motivated behaviors, or the nest itself, should indicate a substantial alteration in the environment or the animals themselves (Gaskill et al., 2013).

Some study limitations encountered during the project include data collection through only a single time observation for each cage, and there was no follow-up of the project after my departure. Additionally, the project was entirely overseen by only one operator, which could be a limiting factor.

Conclusion

The ideal solution to achieve a perfect nest would be to have all the factors discussed earlier under the most optimal conditions possible, both for the mice and the laboratory technicians. A couple of mice in good health conditions inside a cage with good but not excessive hygiene conditions usually give birth to stronger pups. In the right environment with the correct temperature and adequate enrichment provisions, stress levels are low, allowing them to engage in their natural behaviours, such as nest building. The primary responsibility for mouse nest building lies with laboratory workers. Everyone must ensure they provide the necessary interventions with care and materials to maintain the well-being of these animals.
From my observations, I have concluded that the best solution is to provide two different types of nesting material inside each cage: one composed of long and resistant strands, such as Sizzlenest®, and one composed of shorter and softer filaments like paper rolls or strips, such as Paper shavings or Enrich-n'Nest®. Among these latter two nesting materials, I would opt for Enrich-n'Nest®, which can be reused multiple times, thus considering the economic factor as well. It is more cost-effective not only for purchase but also for the animals themselves, as they have the opportunity to encounter familiar odors. By using these materials together, a perfect combination of warmth is achieved, helping to maintain adequate body core temperature, and structural integrity, facilitating the construction of a nest both in height and width with minimal effort while still stimulating the animal through a form of enrichment, two essential characteristics for the construction of a complete nest.

It is essential to direct attention towards a holistic evaluation of the overall well-being of mice, encompassing a thorough consideration of multiple facets. The pinnacle of animal welfare can be envisioned when meticulous care is applied to every aspect that impacts it.

Figure 20. Nesting made of Paper Wool® and Sizzlenest®

Figure 21. Nesting made of Paper Wool®

Figure 22. Nesting made of Paper Shavings®

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Sitography

- https://mousebehavior.org/nesting-behavior/
- https://www.datesand.com/

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