The use of Home Cage monitoring to determine whether individual male mouse activity patterns correlate with nest complexity

Joanna Moore[1] and Hilary Lancaster[2]
GSK, Stevenage, UK
Laboratory Animal Medicine[1], In Vivo Science and Delivery[2]

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals

Introduction

It is well established that nesting materials are an important inclusion for mouse cages. We wanted to determine whether there was a significant difference in mice activity when offered three different material choices and whether there was any correlation between activity in the cage and nest complexity. We used the established home-cage monitoring system to determine whether there were differences in the activity pattern of mice depending on the nesting, and whether these related to the complexity of the nest. The aim of this study was to show that a combination of materials enabled mice to create a more complex nest, which is considered to be an indication of better welfare.

Methods

We individually housed six black Sik2, one albino and two agouti Prm1 adult exbreeding male mice; in GM500 Digital Ventilated Cages (DVC®), (Tecniplast S.p.A). Each mouse had the facilities' standard enrichment of a mouse Igloo (LBS), cardboard fun tunnel and aspen chew block and were housed on Lignocel wood bedding (IPS). The nest placement is outlined in Table 1.

Activity	Option 1 (Shred Paper)	Option 2 (Lignocel Large)	Option 3 (Combined Shred Paper and Ligocel Large)
Cages and nesting change	Monday 23rd Dec 2020	Monday 30th Dec 2020	Monday 06th Jan 2020
Nest scoring dates	Monday 30th Dec 2020	Monday 06th Jan 2020	Monday 13th Jan 2020

Table 1; Dates for nest and cage changing and nest scoring for all study animals.

Nesting was placed in the back left of the cage, and the igloo on the back right. The mice were given one of three nesting options for a six-day period: 9gms of shredded paper, autoclaved Lignocel (IPS) Large (Wood Wool) or a combination of Lignocel and shredded paper (Combined) as well as a red igloo and cardboard fun tunnel, and chew block (Datesand) (see Figure 1). At the end of each seven day period the nest was scored (see Table 2).

Figure 1; Nesting options (and starting position) offered to mice, from left to right: Shred Paper, Wood Wool, Combined.

2	3	4	5
At least quarter of the product move from original position flattened with slight dip in centre, no sides to nest	Most of product used, nearly halve used for the nest, some sides showing and a clear dip in the centre of the nest.	Almost all product used, with clear walls to the nest and a deep dip in the middle. Usually all nesting is in one part of the cage.	Full use of all material, all nesting in one part of the cage, a round enclosed nest is visible.

Table 2; After seven days the nest was given a score in terms of complexity using the method published by Jirkof et al (2013). Score scale between 0-5.

Study design Rational

We only had the DVC for a relatively short assessment and with the added pressure over the Festive break we did not use the more complicated study design that also accounted for time. Given that the animals were kept singly housed in a controlled environment with a very rigid husbandry routine we felt that a simplified design, ignoring time effects, would enable us to get indications of how nesting can influence mouse activity. For any future studies of this type we will use a cross-over design which is the method we recommend is generally used for this type of study.

Activity sampling

We analysed the activity pattern within each nesting type for the two-hours after bedding change timeframe and the 20.00-22.00hrs timeframe. We calculated the change from baseline in the following way:

Two-hour time interval change from baseline activity = **Two-hour time interval activity on Wednesday – Two-hour time interval activity on Monday**

This measurement shows whether activity for each cage has increased or decreased two days after bedding change compared to the day of bedding change. We then compare the change from baseline for each cage between each nesting option.

Results

Exploratory analyses comparing the activity of the two strains showed that Prm1 mice tended to be more active compared to Sik2 males. There was no particular trend in activity during the night period across any nesting options (see Figure 2).

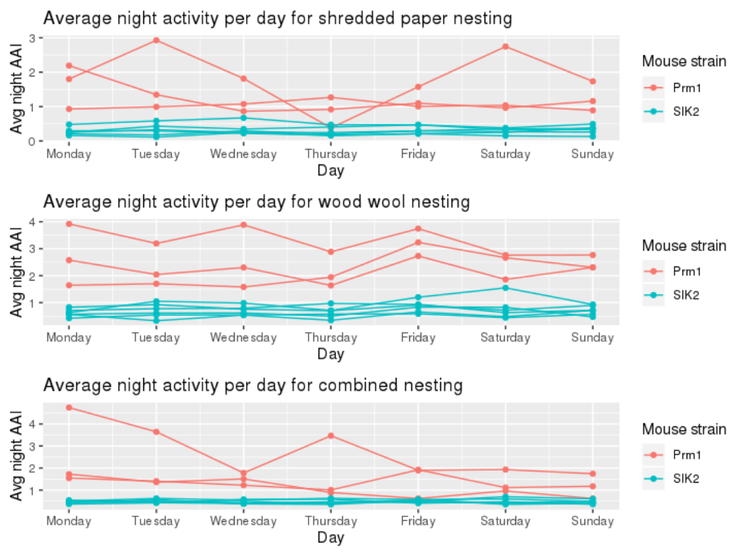

Figure 2; Average dark phase activity per strain

There was an increase in activity across all three nesting options immediately after bedding change when compared to the same time frame for the subsequent days (see Figure 3).

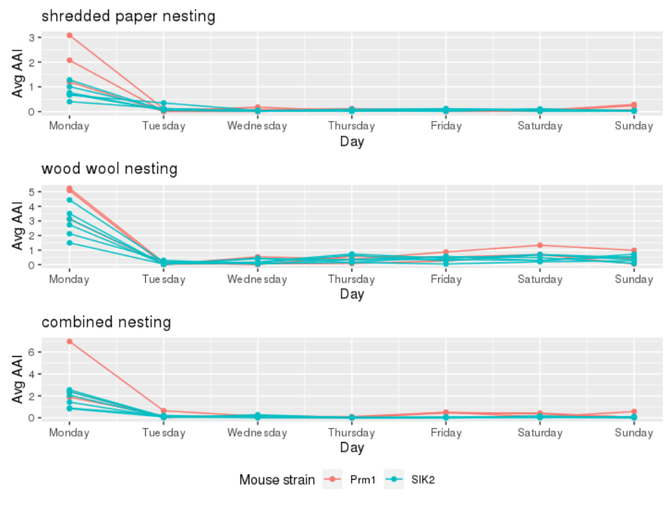

Figure 3; Average activity two hours post nesting changes.

A repeated measures ANOVA showed there was a significant effect for type of nesting material and mouse breed on the change from baseline measurements. A post-hoc test was used to identify which nesting material has an effect on change from baseline (see Table 3)

Contrast	Estimate	P-value
Shred Paper – Wood Wool	2.02	**0.004**
Shred Paper – Combined	1.07	**0.046**
Wood Wool – Combined	-0.95	0.079

Table 3; Results of post-hoc test.

There was no identifiable effect of nest material on mouse activity at the 20.00-22.00hrs analysis. Prm1 mice were significantly more active than Sik2 (P=0.001). We found that mice tended to spend more time at the back of the cage.

To evaluate the nest scoring we averaged by nesting type across all mice and found that Wood Wool is consistently better than Combined; despite the average being the same, the median was higher. (See Figure 4).

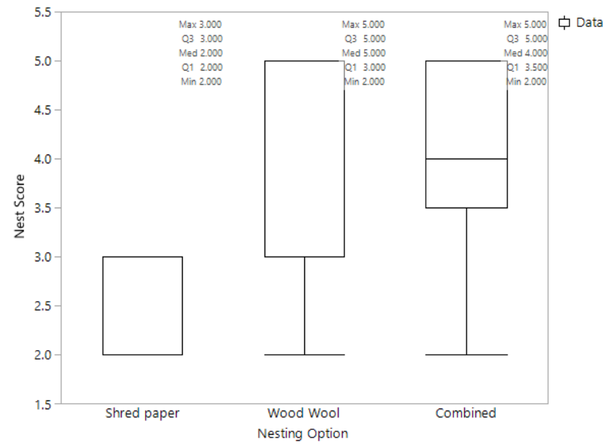

Figure 4; Results from nest scoring.

Discussion

The use of home cage monitoring indicated that the activity pattern of male mice (1) was affected by poor nesting material such as Shred Paper (2) that nest building starts as soon as the nesting material is offered (3) low activity in week one is also reflected by the poor nest score achieved. The Wood Wool alone was the material where we observed the most activity and gave the highest nest score, which may have been due to it being a long stranded natural product which may have enabled better nest building. However, the results in the second week may have been confounded by the staff returning from the festive break. Studies in large animals show that their behaviour can be impacted by the change in routines over the long festive break, which makes it difficult to be certain that there was a true difference in the reaction to the mice for the nesting in week two. An Examples of our scores are in Figure 5.

Figure 5; Examples of scored nests.

In similar studies all potential nesting materials are removed, whereas we left our standard enrichment in the cages, indeed we found some mice used their cardboard tunnel as part of the nest and thus included this in their nest whereas others only used the material provided. It would be interesting to see if there is similar reaction with pairs of female mice, and to carry out a similar study which avoids the festive break. Overall, with the testing we completed, we found that it was likely the bulk of nest building is completed within the first two hours of it being offered to mice and activity of male mice seemed to be affected by nesting material. Mice are highly motivated to build nests (Rock *et al,* 2014; Jirkof *et al*, 2013). This study may indicate that the understanding the motivation behind increased activity is integral for drawing conclusions. A complex nest is likely to be the result of a large part of the time budget being spent on nest building which is an indication of better welfare in mice.

References

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