POSTER PRESENTATIONS

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Using home cage monitoring to determine the impact of repeated timed mating on male mouse welfare

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Introduction

Some of our Genetically Altered mouse breeding programmes include the use of a strain of sterile Prm1 male mice. These males are used to induce pseudopregnancy in female mice and are often kept up to nine months under singly housed conditions. A previous refinement was to house these males with a companion female. During the timed mating period the companion is swapped for a naive female. We wanted to determine whether there was a significant impact on their welfare when their stable pair was disrupted.

We hypothesised that the impact in cage activity would significantly increase when a companion female is replaced with a new female, rather than when the companion female is left with the male. We used an established home-cage monitoring system to determine how much disruption was created to the activity pattern in cages when a companion female is swapped, compared to when she is just removed and replaced in the pair's home-cage.

Method

An allocation of 20 pairs of established and proven sterile *Prm1 males with their companion females and 20 naïve females that were weight matched to the companion they would be exchanged with. Upon arrival to the animal room, the mice were checked carefully and acclimatised for five days prior to the start of the study. Mice were kept on a 14:10 hour light: dark cycle.

*Prm1 Genetically Sterile Protamine-1 (Prm1) EGFP Transgenic mouse obtained under licence from Dr Pawel Pelczar, University of Zurich, Switzerland (Haueter *et al,* 2010)

Activity sampling

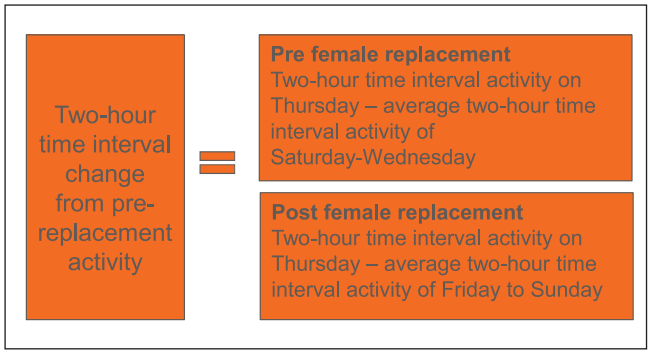
We focussed on the activity during four x two-hour intervals per comparison day: 8.00 - 10.00, 10.00 -12.00, 12.00 -14.00 and 20.00–22.00 hours.

For each time interval we calculated the change from pre female replacement for each cage to determine

Date of activity	Activity to be completed
Friday 6th December	All selected cages were cage changed.
Monday 9th December	The cages were randomised by naïve female weight to the Group One Companion.
Thursday 12th December 07:00-08:00hrs	Each male mouse in group one had his companion female replaced with a similarly matched by weight female. A note of the time the last cage was replaced on the rack was made. – Group 1: the cage was removed from the rack and the companion. female replaced with a naive female – Group 2: the cage was removed from the rack, each female was removed from the cage then put back with the male.
Monday 16th December	All females were replaced with the original companion female. Study ends.

Table 1. Outline of study protocol.

the activity increase or decrease on the day of female replacement compared to the previous days in the same time frame.



Results

The analysis focussed on the pre-female and post-female replacement. Comparing:

1. Activity for each cage on the day of female replacement (Thursday) to the average activity of the previous five days.
2. Activity for each cage on the Thursday to three following days.

Pre female replacement

There was more variability in the activity data sampled between 8.00 – 10.00. Figure 1 shows a slight increase in activity for Group 1 during 10.00 – 12.00 on the Thursday compared to previous days (see Figure 1).

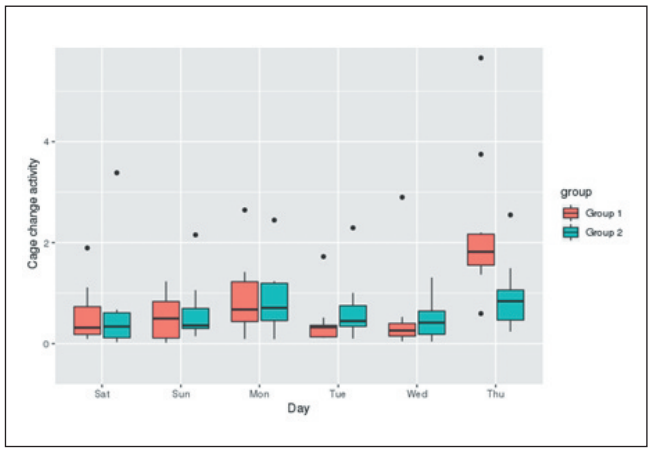


Figure 1. Average activity per day and cage between 10:00 – 12:00 hours.

We completed a one-way factorial ANOVA on the change from baseline measurement for each time interval comparing the average activity of the previous days within the same timeframe. We found no significant difference in activity between 8.00 – 10.00 hours, nor between 12.00 – 14.00 hours and 20.00 – 22.00 hours. There was a significant increase in activity (P=0.0198) between the groups during the 10.00 – 12.00 period (see Figure 2).

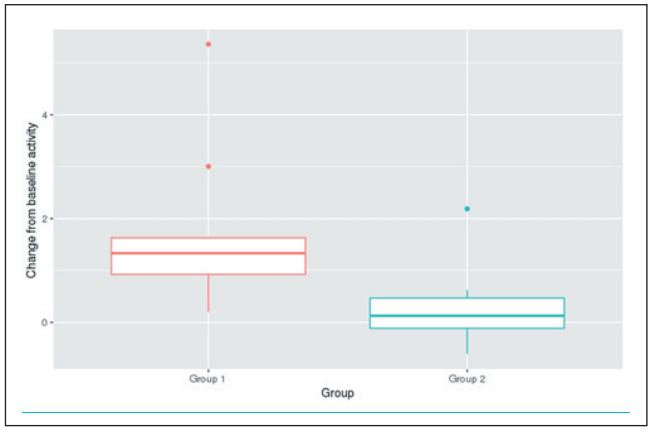


Figure 2. Change from baseline between 10.00 – 12.00 hours.

Post-female replacement

There was more variability in the activity data sampled between 8.00 – 10.00 hours. Figure 3 shows decreasing activity for Group 1 between 10.00 – 12.00 hours for each subsequent day (see Figure 3).

We completed a one-way factorial ANOVA on the change from baseline measurement for each time interval comparing the average activity of the following days in the same timeframe. We found no significant difference in activity between the 8.00 – 10.00 or the 20.00 –

22.00 time frames. There was a significant increase in activity between the groups during the 10.00 – 12.00 time frame (P=0.0036), (see Figure 4) and 12.00 – 14.00 hours (P=0.023), (See Figure 5).

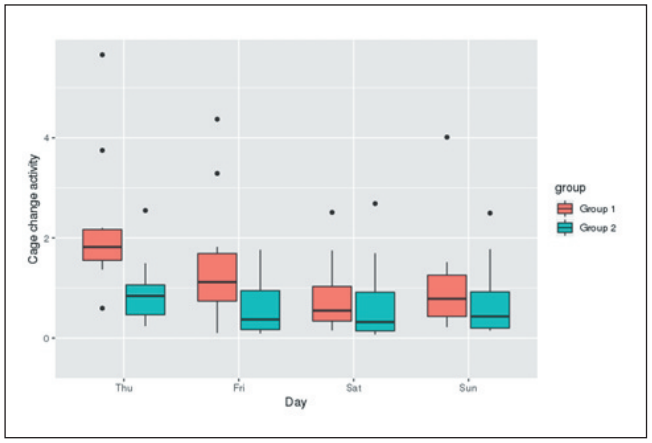


Figure 3. Average activity from 10.00 – 12.00 hours per day per cage.

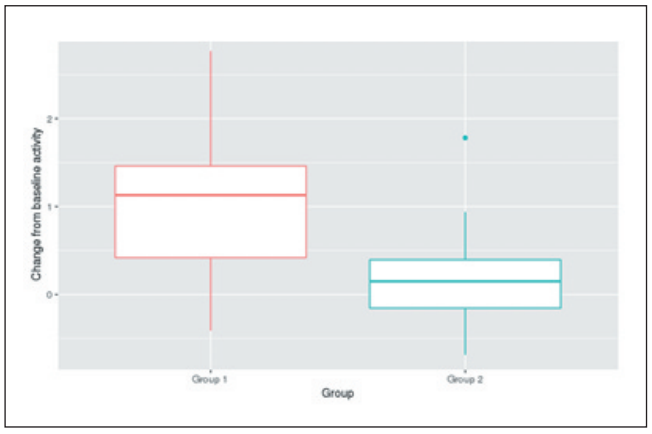


Figure 4. Change from the baseline between 10.00 – 12.00 hours.

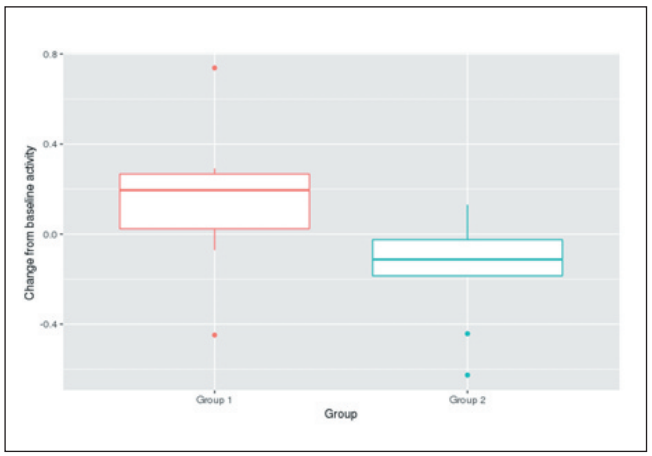


Figure 5. Change from the baseline between 12.00pm – 14.00 hours.

Discussion

When time mating animals (females are placed with a male overnight and then removed to accurately assess stage of pregnancy), the male is often individually housed up to nine months. Using the sterile male mice, we are already able to house them with a companion.

During our initial study the activity caused by the replacement female was still visible when completed at the same time as cage changing (unpublished data) in the two hours directly after the activity which is in agreement with the results of this study.

We found more variability in the activity data sampled between 8.00 – 10.00 likely due to staff presence in the room. Between 20.00 – 22.00 the decrease in activity from group one compared to group two (after female replacement) is likely to be that the mice being more active during the light phase and possibly fatigued during the dark phase.

Regardless of the impact of timed mating itself on male mouse welfare (it could be seen as a positive), the disruptions caused by the intense activity seen in group where female were replaced, during their usually inactive hours followed by periods fatigue during active hours, is likely to have an effect on their cycadean rhythm.

This effect likely leads to poor welfare in the replacement group versus the group where the companion was left with the male. It would be interesting to see if the increase in activity was mirrored when the companion female is replaced or if timed mating was carried out at the end of the working day, nearer to the active time of the mice.

Home cage activity monitoring gives us the unique ability to increase our understanding of how the work we do can impact the welfare of animals, thus giving us an opportunity to refine our processes to further meet their needs.

Acknowledgements

Guido Gottardo, Fabio Iannello, Tecniplast SpA. Kay Dowse, IVSD, GSK, Steve Barrett, Research Statistics, GSK, Steve Wilson, IVSD, GSK.

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1 **Haueter, S., Kawasumi, I., Brykcznska, U., Cinelli, P., Moisyadi, K.,** *et al,* (2010). Overexpression of Prm1-EGFP fusion protein in elongating spermatids causes dominant male sterility in mice. *Genesis, 48 (3) 151-160. doi.org/10.1002/dvg.20598.*

Taking blood from a Göttingen minipig while placed in a sling

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Abstract

Traditionally Göttingen minipigs are restrained in dorsal recumbency to have access to the blood vessels in the neck (see figure 1).

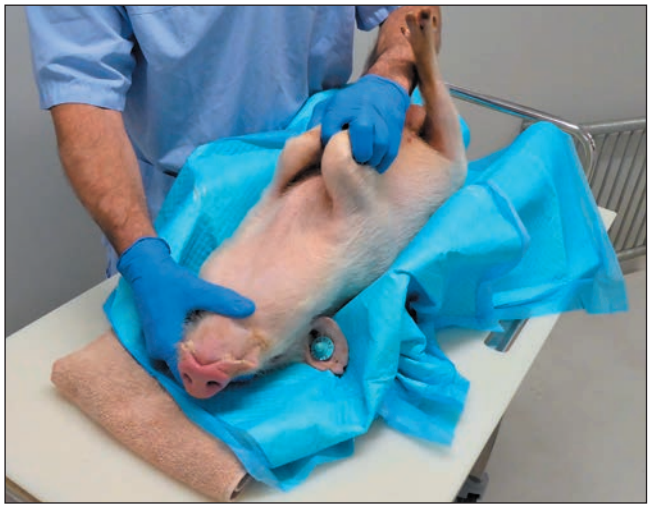

Figure 1. Minipig in dorsal recumbency.

Figure 2. Minipig in sling.

Minipigs, like any other animal do not particularly like to be restrained and being turned on their backs with their belly exposed. With increasing age and weight this method also creates some physical challenges to the technicians. The force needed to control a resisting minipig can be considerate. Lifting and turning a larger minipig on its back could also infringe some occupational health regulations. The sling has been proven to be a valuable restraint for various procedures with the mini pig. This study showed that with slight modification the sling can be used to restrain minipigs for blood sampling and thus reduce the strain on and on animals and personnel.

Materials and methods

The aim of this study was to test whether the sling could be used as a restraint in various blood sampling situations. A standard sling was modified and several options explored to find the most satisfying design.

As there was an electric, height adjustable table in the facility a frame was custom-made to fit this device. The

Figure 3. Adapted height adjustable table.

actual sling is stretched quite tightly in the frame and the cutout was made at the head end to such a degree that the manubrium sterni of the minipig is exposed when it hangs in the sling. Once the minipig is placed in the sling, the head is supported by an assistant and the table can be raised to give easy access to the lower neck. The minipig head is lifted so the net is nicely stretched and exposed. Sitting in a low chair the technician can now obtain a blood sample.

Figure 4. Sling showing adaption for access to the neck of the animal.

To have an alternative we fitted an industrial fork-lift that can carry the sling and allows the forks to be lowered to the ground which provides the option to train the Göttingen minipig to step into the sling by itself and to be raised to a comfortable working position.

Figure 5. Shows adapted sling in place.

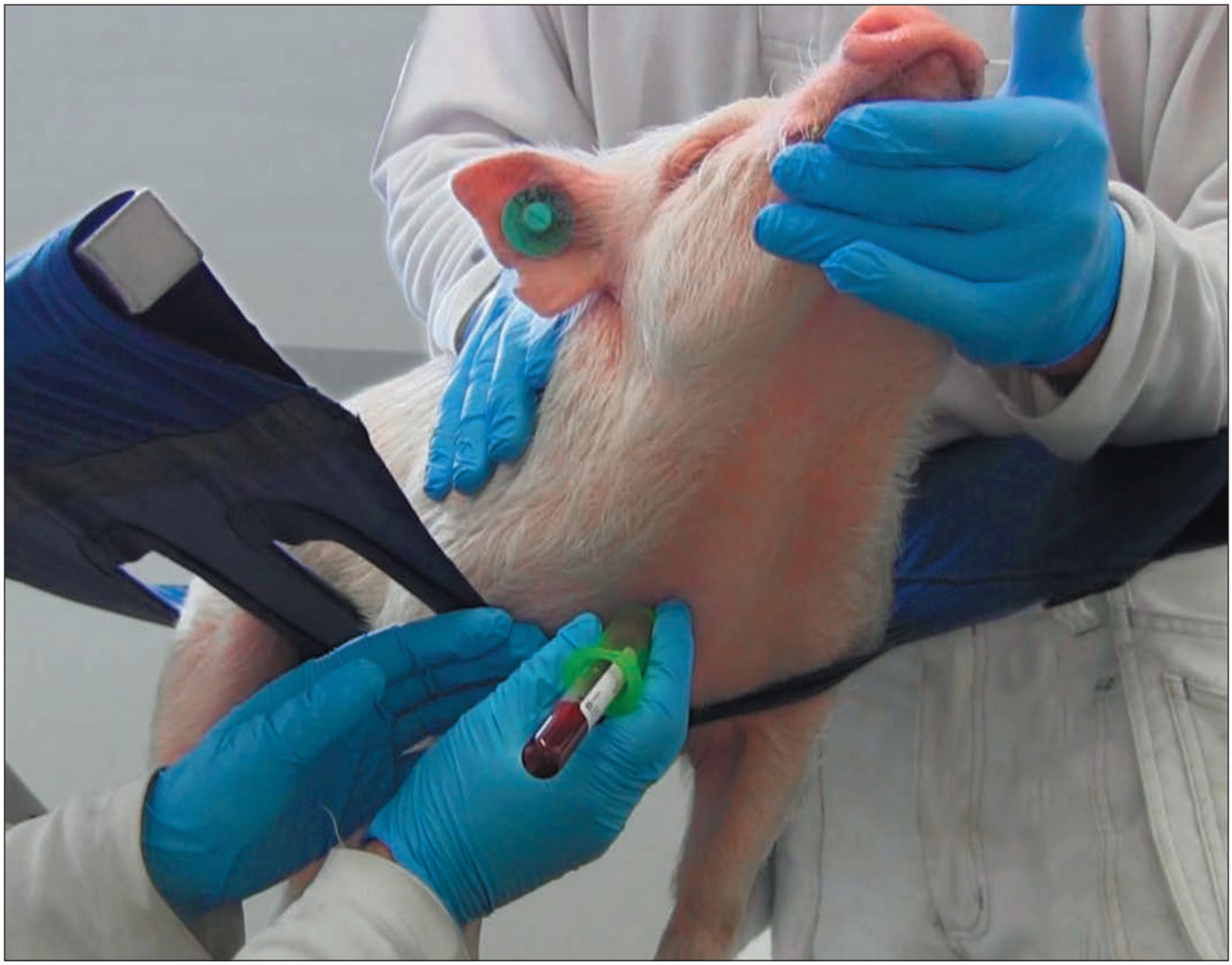

Figure 6. Showing blood sampling of pig.

Figure 7. Industrial fork lift.

The sling was modified so that the head is lifted and an extra hole was placed centrally, halfway between the openings for the front legs. The raised head makes it easier to palpate the anatomy of the neck through the central opening and to find the right site for puncture. The hole was fitted with the flap that covers the opening whilst placing the minipig. This helps prevent the minipig from putting its snout through the hole. When the flap is opened, the site for sampling is exposed.

Figure 8. Moditional sling showing additonal access hole with flap in place.

Figure 9. Fork-lift with sling in place.

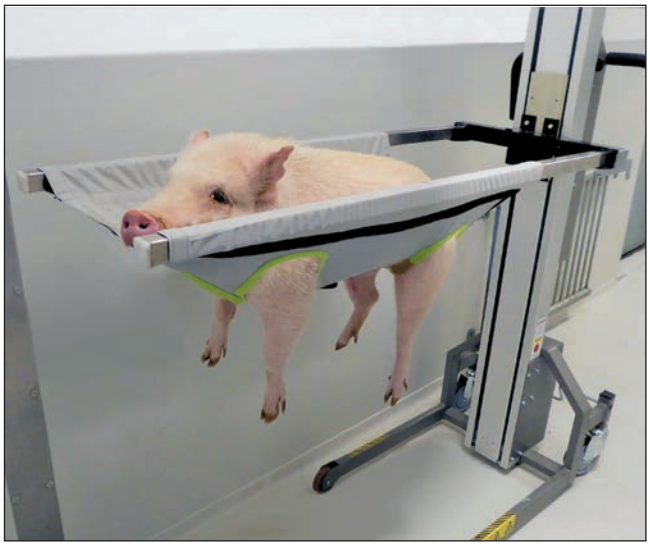

Figure 10. Fork-lift with sling and minipig in position.

Figure 11. Technician in position to take blood sample position.

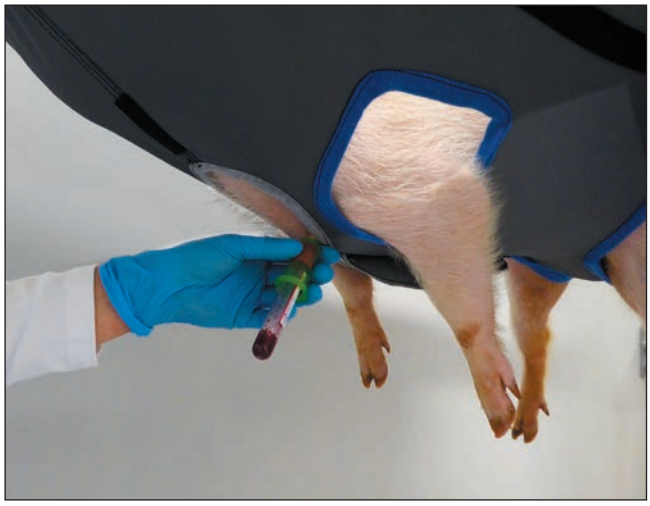

Figure 12. Venepuncture in progress.

Results, conclusion and discussion

We developed two methods that work flawlessly in most cases if the minipigs are properly acclimatised. The minipigs are calm, do not appear to be stressed and no vocalisation was observed. Less manpower than usual was required and we were able to take a sample every two minutes with time to spare.

Figure 13. Sitting technician holding small Gottingen minipig for blood sampling.

The systems were tested on males and females ranging from 5 to 35 kg. It proved to be particularly successful in the range >10 kg, however after a certain size it is advised that two people lift and place the minipig in the sling.

Minipigs in the lower weight range are generally a bit more nervous or unsettled and might be restrained by a sitting technician (figure 13) or in a traditional method on the V bench.

Göttingen minipigs adapt very well to the sling and require minimal training for that procedure. However, it is imperative to take your time when placing the minipig in the sling. You need to give this procedure the utmost attention when you do it the first time with the minipig because the outcome of this first attempt will define the character of the subsequent sling placements.

The technique of the actual sampling needs to be adapted to the new position. Practice has shown that technicians adapt quickly to the new angle of view and even less experienced technicians have no problems obtaining a blood sample with this type of restraint. The feedback from the technicians is positive throughout, they experience less stressed animals and need less man-hours. Overall it is a true contribution to animal welfare and is a refinement in the sense of the 3Rs.

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

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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 ex-breeding male mice;[1] in GM500 Digital Ventilated Cages (DVC®), (Tecniplast SpA). 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.

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).

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.

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 half 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).[2] 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 Christmas and New Year 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).

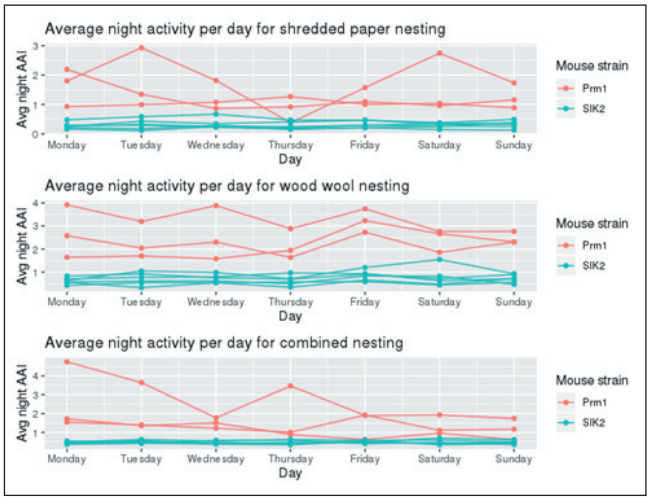

Figure 2. Average dark phase activity per strain.

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).

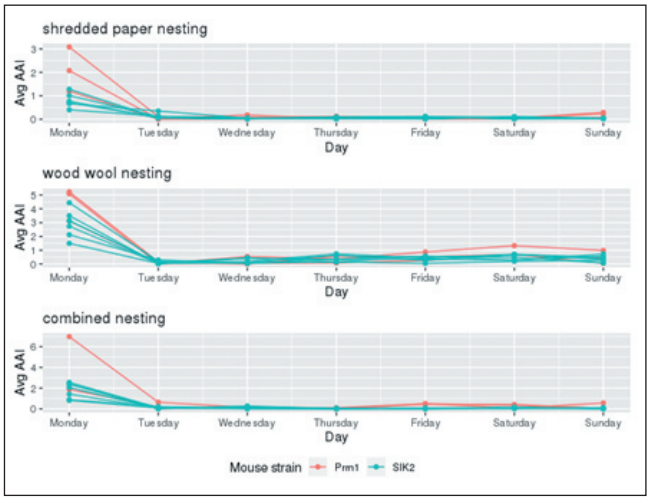

Figure 3. Average activity two hours post nesting changes.

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).

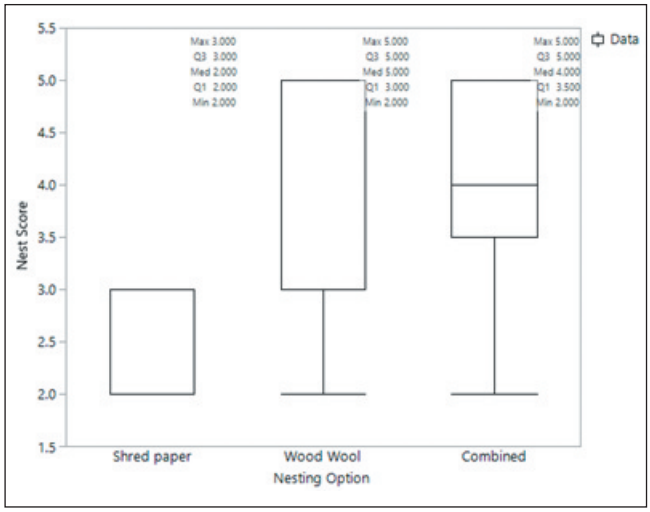

Figure 4. Results from nest scoring.

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 Christmas 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. Examples of our scores are in Figure 5.

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 Christmas period.

Score = 2

Score = 3

Score = 4

Score = 5

Figure 5. Examples of scored nests.

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 (Jirkof *et al*, 2013Rock *et al,* 2014;).[2,3] 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.

Acknowledgements

Guido Gottardo, Fabio Iannello and Mara Rigamonti, Tecniplast SpA.
Eloisa Brook, Giulia Del Panta, Steven Barrett, Research Statistics, GSK, Steve Wilson and Kay Dowse, IVSD, GSK.

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A method to improve the housing of breeding rats used to produce pups for tissue

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Introduction

Our facility houses a small colony of Sprague Dawley rats whose purpose is to supply pups at specified time points after they are born for neurophysiology and related research. Historically, a male and female were put into a grid floor cage for seven days or until the female plugged. The male and female were then separated and housed individually in solid floor cages with a wood shaving substrate, The male remained on his own until used for further matings and the female remained alone until she littered down. Once her pups were weaned the dam was single housed until she was put back in the grid floored cage to mate.

The system had been set up originally to allow plug date identification, but when we talked to the scientists it was clear that they did not need this information. They simply needed an idea of when litters might be born and therefore when the pups would be available for *ex vivo* work.

What did we want to do and why?

Rats are considered highly social animals (Research Animals Department, RSPCA).[1] In the wild, they live in groups and develop complex social structures. Re-grouping rats is considered stressful (Suckrow, 2015).[2] We therefore wanted to keep animals in monogamous pairs so that they could develop a social bond that was not being repeatedly disrupted and where they could be housed on solid floors all the time, as we felt that this would provide better welfare for the animals (Manser, 1995).[3]

We had to be able to produce pups efficiently. We had to identify whether or not the females were pregnant and their likely date for littering. In order to help with planning, and so as to provide tissue regularly, the scientists wanted two litters born a week, ideally with several days between them.

What did we do?

We bought in six-week-old male and female Sprague Dawley rats from Charles River Laboratories (Margate). They arrived in boxes of ten. Animals were housed in the groups that they had arrived in, until they had recovered from transport, were acclimatised and had reached a size and weight considered big enough to breed (as judged by an experienced technician).

We kept some animals on the previous system, to ensure that a continuing supply of pups was available for the scientists, whilst we set up an initial group of three stable pairs. These animals were housed in RC2R cages (NKP Isotec). Over time, additional animals were set up as pairs as the trial progressed and currently there are eight monogamous pairs in use.

We tried two methods of assessing pregnancy: manual palpation and visual inspection. The latter was sufficiently accurate for the needs of the scientists and was considered a lot less stressful for the animals. Health and welfare of the animals, pregnancy rates and number and sizes of litters born were monitored.

In order to increase the area available to a pair of rats, two cages were linked together by means of a polycarbonate red rat tube (Datesand Ltd). To do this, the end of the tube was placed against one side of a cage base 3cm from the back of the cage and was drawn around with a marker pen. The same was done on the next cage base but on the opposite side. A 5mm drill bit was used to drill a hole big enough to insert a jigsaw blade and then the marked circles were cut out, ensuring that they were cut accurately to be opposite

each other so the tube could be inserted from inside one cage and across to the other. The holes were filed to ensure they were smooth and that the tube fitted snugly. The tubes are not fixed in place and can be easily slid into place and then pulled back out when cages need to be separated for cleaning.

Figure 1. Cages on rack are joined using red tubes inserted through holes cut out of the side of the cage bases, towards the back.

Figure 2. Female with pups in double cage, with male sleeping in adjoining cage.

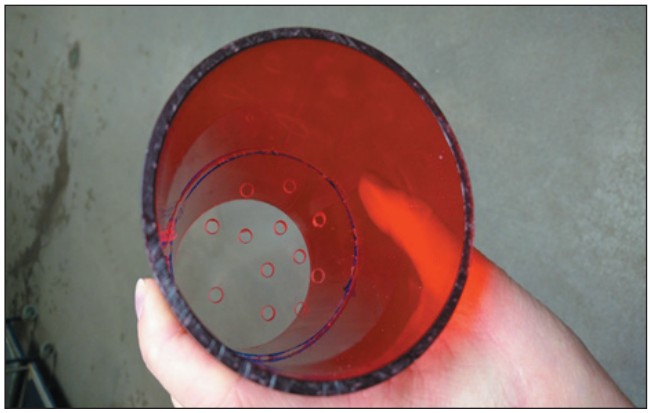


Figure 3. Plastic grid in the tube to join two cages.

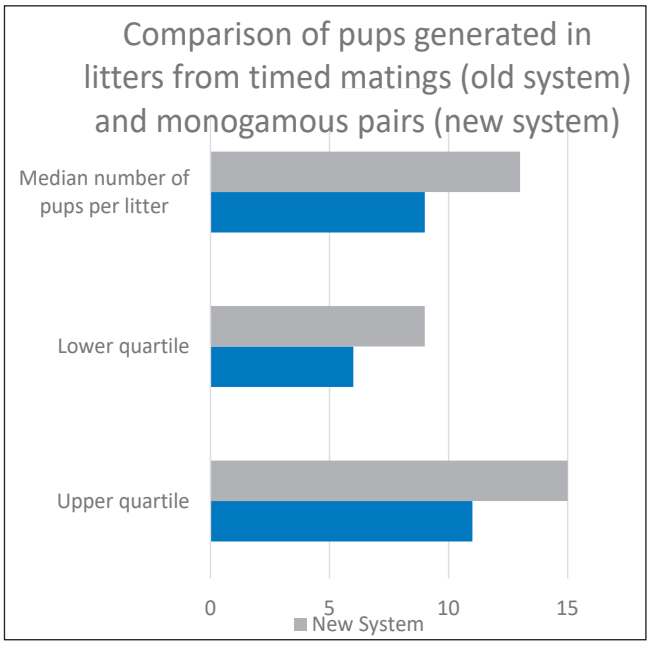
It was noted that females in pairs began to synchronise their oestrus cycles and consequently, when they gave birth. To avoid separating and reintroducing the pairs, with associated risk of aggression between the pair or the male and the pups, we used a plastic 'grid' in the tunnel joining the cages to allow contact but not mating (Figure 3).

Results

Females in the monogamous pairs housed in standard cages suffered from hair loss around their nipples and underbelly and some of the males had hair loss on their cheeks. The Named Veterinary Surgeon (NVS) considered the most likely diagnosis was excessive grooming by the pups. The cages also became damp rapidly with the numbers of animals present. Increasing cage change frequency for pairs with large litters from once to twice a week was unsuccessful in reducing the over-grooming.

We increased the space available to pairs with pups by linking two cages together (Figures 1 and 2) to see whether this benefited their welfare, as judged by external signs. All animals regrew lost hair and no further hair loss was seen. Technicians caring for the rats noted that the adults were much easier to handle, calmer and interacted more with the handlers.

Data generated under the previous and new systems were compared.



For the time-mated animals, median litter size was 9 (inter-quartile range 6-11; 40 observations) and in the monogamous pairs, the median litter size was 13 (inter-quartile range 9-15; 52 observations). Thus the median litter size was about 50% higher in the monogamous

pairs. It was difficult to compare the data directly beyond these basic figures as mothers were at different stages in their reproductive life.

Dividing grids have been used in 7 pairs for 11 matings to date. On one occasion, one female did not become pregnant, was divided from the male for a second time, was reintroduced to him and became pregnant. The median litter size for these matings is 15 (inter-quartile range 11-15; 10 observations). On average pups are born 35 days after reintroduction of the male (range 24-43 days).

Conclusion

The change to monogamous pairs was very successful, resulting in marked increase in median litter size. This allowed us to reduce the number of adult rats with related decreased costs and husbandry-related time. By joining two cages together, thus providing a greater floor area, there were improvements in outward signs of welfare and docility of the animals.

It appears that we can control the tendency for females to synchronise their oestrus cycles, which otherwise could result in an uneven supply of pups, by allowing the male and female in each pair to remain separated but in communication with each other using a grid and then reintroducing the pair around 6 to 7 weeks before pups are needed.

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