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Weighing used for the automatic registration of preferences when testing rats

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Summary
The preference test is one of the only behavioural test, which gives the animal an opportunity to make a free choice or indicate what it prefers and, therefore, it is widely used to evaluate whether an animal prefers one set-up to another. Providing what the experimental animal prefers (eg grids) will reduce stress, good for both experimental reliability and animal welfare.
In the present study the rat's preference for different cages was registered and recorded by digital weights. This study showed that this relatively simple set-up was applicable for registration of the preferences for different housing conditions, such as bedding or grid.

Introduction
The preference test is one of the only behavioural test, which gives the animal an opportunity to make a free choice and indicate what it prefers here and now, and, therefore, it has been widely used in many different species to evaluate whether an animal prefer one set-up to another (*Hughes, 1976*; *Blom, 1993*; *Manser et al., 1995*; *Held et al., 1995*). It is also one of the most controversial tests used in animal welfare analysis, and its advantages and disadvantages have been discussed in several previous papers (*Dawkins, 1976*; *Duncan, 1978*; *van Rooijen, 1982*; *Dawkins, 1983*; *Fraser et al., 1993*). Three conclusions can be drawn from these discussions. Firstly, the animal cannot consider and evaluate the long-term consequences of the choice it makes; secondly, the preferred option may simply be the lesser of two evils; finally, the animal is only able to make a choice between those options given. Keeping these reservations in mind, the preference test can, however, be a very useful tool to evaluate different housing conditions, as long as conclusions are drawn in connection with other behavioural and physiological tests.
Different preference test set-ups have been designed for evaluation of housing conditions for rodents (*Baumans et al., 1987*; *Manser et al., 1995*; *Chmiel and Noonan, 1996*; *Patterson-Kane et al., 2001*). Commonly, the activity is recorded on video and analysed afterwards, or the set-up is equipped with micro-switches detecting the animal entering and leaving each cage. The former approach is very time consuming, while the latter approach requires some technical skill and equipment. In the present study a very simple and cheap set-up for registering the animal's choice was designed. The two different cages for the animal to choose between were placed upon digital weights, which registered and recorded the presence or absence of the animal. The aim was to evaluate whether this set-up was applicable for evaluating housing preferences of rodents. For that purpose three studies were designed and carried out. The results were compared to results from previous studies testing the same options in rats.

Material and Methods
Ten rats (Mol:SPRD Han), five females weighing 150-250 g and five males weighing 200 -299 g, were used for each study. Prior to testing, the rats

were housed in four U1400 cages (Tecniplast, Italy) in groups of two or three for at least one week to allow them to acclimatize. Bedding (Tapvei, Finland), wood blocks (Tapvei, Finland) and woodwool (Tapvei, Finland) were used and the cages were changed twice a week. Food (Altromin 1324, Brogården, Denmark) and water were given ad libitum.

The preference set-up was constructed as follows: Four Type III cages (Tecniplast, Italy) 18 cm high were connected two by two, in total two set-ups (Figure 1). All four cages were equipped with water bottle and food pellets. One rat was placed in each set-up and always initially placed in the left cage. In each cage a hole (diameter 7 cm) was connected to a PVC tube (diameter 7 cm, length 7 cm). Each cage was placed on a digital weight (EA6DCE-L, Sartorius AG, Goettingen, Germany) and a software program (WinWedge 16 Pro, TAL Tech, Philadelphia, US) stored the data from the weights every 15 sec. on a PC (Pentium 2, 233 MHz, 32 mb Ram, Datafilen, Denmark). By the use of Excell (Microsoft Corporation, US) and the function frequency, the data were divided into two periods, a light/day period (0600 hr – 1800 hr) and a dark/night period (1800 hr – 0600 hr). A registration under 100 g and over 100 g on each weight were counted as absence or presence, respectively. Three night and two day periods were analysed for each rat in each study. Finally, the results were statistically analysed by the use of a t-test (Minitabs ver 12.1, Minitab Inc, US) testing whether the distribution between the left and right cage was 50/50. A power analysis was used to calculate the differences that it would be possible to show on the basis of the number of animals used and the variation observed, setting the power to 90%. The set-up was placed in a separate room with no other animals and with automatic day/night light shift, room temperature at 23±1 °C and relative humidity at 45±5 %. The room was ventilated 10-15 times per hour.

In study 1 the set-ups were placed on a rack in the room, one set-up on the second shelf and one set-up on the third shelf. All cages were with bedding.

In study 2 the set-ups were placed inside a ventilated cabinet (Scantainer, Scanbur A/S, Denmark), one set-up on the upper and one set-up on the bottom shelf (Figure 1). The cabinet was ventilated 70 times per hour. All cages were with bedding.

In study 3 the same set-ups as in study two were applied. Of the two cages in each set-up one was with bedding and one was with a grid floor inlet (Tecniplast, Italy). The grid inlet was randomly distributed to either the left or the right cage.

Results

The results for the three studies are illustrated in Figure 2-4. The p-values from the t-tests are given in Table 1.

Study 1 showed a 50/50 distribution between the left and right cage, although there was a slight, non-significant preference of the right cage (Figure 2 and Table 1). Study 2 showed a 50/50 distribution between the left and right cage (Figure 3 and Table 1).

Study 3 showed a significant preference for bedding both at day and night for the females and a significant preference for bedding for males at day and for grid at night (Figure 4 and Table 1).

The power-analysis proved it possible to detect a difference of 6% and 9% for females at day and night, respectively, and 15% and 9% for males at day and night, respectively.

Discussion

The present study verifies that ordinary digital weights with logging function can be used as a method of regististration in which cage the animal is present in a preference study. Therefore, the present set-up can be used as registration in a preference test in the same way that video or micro switches have been used previously (*Baumans et al., 1987*; *Manser et al., 1995*; *Chmiel and Noonan, 1996*; *Patterson-Kane et al., 2001*).

In study 1 there was a problem with a slight preference for the right cage, and therefore it was decided to place the set-up in a ventilated cabinet as done in study 2. When the set-up was placed on an open shelf, external non-experimental factors such as draft in the left cage, differences in light, or some other non-controllable factors, might cause the animals to prefer the right cage above the left, although it was not significant. By placing

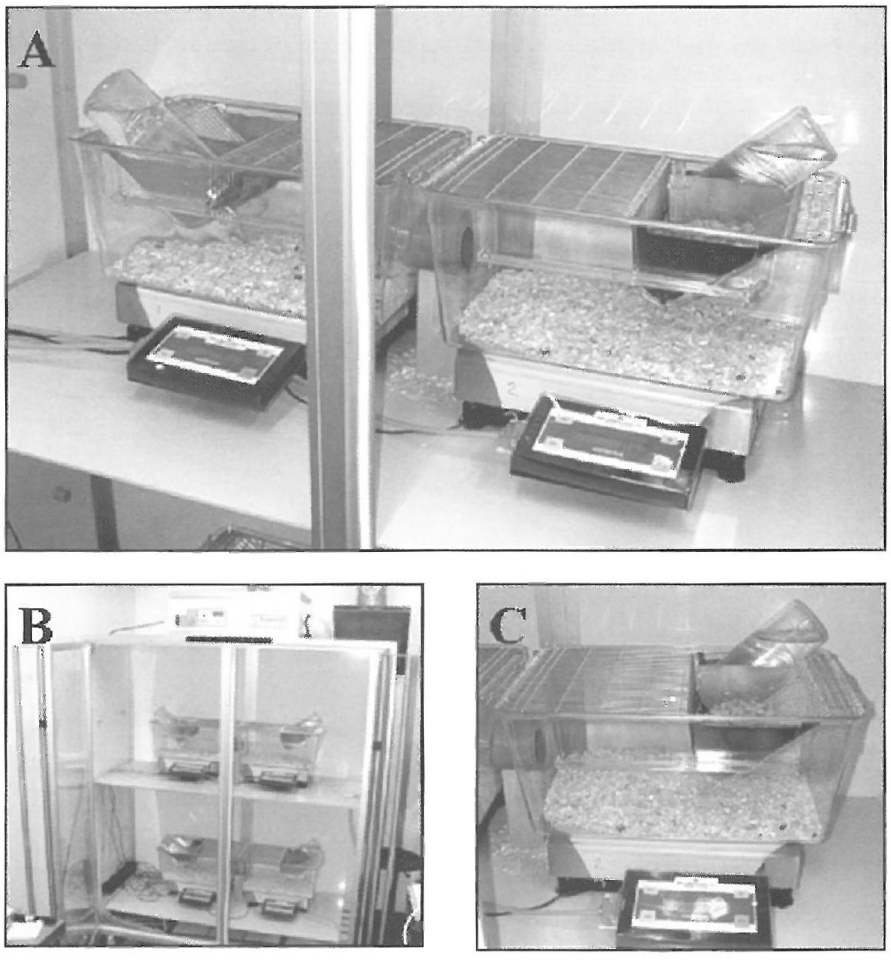

Figure 1. The preference set-up used in the present study. A) The interconnected cages with the connection tube between the two cages and each cage placed on a digital weight. B) The full set-up placed in a Scantainer as used in study 2. C) One of the cages placed on a digital weight and showing the connection tube to the left. The cage is fully equipped with bedding, food and water.

Table 1. The p-values for the three studies at day and night for male and female Sprague Dawley rats. The results were statistically analysed by the use of a t-test to see if the distribution between the left and right cage was different from 50/50.

	Male		Female	
	Day	Night	Day	Night
Study 1 (open shelf - bedding vs. bedding)	p = 0.22	P = 0.055	p = 0.36	p = 0.92
Study 2 (Scantainer – bedding vs. bedding)	p = 0.71	p = 0.11	p = 0.12	p = 0.62
Study 3 (Scantainer – bedding vs. grid floor)	p = 0.0042	p = 0.047	p = 0.0000	p = 0.026

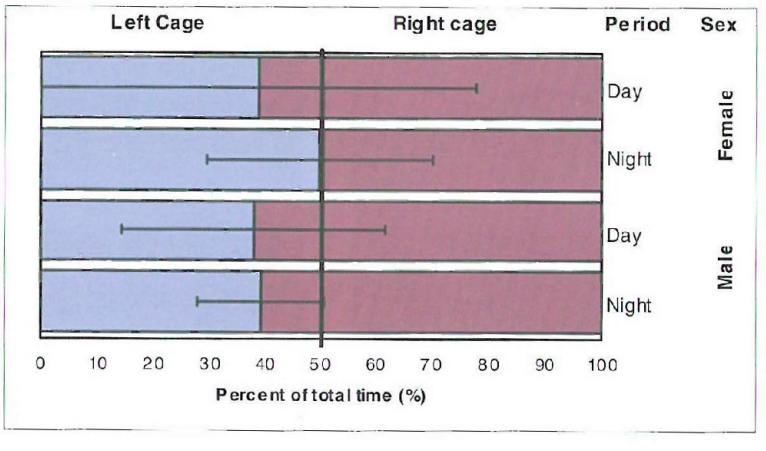

Figure 2. The results from study 1 where the preference set-up was placed on an open shelf and two equal options were tested. The figure shows the distribution of dwelling time for Sprague Dawley rats between left and right cage for day and night when both cages are with bedding. Also results for both male and female rats are shown. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked.

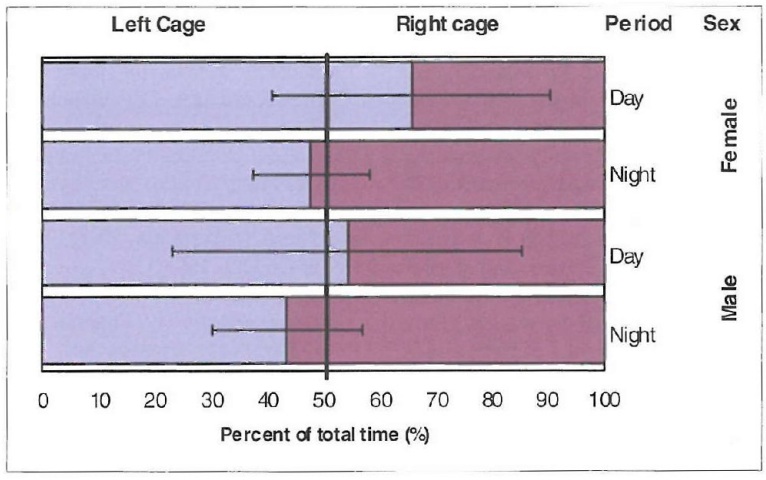

Figure 3. The results from study 2 where the preference set-up was placed in a ventilated cabinet and two equal options were tested. The figure shows the distribution of dwelling time for Sprague Dawley rats between left and right cage for day and night when both cages had bedding. Also results for both male and female rats are shown. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked.

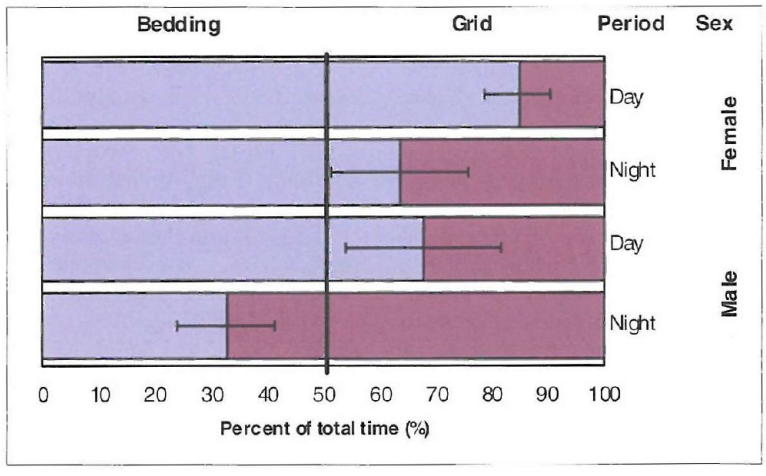

Figure 4. The results from study 3 where the preference set-up was placed in a ventilated cabinet and two different options were tested. The figure shows the distribution of dwelling time for Sprague Dawley rats between bedding and grid floor for day and night. Also results for both male and female rats are shown. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked.

the set-up in the Scantainer some of these factors may be eliminated.

When testing the preference for one of two *identical* options as done in studies 1 and 2 the standard deviation should turn out to be rather large, if the two options are equally preferred by the animal, as the animal must necessarily be inside one of the cages, and probably have a preference for one of the cages although identical. So, if one animal prefers the left cage and another animal prefers the right cage, the standard deviation will be very large when analysing data on group level, as also shown in a previous study validating a preference test system (*Blom et al., 1992*). On the other hand, if the animal must choose between two different options, the standard deviation should turn out to be small, as the animals in the same group should prefer the same cage.

The preference for bedding over grid in daytime confirms a previous study (*Manser et al., 1995*). Another study has shown that rats housed on grids respond by a rise in blood pressure and heart rate (*Krohn et al., 2001*). The difference in preferences for the males and females at night might be explained by general differences in behaviour between the sexes. Also other studies showed that males compared to females had a higher preference for grids (*Blom et al., 1996*). The higher preference for grid by the males may be due to generally more explorative behaviour by males compared to females, whereas the females' preference for bedding, may be due to general higher level of nesting behaviour by the females.

In conclusion, our studies showed that by placing the cages on digital weights, it is possible to register in which cage the rat is present, which is a very simple way of measuring the rat's preference for different housing conditions or situations; and that by being simple and apparently reliable, it may well have some advantages over the previously described (video and micro-switch) procedures.

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