Rating enrichment items by group-housed laboratory mice in multiple binary choice tests using an RFID-based tracking system

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Abstract

There is a growing body of evidence that enrichment of the housing conditions of laboratory animals has positive effects on daily behavior, growth, and health. Laboratory mice spend most of their lives in their housing rather than in experimental apparatus, so improving housing conditions is a first-choice approach to improving their welfare. Despite the increasing popularity of enrichment, little is known about whether it is also perceived as being beneficial from the animal's point of view. This is especially true due to the fact that 'enrichment' has become an umbrella term that encompasses a wide variety of different elements. We categorized enrichment items according to their prospective use into the categories 'structural', 'housing', and 'foraging' and let female C57BL/6J mice chose within the categories according to their preference. Preferences were tested over a 46-hour period in a home cage system consisting of two interconnected cages equipped with the respective items to be compared. A new analysing method combined binary decisions and ranked the enrichment items within each category by calculating worth values. The assignment of worth values to the items to be compared led to a further gain in information, estimating importance from the mice's point of view in addition to pure ranking. Given the known beneficial effects of enrichment, these data will help in deciding how to provide appropriate enrichment elements to improve animal welfare and refine animal experimentation.

Keywords: laboratory mouse, environmental enrichment, housing conditions, animal welfare, preference testing, C57BL/6J mice

Introduction

Attitudes toward animals as fellow living creatures have changed significantly in recent decades. There is growing concern about the conditions under which laboratory animals are kept, and it is therefore not surprising that legal requirements are also becoming increasingly demanding. In Europe, minimum requirements for housing animals are set out in EU Directive 2010/63, which stipulates that animals must be housed according to the specific needs and characteristics of each species. Experimental animals should be provided with 'space of sufficient complexity to allow expression of a wide range of normal behavior'. While the available space itself is a pressing issue for future improvements, the issue of complexity is usually approached through what is known as enrichment of housing conditions. It is reasonable to assume that additional enrichment opportunities in barren cages will create a more complex environment, which is likely to be appreciated by the animals. However, it is important to note that 'enrichment' has become an umbrella term that encompasses a wide variety of different elements. Therefore, it must be kept in mind that by no means a uniformly accepted enrichment is meant when speaking of effects of enrichment [1]. This being said, many research groups have indeed shown the benefits of enriched environments relative to conventional housing on wellbeing parameters in mice [2]. Abnormal repetitive behavior expression, behavioral measures of anxiety, as well as growth and stress physiology were influenced positively by providing mice with a more varying environment using enrichment items [3]. Access to enrichment lead to improved learning and memory function [4,5], increased hippocampal neurogenesis [5,6], attenuated stress responses and enhanced natural killer cell activity [7]. Importantly studies showed no influence of a more diverse environment on variability of important parameters in biomedical research in mice [8–10] and the workload of animal caretakers but enhanced wellbeing in laboratory rodents [11]. Vice versa, there is increasing evidence, that keeping animals in standard housing conditions may be the negative factor in the development of behavioral disorders because of its impoverished character [12].

To create a more varied and stimulating environment, the size of the home cage can be enlarged, the group size increased, and stimulating elements can be provided [13,14]. However, the human perspective does not necessarily reflect the wants and needs of mice. Therefore it is essential to ask the animals themselves about the adequacy of the enrichment items [15,16]. To determine how different items are perceived by the animals themselves [17], animal centric strategies like preference tests will help to assess and rate different items [18,19].

From the three typically used preference testing designs [19], T-Maze, conditioned place preference, and home-cage-based preference tests, the last one seems to be the most appropriate for rating enrichment items. Especially when it comes to the avoidance of frequent animal handling and the opportunity to extend testing periods up to a full circadian rhythm or longer [19]. Additionally, choice tests conducted within the home cage without the influence of an experimenter [20,21] correspond better to real laboratory keeping conditions.

Home-cage-based testing systems usually consist of two [22,23] or more [23,24] connected cages with or without a center cage. In such tests mice are able to stay in their preferred surrounding and the cage that is chosen more often or with the longer period of stay is regarded as the preferred one. Or, in case of aversive properties, as the least avoided [19].

For our preference test, we used the Mouse Position Surveillance System (MoPSS), a new test system constructed in our laboratory [25]. The MoPSS allows automatic long-term calculation of time spent in each of two interconnected cages for each individual mouse in a group. The determined dwelling time is used to conclude the choice between different enrichment items from the point of view of a mouse. The offered items were categorised and tested by their intended purpose of structuring the cage (structural enrichment), stimulating foraging engagement of the mice (foraging enrichment) and providing an alternative resting place (housing enrichment). To rank multiple items, we combined multiple binary choice tests and calculate worth values [26]. In order to further evaluate the quality with regard to strength of preference and consistency of choice among individual mice we used a recently developed method (Lewejohann, Talbot et al., in prep; see https://talbotsr.com/simsalRbim/index.html). The overall aim of assigning worth values to specific enrichment items by comparison, is to provide scientifically based assistance for improving housing conditions of laboratory mice and thus increase animal welfare.

Materials and Methods

Ethical approval

All experiments were approved by the Berlin state authority, Landesamt für Gesundheit und Soziales, under license No. G 0069/18 and were in accordance with the German Animal Protection Law (TierSchG, TierSchVersV). The study was pre-registered in the Animal Study Registry (ASR, DOI 10.17590/asr.0000162).

Animals and housing condition

Twelve female C57BL/6J mice were purchased from Charles River Laboratories, Research Models and Services, Germany GmbH (Sulzfeld). The mice were 7- 8 weeks of age upon arrival in the animal facilities. During the first three weeks the animals were housed in groups of four animals in type III Makrolon cages (L x W x H: 425 x 265 x 150 mm, Tecniplast, Italy) with aspen bedding material (Lignocel FS14, spruce/fir, 2.5-4 mm, JRS, J. Rettenmaier & Söhne GmbH + Co KG, Germany), paper (cellulose paper unbleached 20x20 cm, Lohmann & Rauscher International GmbH & CO KG) and cotton roll nesting material (dental cotton roll size 3, MED-COMFORT), a 15 cm transparent plexiglas tube (Ø 4cm PMMA xt®, Gehr®) and a red triangle plastic house (mouse house, TECNIPLAST®). They were provided with regular rodent food (autoclaved pellet diet, LAS QCDiet, Rod 16, Lasvendi, Germany) and tap water *ad libitum*. Room temperature was maintained at 22 °C (+/- 2), room humidity by 55 % (+/- 15) and a 12/12 light/dark cycle regimen (lights off 20:00) with simulated sunrise between 7:30 and 8:00 using a Wake-up light (HF3510, Philips, Germany). To implement refinement procedures according to the 3Rs [27] all mice were trained to tunnel handling [28] daily during this habituation phase which was used throughout the whole experiment.

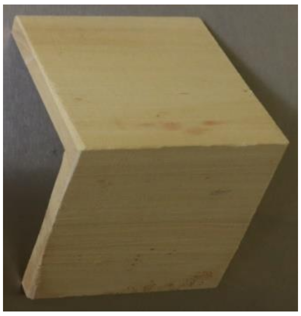
At the age of eleven weeks mice were provided with cage enrichment. Cages were cleaned weekly and each mouse was subjected to a visual health check. The enrichment scheme consisted of permanently provided items (running disc with mouse igloo, paper nesting, cotton rolls, Table 1) and five weekly rotating items from structural, housing, nesting and foraging categories (See Table 1 and 2). These enrichment items were randomly exchanged during the weekly cage cleaning. Randomization of the enrichment combination was done with the use of the function randomize() in the software R (version 4.0.4). To motivate the mice in solving the riddles of the foraging enrichment category, a small amount of millet was provided in the morning daily inside the riddle during the animal health check. Prior to the preference experiments, the mice were used in another experiment (Hobbiesiefken et al. subm.) but stayed in the above-mentioned housing conditions.

Table 1: Used enrichment items

deployment	enrichment item	
standard house in MoPSS experiment	**triangular house** (mouse house, TECNIPLAST®)	
housing used in husbandry period	**running wheel** (fast-trac + mouse igloo, Bio-Serv®)	
permanently available (husbandry and MoPSS experiment)	**paper nesting** (cellulose paper unbleached 20x20 cm, Lohmann & Rauscher International GmbH & CO KG)	
	cotton roll (dental cotton roll size 3, MED-COMFORT)	
nesting used in husbandry period in homecage	**fine wood wool** (H0234-NBF, ABEDD®)	
	coarse wood wool (H0234-NBU, ABEDD®)	

	square hemp pads (H3279-10 eco- hemp, ssniff Spezialdiäten GmbH)	
	folded paper strips (sizzlenest®, datesand Ltd)	
	mid coarse wood wool (NBGE012, ABEDD®)	

Table 2. Tested enrichment items

category	enrichment item	
housing	**house ball** (crawlball, Bio-Serv®)	
	floor house (safe harbor, Bio-Serv®)	
	paper house (LBS Serving Biotechnology)	
	wooden angle (climbing roof, ABEDD®)	
	holed wooden angle (holed climbing roof, ABEDD®)	

structural	**second level, 1 hole** (1 hole lying boards for cage type III, ABEDD®)	
	second level, 2 holes (2 hole lying boards for cage type III, ABEDD®)	
	clip with paper tube (38 x 1.25 x 75 mm play tunnel and tunnel clip, Datesand Ltd)	 
	clip with plastic tube (Plexiglas tube transparent 70mm Ø, KUS and tunnel clip, Datesand Ltd)	 
	mouse swing (single mouse swing, Datesand Ltd)	
	mouse swing double (double mouse swing, Datesand Ltd)	
	rope (jute yarn 6-ply, 6mm, Rayher 4200531)	

	treat ball (self-designed and printed with Filament world PLA 2,85 mm, Ultimaker extended 3)	
	sliding puzzle (Interactive Smart Toy, Living World® green)	
foraging	**tube with stones** (mouse tunnel, Bio-Serv® and white marble pebbles 15 – 25 mm Ø, Min2C Natural Minerals)	
	lattice ball with ball chain (Hol-ee Roller® size mini, JW®)	
	flap puzzle (self-designed and printed with Filament world PLA 2,85 mm, Ultimaker extended 3)	

Animal identification

For individual animal identification, all animals were provided with a microchip transponder (ISO 11784/85, FDX-B transponders, Planet ID®) under the skin of the dorsal neck region in rostro caudal implantation direction. This procedure took place at the age of 9-10 weeks under general isoflurane anaesthesia and pain reliever (Metacam ®).

Additionally, all mice were color-coded weekly on the tail with a permanent marker (Edding® 750) to distinguish them in video observations.

Preference testing

After 43 weeks in the enriched housing condition, preference tests were conducted using the MoPSS [25]. The system consisted of two macrolon type III cages, connected with a 30 cm plexiglas tube. Two circular RFID antennas were attached outside the tube. Inside the tube, plastic barriers were installed in order to slow down mouse movement (see Figure 1: The Mouse Positioning and Surveillance System). The RFID antennas were connected to a reader, which recorded the mouse movements between the left and right cage through detection of the implanted microchip.

The mice remained in their group of four animals and three preference systems were used in parallel. The systems were positioned in a row on a steel table (see Figure 2: Experimental setup), in an experimental room with the same environmental conditions as during the housing period.

To achieve same lightning conditions for the left and right cage of the preference system, four LED lights (Brennenstuhl® Dinora 5000 Baustrahler 47 W 5000 lm Tageslicht-Weiß 1171580) on tripods were set up pointing towards the ceiling. Light intensity in both test cages was checked with a lux meter (voltcraft® light meter MS-1300).

The testing cages were outfitted with 150 g aspen bedding (Polar Granulate 2-3 MM, Altromin), a red translucent triangular plastic house, three uncoloured paper towels, two cotton rolls, and water and rodent food (autoclaved pellet diet, LAS QCDiet, Rod 16, Lasvendi, Germany) *ad libitum* with same amount on each side (see Table 1 for equipment details).

Enrichment items of one category each were randomly presented twice for 23 hours starting at 10:00 am until 9:00 am the following day. Between the two sessions using the same items, the enrichment items were switched between the cages to counteract possible side preferences. In addition also the nesting material and bedding was mixed between the left and right cage and the mice were supplied with their daily amount of millet. The first category tested was the 'structural enrichments' followed by the 'foraging enrichments' and the 'housing enrichments'. Two days before the first preference test, the mice were introduced to the experimental setup including the MoPSS for habituation purpose.

Figure 1. The Mouse Positioning and Surveillance System (MoPSS)

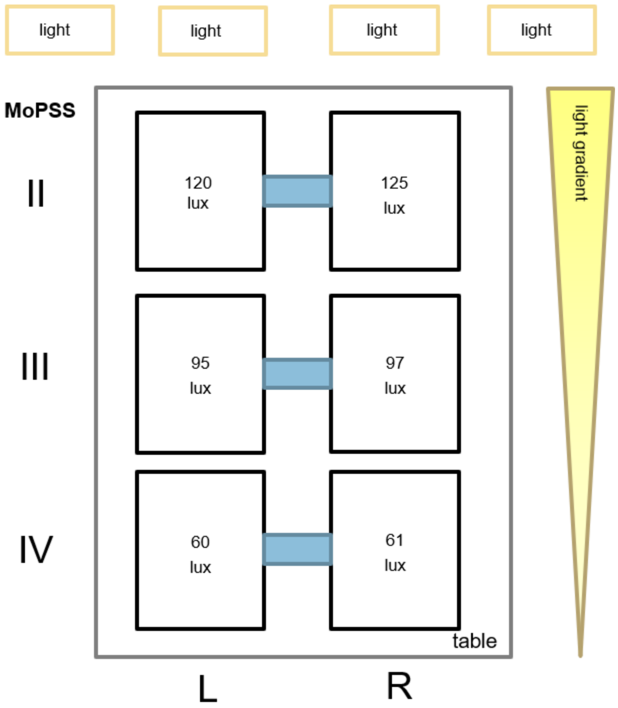


Figure 2. Experimental setup

Analysis of preference

The mouse tag detections were automatically saved onto a microSD card during the experiment and each detection was marked by a current timestamp with the antenna number (left/right) and the individual mouse RFID tag number. Data analysis and sanity checks with logical correction of missing detections were done using a data evaluation script in the software R (R version 4.0.4, R Studio version 1.3.959) specially developed for MoPSS data analysis [29]. Stay times for each of the twelve mice in each cage side were calculated as times between cage changes when a mouse tag was detected at a new cage. It has been shown that the time spent in the tube is neglectable for preference calculation [29] and therefore we did not subtract the time spent in the passaging tube. Stay durations over the 46 hours testing period of each single experiment were summed up for each mouse and then calculated as percentage of the total time. All data was analysed both at group/cage level and in relation to the length of stay of all individual mice over the total period of 46 hours and over the light and dark phase representing the activity phases of the mice. The calculated percentages of stay durations were then used for comparison of side preferences (left vs. right cage) for enrichment one and enrichment two including a side switch of the presented items. The raw data with stay durations in percentage during the hole 46 hours testing period and divided into the active and inactive time period can be found in the supplementary material (Supplement: Table 1,2,3).

To rank the tested enrichment items regarding the strength of the preference for each item, a method developed by Hatzinger et al. 2012 [26] of combining the multiple single binary choices to a ‘worth value’ was performed using R and the package simsalRbim (https://talbotsr.com/simsalRbim/index.html). A similar method was used by Hopper et al. 2019 to determine the worth value of different items of food rated by a male gorilla [30]. In short, to estimate the position of an item, the ‘worth value’ of each enrichment item was calculated based on the prefmod package [26] with its fit to a log-linear Bradley-Terry model (LLBT). The LLBT was specifically made for paired- comparison testing and estimates a subject´s relative ‘worth value’ for each choice on a preference scale that sums to 1 [26]. Greater preference is represented here by a higher ‘worth value’. To determine the meaningfulness of the 'worth value' for each ranked enrichment item and its estimated position on the scale, a consensus error was also calculated using the simsalRbim. The consensus error reflects the extent of agreement that the mice showed regarding the preference for a certain enrichment in binary choices over the other tested enrichment items. A value of 0 % points out a perfect agreement of a ranking position and 100 % indicating a full disagreement.

All analyses were run in R version 4.0.4 using RStudio (Version 1.3.959).

Sample size

It is debated whether or not group housed animals can unequivocally considered to act independently in their choice and therefore each cage would have to be considered as one independent sample [25,31–33]. This presents a dilemma because the mice would either have to be housed individually or the total number of experimental animals would have to be increased by the use of additional cages. As we are explicitly interested in the preference for enrichment items under common social conditions, housing mice singly was not an option. With regard to keeping the overall number of experimental animals as low as possible in the light of the 3Rs, we calculated that 12 mice would be a reasonable sample size if they indeed act independently. In order to demonstrate that individual preference was an independent choice, we conducted a follow and influence behavior analysis using R (Version 4.0.4) with our obtained experimental data from the MoPSS system. A *follow event* was defined as a transition of one mouse directly detected within one second after another mouse. The leading mouse detected in this constellation received an *influencer event*. We further calculated a *follow rate* and *influence rate* as follows:

$$follow\ rate = \frac{follow\ events}{transitions}$$

$$influence\ rate = \frac{influence\ events}{transitions}$$

Results

Preference testing

The relative preferences (worth values) and consensus errors of all 12 mice for the enrichment items of the categories foraging, structural and housing during are given in Figure 3.

Mice preferred the lattice ball over all other *foraging enrichment* items during the 46-hour testing interval (worth value (WV): 0.51; consensus error (CE): 29.17 %), both during active (WV: 0.47; CE: 33.3 %) and inactive time (WV: 0.42; CE: 45.83 %).

Over the total time of 46 hours, the highest worth values regarding the *structural enrichments* was achieved by the rope (WV: 0.42; CE: 45.83 %). However, during the active time the second plane (WV: 0.42; CE: 45.83 %) was preferred while during the inactive time both, the second plane (WV: 0.25; CE: 75 %) and the rope (WV: 0.25; CE: 50 %) had the highest worth values.

Out of the *housing enrichments* all mice preferred the floorhouse over 46 hours (WV: 0.27; CE: 45.83 %) and within the active time (WV: 0.34; CE: 45.83 %). During the inactive time the floorhouse (WV: 0.21; CE: 79.17 %) and the houseball (WV: 0.21; CE: 79.17 %) were equally favored.

Figure 4 illustrates the relative preferences (worth values) and consensus errors of the four mice of *group 1* for the enrichment items of the categories: foraging, structural and housing. During the whole testing cycle and during the active and inactive phase.

Group 1 ranked the lattice ball (WV: 0.36; CE: 0%) and the tube with stones (WV: 0.36; CE: 0%) the highest out of the *foraging enrichments* during the 46-hours cycle. Within the active time the lattice ball (WV:0.36; CE: 0%) and tube with stones (WV: 0.36; CE: 0%). Whereas the sliding puzzle (WV: 0.49; CE: 25%), achieved the first rank within the inactive time. From the *structural enrichments* the rope (WV: 0.35; CE: 0%) and second plane (WV: 0.35; CE: 0%) were ranked on the first position in 46 hours. The rope (WV: 0.37; CE: 0%) also achieved the first position within the active time whereas the second plane (WV:0.49; CE: 25%) was ranked first during the inactive time by group 1. *Housing enrichments* with the first ranking position in group 1 were the floorhouse within 46 hours (WV:0.34; CE:12.5%) and during the active time (WV:0.29; CE: 25%), whereas the houseball (WV:0.38; CE: 50%) achieved this position in the inactive time.

In Figure 5, the relative preferences (worth values) and consensus errors of the four mice of *group 2* for the enrichment items of the categories foraging, structural and housing are shown. Of the *foraging enrichments*, the lattice ball was ranked on the first position in 46 hours (WV:0.41; CE: 12.5%), as well as in the active time (WV:0.35; CE: 0%) by group 2 and the flappuzzle achieved the first rank in the inactive time (WV:0.54; CE: 12.5%). Group 1 ranked the rope from the *structural enrichments* on the first position both in 46 hours (WV:0.28; CE: 50%) and in the inactive time (WV:0.33; CE: 37.5%), whereas the clip with papertube (WV:0.41; CE: 25%) was first ranked in the active time. From the *housing enrichments* the paperhouse was ranked on the first position by group 2 all the analysing periods 46 hours (WV:0.49; CE: 25%), active time (WV:0.57; CE: 12.5%) and inactive time (WV:0.34; CE: 37.5%).

Figure 6 depicts the relative preferences (worth values) and consensus errors of the four mice of group 3 for the enrichment items of the categories foraging structural and housing during the hole testing cycle of 46 hours and during the active and inactive phase of the 46-hour testing cycle. *Group 3* ranked the lattice ball from the *foraging enrichments* on the first position during the 46-hour analysis (WV:0.6; CE: 0%), the active time (WV:0.67; CE: 0%) and the inactive time (WV:0.5; CE: 25%). The highest ranking from the *structural enrichments* achieved the clip with plastic tube in 46-hours (WV:0.47; CE: 12.5%), active (WV:0.33; CE: 12.5%) and inactive time (WV:0.40; CE: 0%) by group 3. From the *housing enrichments* firstly ranked during 46 hours by group 3 was the wooden angle (WV:0.49; CE: 25%), during the active time the wooden angle (WV:0.44; CE: 21.43%) and floor house (WV:0.37; CE: 0%) were ranked first and in the inactive time the mice ranked the paper house (WV:0.28; CE: 50%) first.

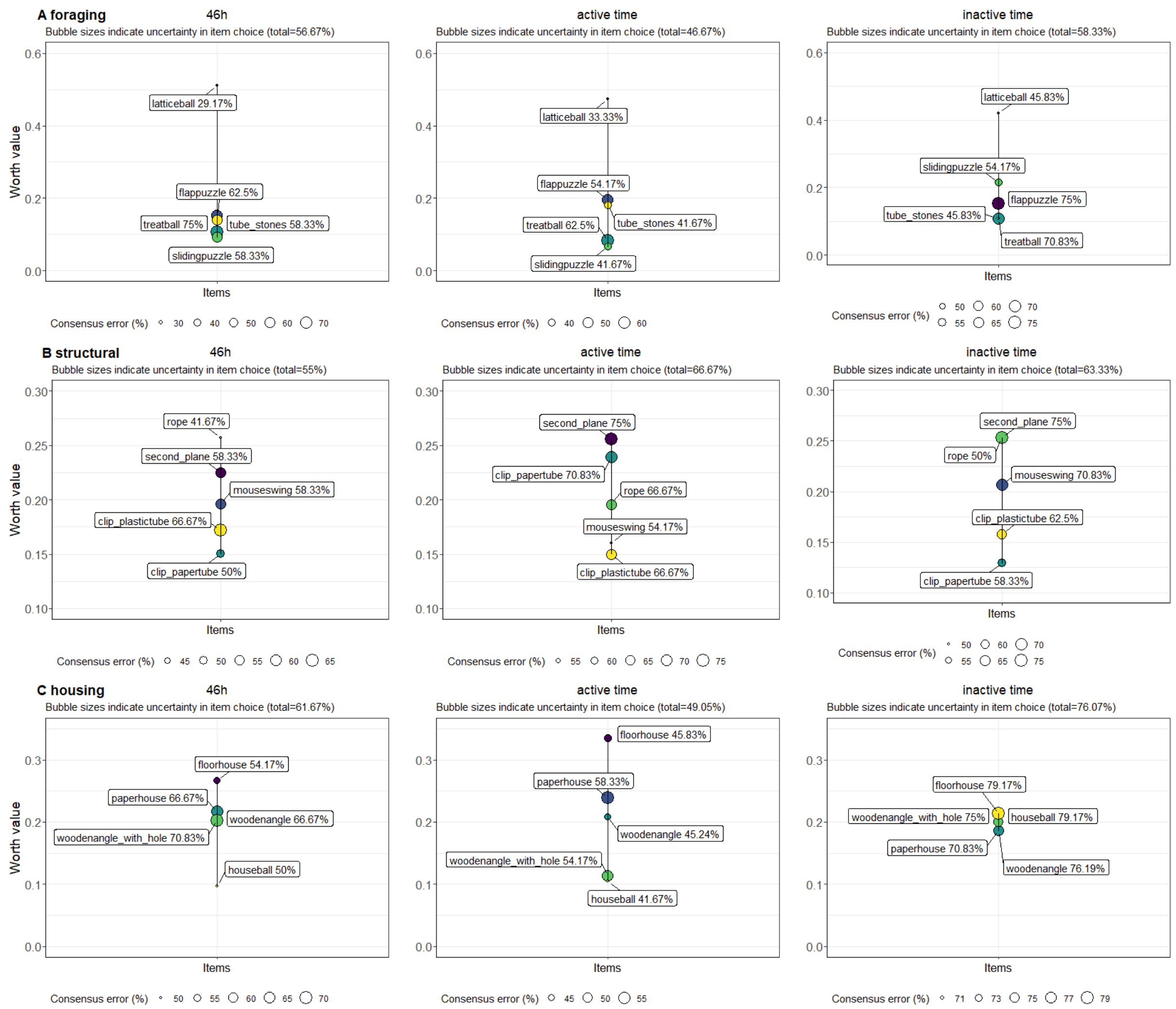


Figure 3. The relative preferences (worth values) and consensus errors of all mice (n=12) for the tested enrichment items from the categories foraging, structural and housing in the single paired comparisons. The 46-hour period depicts the hole testing cycle whereas the active time depicts the dark phase of the testing cycle and the inactive time depicts the light phase of the testing cycle.

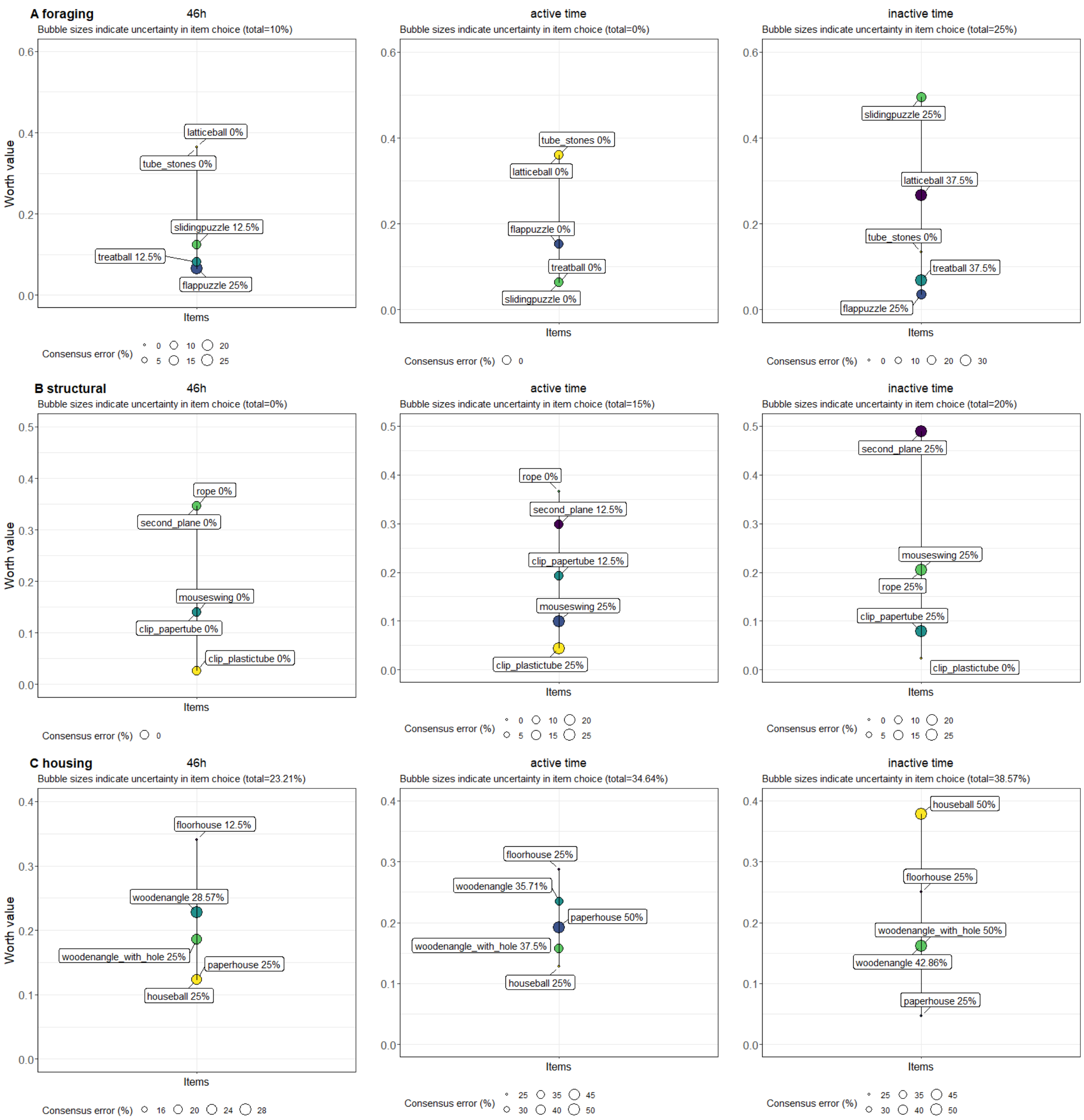


Figure 4. The relative preferences (worth values) and consensus errors of *Group 1* mice (n=4) for the tested enrichment items from the categories foraging, structural and housing in the single paired comparisons. The 46-hour period depicts the hole testing cycle whereas the active time depicts the dark phase of the testing cycle and the inactive time depicts the light phase of the testing cycle.

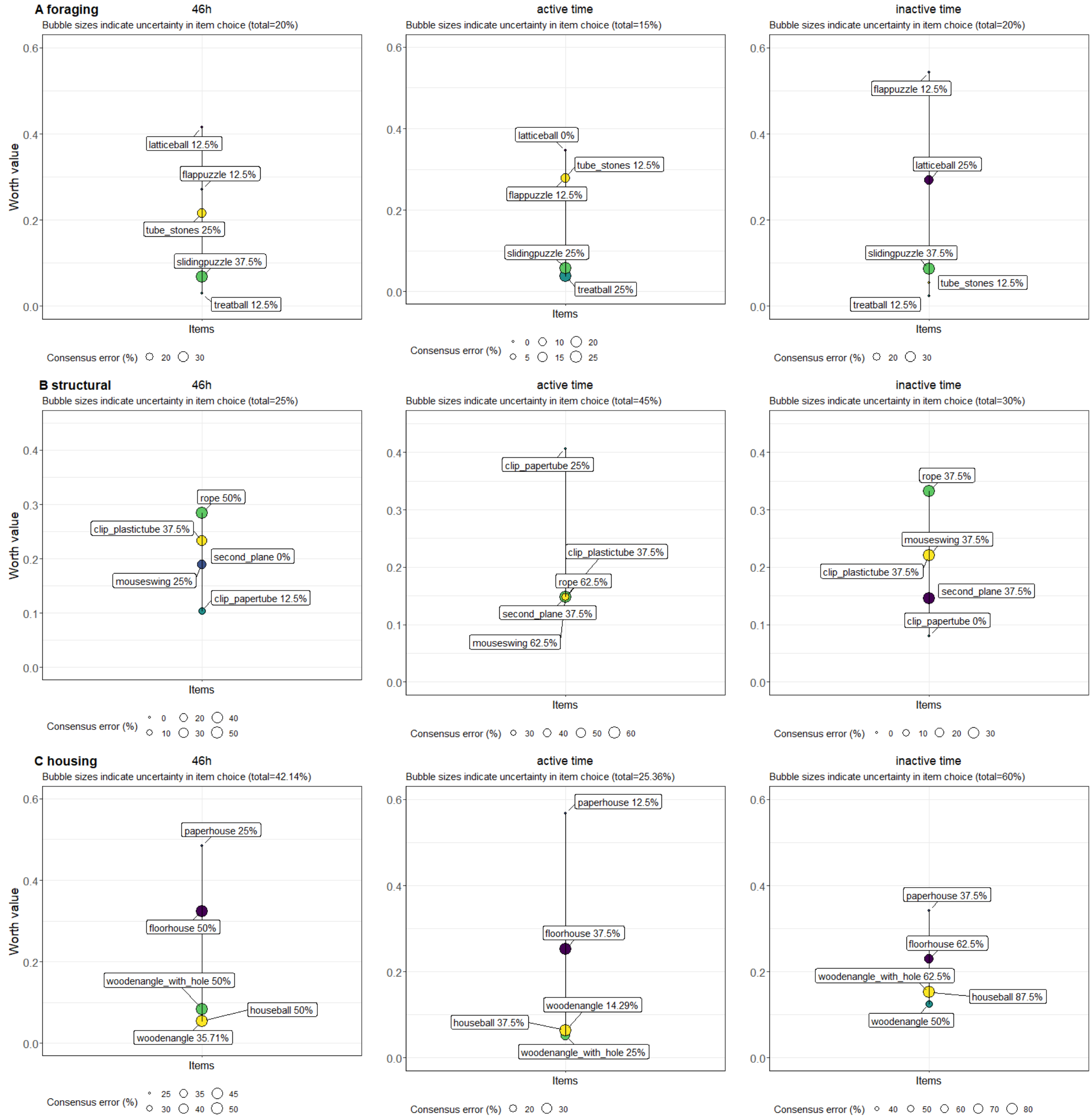


Figure 5. The relative preferences (worth values) and consensus errors of *Group 2* mice (n=4) for the tested enrichment items from the categories foraging, structural and housing in the single paired comparisons. The 46-hour period depicts the hole testing cycle whereas the active time depicts the dark phase of the testing cycle and the inactive time depicts the light phase of the testing cycle.

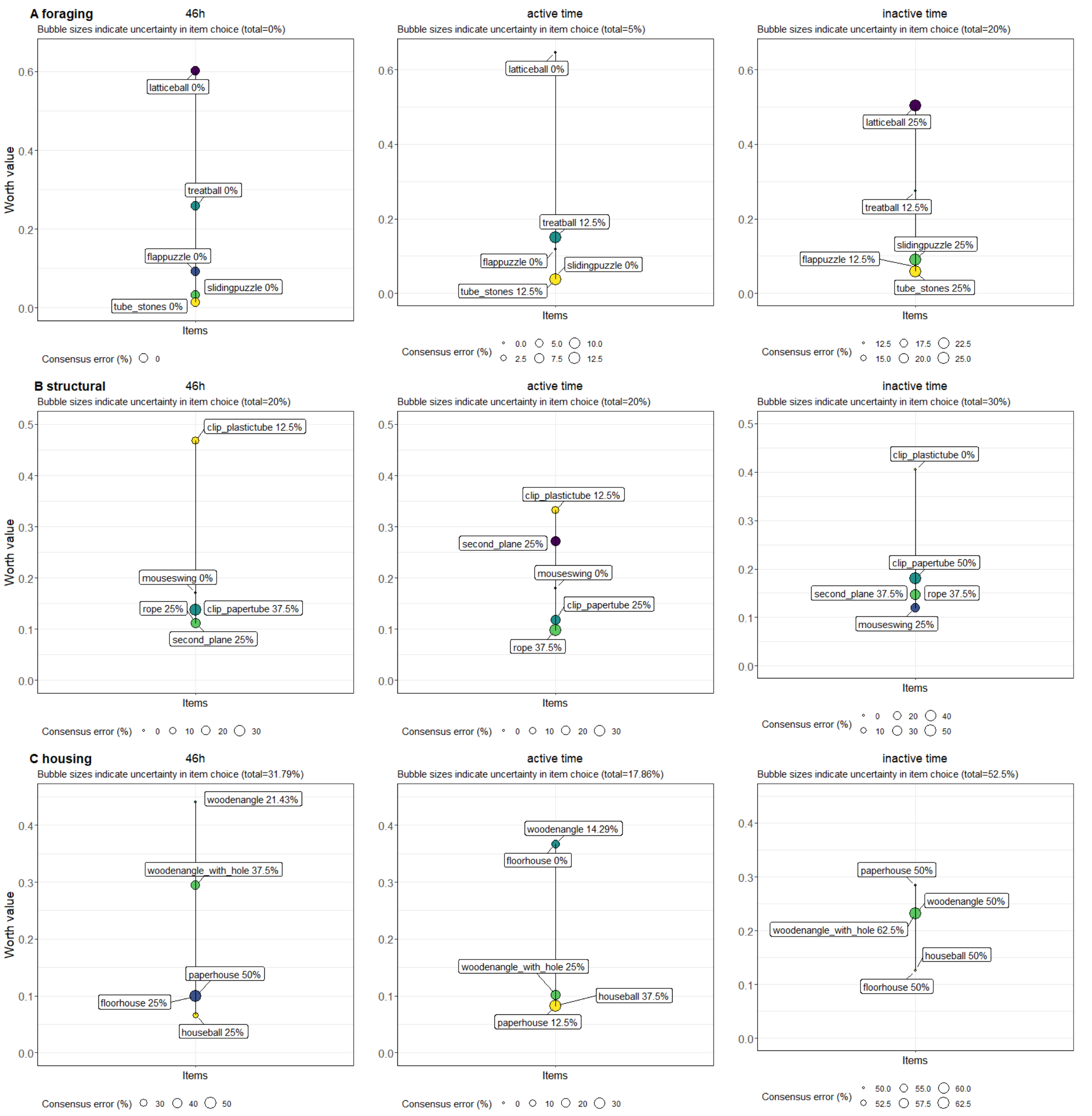


Figure 6. The relative preferences (worth values) and consensus errors of the *Group 3* mice (n=4) for the tested enrichment items from the categories foraging, structural and housing in the single paired comparisons. The 46-hour period depicts the hole testing cycle whereas the active time depicts the dark phase of the testing cycle and the inactive time depicts the light phase of the testing cycle.

Sample Size

Table 3 presents the results of the follow events, the influence events and the proportion of follow events and influence events of the transitions per mouse.

The mean proportion of follow events in the transitions was 1.39% and the proportion of influence events in the transitions was 1.31%. If the follow interval was increased to 3 s, the proportion of follow events was increased to 4,73 %.

Figure 7 depicts the ratio of follow rate and influence rate per mice. Six mice showed similar influence ratio and follow ratio and assembled near the zero line. Whereas the other six mice diverged from the zero line either towards higher influence ratio or higher follow ratio.

Table 3. The results from the follow and influence behavior analysis of 12 mice from the three experimental groups (1,2,3) of the complete data set.

Group	Mouse	Transitions	Follow Events	Follow %	Influence Events	Influence %
1	1	11608	69	0.594	68	0.586
1	2	11132	54	0.485	73	0.656
1	3	10919	77	0.705	58	0.531
1	4	9955	58	0.583	59	0.593
2	1	10387	229	2.205	183	1.762
2	2	10224	199	1.946	193	1.888
2	3	8442	207	2.452	213	2.523
2	4	7557	143	1.892	189	2.501
3	1	7480	93	1.243	81	1.083
3	2	8440	97	1.149	96	1.137
3	3	7428	88	1.185	89	1.198
3	4	6013	64	1.064	76	1.264

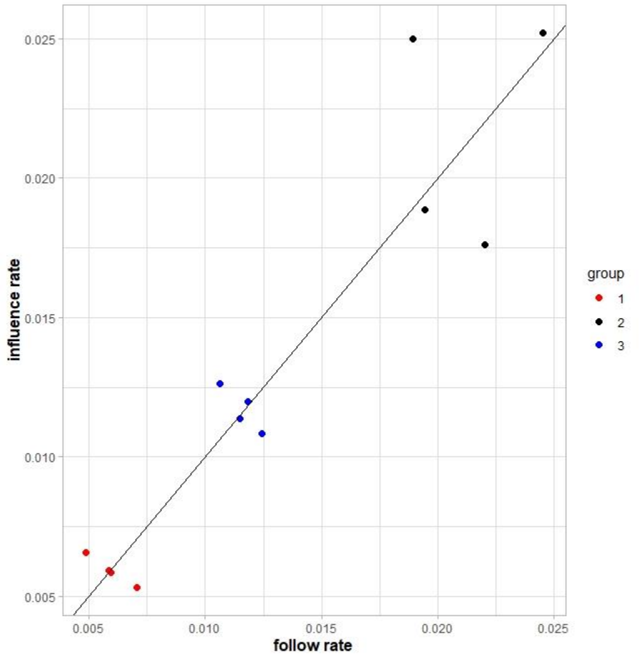


Figure 7. The ratio of the follow rate and influence rate from the follow and influence behavior analysis of 12 mice from the three experimental groups (1,2,3) of the complete data set.

Discussion

In terms of "applied refinement", the aim of this study was the evaluation of enrichment elements from the perspective of group housed female C57BL/6J mice. In a battery of binary comparisons conducted in a homecage-based preference test mice could choose between different items. The combined data was used to rank the items according to their worth value.

All choice tests were performed while the mice were in their respective social group in one out of three cages with four mice each. In order to evaluate whether and if individual behavior was determined by other members of the group, we conducted a follow and influence behavior analysis. Data revealed that the three cages indeed did not come to the same conclusion. However, we also demonstrated that there was no considerable attraction to individual mice that could explain the preference as a trend triggered by individual influencer mice. Overall, a mean follow rate of 1,39 % is reflecting a negligible impact on individual choices. Even if a more conservative follow interval were applied, more than 95% of all cage changes were not directly related to an influencer.

Since mice as mostly socially living animals are rarely housed individually under experimental conditions and in accordance to underlying legislation, testing mice individually for their preference for enrichment items is hardly transferable to real laboratory conditions. Thus, we decided against testing individual animals and used the option of the home cage-based choice experiment to study the mice as socially living animals within the group [34].

To investigate whether the mice agreed in their choice of preference we generated a consensus error to display the amount of agreement. Low scores indicated a high agreement. Analysing the different rates of the consensus error, it can be concluded that mice within a group in average reached lower consensus errors displaying a higher agreement in choice. This can be explained partially by dominance and following behavior. Evaluation at the level of all mice of the three groups resulted in an average higher consensus error and thus a lower agreement in choice indicating the different perceptions of which enrichment was preferred within a group of mice.

The foraging enrichments were ranked by a relatively low consensus error on group level and analysing all mice together, this can be explained by the high visual and functional difference of these elements. Here the lattice ball was fixed at the cage top and serves better for climbing and gnawing, the tube filled with stones fulfilled the luxury behavior of burrowing, the treatball as well as the flappuzzle and sliding puzzle allowed foraging behavior, climbing and gnawing. The lattice ball, filled with paper stripes and millet reward, was fixed with a metal chain to the cage lid and achieved a high worth value on group level in all three testing groups with a low consensus error indicating a high agreement in choice. This high worth value was then reflected in the first position in the analysis of all mice decisions. This could be due to the fact, that after pulling paper out of the ball and eating the millet, the mice were still able to interact with the ball as an moving object to gnaw at or to climb on. Whereas the other items from the same category were also used for climbing and gnawing, displaying a more rigid less interesting character. A previous study investigating the use of the lattice ball in video recordings showed that this design element was used less than other foraging elements of the same category (Hobbiesiefken et al., subm.). Here, however, only a snapshot of 30 minutes and in the presence of other enrichment elements from other categories was recorded, so the analysis over two circadian cycles in a binary choice test might be more conclusive.

Treatball, sliding puzzle, flap puzzle and the tubes filled with stones led to inconclusive worth values and thus low ranking positions in the evaluation at individual group and overall level. Here, the high consensus error, both at group and overall level, symbolises a disagreement regarding the preference for a specific design element. Again, since the lattice ball is attached to the cage lid compared to the other elements, this may have made it easier for the mice to distinguish it from the other elements found on the floor.

With regard to the structural elements, low worth values with partly different ranking positions of the same element between the groups tended to be found at the overall level. This allows conclusions to be drawn about a strong individual decision, which is supported by comparatively high consensus errors. Again, a strong similarity of the elements, which are all attached to the cage lid and used for climbing and gnawing, may be the decisive factor. This hypothesis can be supported by the high worth value of the second plane, which can be inserted into the cage and thus serves as a climbing element as well as for gnawing and as a refuge place, therefore offers other possibilities for interaction than pure climbing elements. The second plane was highly ranked during both the active and inactive time of the mice. This is supported by a high rate of use, which we already found in a comparative behavioral analysis (Hobbiesiefken et al., subm.). Also highly ranked was the rope, which reached especially high worth values by group one and group two.

The evaluation of the housing enrichments generally showed low, closely spaced worth values, with the greatest disagreement among groups 1 and 3, and elements partially achieving a reverse ranking. An example of this is the comparison of the house ball, which achieved the last ranking place in group 3 and the first ranking place in group 1 during the inactive time of the mice. Again, the strong similarity of the design elements in terms of usefulness as a climbing and refuge site may have led to indecision about the worth value among the mice. Although a high worth value was expected from the paper house as Van Loo et al. [23] found a comparable paper housing to be preferred over a triangular plastic house, this could not be confirmed in our preference test. From the video observation we knew that this item was as frequently used during the mice's active phase (Hobbiesiefken et al., subm.) and after each removal during cage cleaning the gnawed corners were obvious. Apparently, this item is better suited for active engagement than for resting. Therefore, asking the mice for the preference of provided items may lead for some items to a different classification than expected by humans.

Overall, the high consensus error, especially for the housing and structural enrichment, shows the individual importance of the different enrichment elements for the individual mouse. To be able to offer each mouse an interesting enrichment item even within the group composition, it is therefore important to provide variety through regular exchange.

Furthermore, to increase the positive effects on wellbeing in mice by enriching the environment with various items, it is necessary not just to apply preference tests, but also evaluate in which kind of way, the enrichment items are utilized by the mice [17]. Evaluation of the type and amount of interaction via behavioral analysis is an important component to create more species-appropriate housing conditions for mice (Hobbiesiefken et al., subm.). Although we cannot determine a statement on motivational strength[19,35] in this preference test, it could be shown that a ranking of the different design elements by the mice is possible using our experimental set-up. The determination of the motivational strength can be achieved through consumer demand tests and represents the price an individual is willing to pay for access to certain enrichment elements [16,35–37].

It should be the ambition of every experimenter to improve the well-being of laboratory animals and thus also to refine the quality of animal experiments. Legal husbandry regulations[38] should be considered a minimum requirement, rather than an aspiration. The impoverished housing of laboratory animals prevents the animals to live out their natural behaviors[39] and may lead to behavioral restriction[40] and behavioral disorders[41]. Chronically under-stimulated animals are very likely to suffer from an emotion that humans perceive as boredom. Severe chronic forms of boredom share symptoms of learned helplessness and depression and should therefore be treated as an important welfare concern[42]. In laboratory animals, the potential consequences of boredom are not well understood. The physical and cognitive stimulation provided by enriched housing could be a preventative for the development of boredom[43].

Thus, there is little to be said against offering a varied environment to enhance animal welfare and thereby increase refinement [27] of animal experimentation.

Conclusion

In our study, preferences for different enrichment items in female C57BL/6J mice are revealed using a homecage-based preference test system. This easy applicable method in obtaining information about the worth value of different enrichment items for mice are facilitating the decision about application of enrichments in laboratory husbandry and shows that mice notably distinguish between different enrichment items. The mice were “asked” for their opinion and thus were actively implemented in the decision-making process. Rising the complexity of laboratory mice housing towards a stimulating living environment enables them to perform a more species-specific behavioral repertoire and therefore resulting in more reliable animal models in biomedical research.

Supplementary Material

Supplementary Material such as 3D printing templates and raw data tables can be found under: https://github.com/DasDritteR/MoPSS-preference-test-supplements

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Authors contribution

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Conception and design of study: U.H., K.D., L.L.; acquisition of data: U.H.; analysis and/or interpretation of data: U.H., B.U., A.H.

Drafting the manuscript: U.H.; revising the manuscript critically for important intellectual content: K.D., L.L.

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