

Protocol D: Mouse preference test

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About the protocol

This example protocol is a step-by-step guide on how you might prepare for and carry out basic behavioural observations to evaluate enrichment and answer the question:

- Is one type of enrichment preferred over another?

This example protocol will compare two different types of shelter for mice, but it can also be easily adapted to investigate other types of enrichment. For example, whether an animal prefers an enhanced enriched environment over a standard home cage setting, that has a lower level of enrichment. An adaptation of this protocol could also be used to examine preference for non-physical enrichment, such as different light levels.



Image credit: University College London.

Within the protocol ^{*Rand} indicates an opportunity to incorporate randomisation into your study, and ^{*Flex} indicates a part of the protocol that is flexible depending on your circumstances (e.g. how many animals/cages are available to study). For advice on randomisation and blinding see [improving the scientific quality of your study](#).

Advice is given on summarising and visualising the data using MS Excel. If you would like to carry out statistical tests on your data, we recommend consulting someone with statistical expertise before you begin data collection.

This document forms part of the 'Evaluating environmental enrichment' resource, developed by the NC3Rs, IAT and RSPCA to support animal technicians to undertake robust evaluations of enrichment items and assess their impact on animal welfare. For more information, visit www.nc3rs.org.uk/evaluating-environmental-enrichment.

For more information on preference tests see the [‘Preference tests’ page](#) of the ‘Evaluating environmental enrichment’ resource. Published studies that include preference tests can be found on the [‘Example enrichment study protocols’ page](#).

Protocol steps

1. Modify, or join together, cages to create two distinct spaces
2. Keep the distinct spaces as similar as possible
3. Place the cages somewhere that they can be easily observed
4. Place the enrichment into the cages ^{*Rand}
5. Select ^{*Rand} and identify your animals
6. Let the animals settle into the new environment
7. Create a schedule of observations
8. Prepare to collect the data: communication with colleagues
9. Prepare to collect the data: ensure you have everything you need
10. Collect the data, following your schedule
11. Input the data into a spreadsheet



Image credit: University College London.

1. Modify, or join together, cages to create two distinct spaces

Preference tests make assumptions about preference for enrichments based on how much time is spent interacting with, or in the location of, each type of enrichment.

Modify six ^{*Flex} cages so that they are divided into two equally sized areas by an opaque barrier or adjoined to another identical cage using tunnels. See Figure 1.1 for examples of how this might look.

If you are splitting a cage in two, be mindful that the sections should be as environmentally similar as possible. Both sides of the partition should allow the animals to look out of the front of the cage or hide at the back of the cage, so position your partition to allow this.

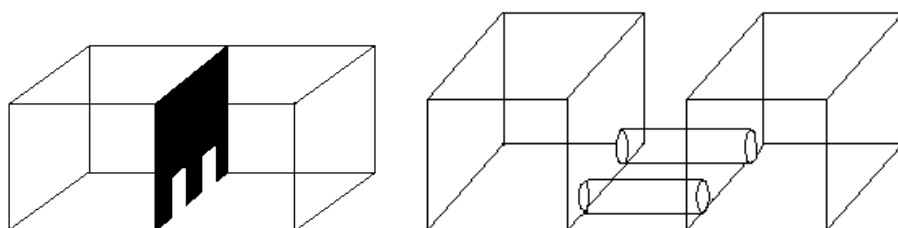


Figure 1.1. Simple examples of how cages may be modified to create two distinct spaces. On the left a partition separates a cage to create two distinct spaces. On the right, two cages are joined using tunnels.

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2. Keep the distinct spaces as similar as possible

Ensure that food, water and nesting material is equally as accessible in each section of the cage (or each cage).

Your investigation focuses on the enrichment, so you do not want behaviour to be influenced by other factors, such as the availability of resources.

3. Place the cages somewhere that they can be easily observed

Be mindful of disturbances, such as being near a door or noisy equipment.

4. Place the enrichment into the cages ^{*Rand}

Each cage has two distinct spaces, and you have two forms of enrichment you wish to trial. We will refer to the two types of enrichment as Enrichment A and Enrichment B.

Each cage will have Enrichment A in one compartment and Enrichment B in the other. We can randomise this order to avoid bias.

For the purpose of this example, we have six cages and want Enrichment A to be on the left an equal number of times as Enrichment B. Therefore, the list will consist of 'left' three times, and 'right' three times.

Input this list into www.randomlist.org to generate a random order. Use the order to assign the position of Enrichment A within each cage. This will decide the position of Enrichment B by default. Create a table, similar to Table 1.1, and keep this with your notes.

Cage	Enrichment A	Enrichment B
1	Right	Left
2	Left	Right
3	Left	Right
4	Right	Left

Table 1.1. Randomly assign when each enrichment item is positioned and keep a record of this to refer to.

5. Select ^{*Rand} your animals

Introduce the mice to the modified cages [using refined handling methods](#). Randomly allocate established, stable groups of mice to the cages ^{*Rand}.

The animals should be individually identifiable by eye (e.g. by using marker pen application).

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Assign each animal a number and select ^{*Rand} a mouse from each cage to be the 'focal individual'; this is the animal you will observe.

6. Let the animals settle into the new environment

Allow the animals to acclimatise to their new environment for a week ^{*Flex}, monitoring them to ensure the enrichment does not have any unexpected harmful effects.

7. Create a schedule of observations

Decide when you plan to record your data each day and for how long for. For example, 8:00, 12:00 and 16:00, Monday-Friday, over two weeks ^{*Flex}. Make a plan that fits your schedule and do not worry if you miss the exact time slot, or if you need to skip an observation. If this occurs, just remember to make a note of it.

8. Prepare to collect the data: communication with colleagues

If you have enlisted the help of your colleagues to record data, ensure that everyone is clear on how to make the observations and record information before you begin. Everyone involved in data collection should have familiarised themselves with the following steps of this protocol and should understand [the importance of keeping good notes](#).

9. Prepare to collect the data: ensure you have everything you need

Position a [data recording sheet](#) and pen in a convenient location that is in sight of the cages but causes as little disruption as possible to the animals being observed.

10. Collect the data, following your schedule

As close as possible to your chosen times, record where (left/right) the focal individuals are for each cage ^{*Rand}. Ensure you know whether this side is Enrichment A or B (refer to the randomisation list created in step 4 if necessary). If the animal is part way between the two areas, this should be counted as being in whichever compartment they are facing. Ensure everyone involved in data collection is clear on this. It may necessary to disturb the animals to find the focal animal; however try to minimise disturbance where possible.

Continue your observations until have the data you set out to collect.

11. Input the data into a spreadsheet

This can be a big job if you leave it to the last minute; try to stay on top of this task and consider enlisting colleagues to implement [blinding](#). Your data will look something like Figure 1.2.

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Cage number	Focal animal number	Date	Time	Observed on side (e.g. left/right)	Enrichment type (e.g. A/B)	Notes
1	2	01/08/22	08:00	R	A	Food added to both sides after observations.
4	1	01/08/22	08:00	R	A	
3	2	01/08/22	08:00	L	A	
2	5	01/08/22	08:00	L	A	
2	5	01/08/22	12:00	L	A	
3	2	01/08/22	12:00	R	B	
1	2	01/08/22	12:00	R	A	
4	1	01/08/22	12:00	L	B	
3	2	01/08/22	16:00	R	B	
2	5	01/08/22	16:00	R	B	
4	1	01/08/22	16:00	R	A	
1	2	01/08/22	16:00	R	A	
3	2	02/08/22	08:00	L	A	Data collected by K.E. today.
1	2	02/08/22	08:00	L	B	
2	5	02/08/22	08:00	R	A	
4	1	02/08/22	08:00	L	B	
1	2	02/08/22	12:00	L	B	
2	5	02/08/22	12:00	R	B	
4	1	02/08/22	12:00	R	A	
3	2	02/08/22	12:00	R	B	
3	2	02/08/22	16:00	L	A	
1	2	02/08/22	16:00	L	B	

Figure 1.2. An example of a completed data table.

Adapting the protocol

- Video record behaviour and use video playback to record the duration that the focal animal spends on either side of the modified cage (e.g. in multiple ten minute periods sampled across the day).
- Switch the enrichment items over halfway through the observation period (e.g. at the end of week one, before the weekend) so that each item has an equal amount of days on the left and right.
- If smart cages with infrared or video technology are available, data on activity level and percentage of time spent in each compartment could be collected automatically.

A limitation of this study is that behaviour is only observed during daylight hours:

- Use video recording equipment to sample over a 24-hr period to gain a more complete picture of preference throughout the day and night.

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Animals can be provided with multiple options when testing preference. However, having more than two choices increases the complexity when it comes to drawing conclusions from the data. Image credit: C Sherwin.

Looking at the data

This guidance will focus on summarising and visualising your data to draw conclusions, rather than statistically testing hypotheses.

If you would like to carry out statistical tests on your data, and you're unsure of how to approach this, it is recommended to consult someone with statistical expertise before you begin data collection. Data interpretation can be off putting for many, but collaboration can help with this. Beginning with data visualisation, and using this to inform your next steps, can also make the task of looking at data less overwhelming and you may even come to find it enjoyable.

Any data presented here has been generated for the purpose of providing guidance and is not real data. The examples have been created using MS Excel 2016. The general principles outlined here will be the same across different versions of Excel, however the steps may differ slightly. There are numerous tutorials for specific versions of Excel available online if you run into difficulty.

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When looking at data it is important to refer back to the question you are interested in. For Protocol D the aim was determine whether one type of enrichment was preferred over another. To approach this question we will take the following steps:

- [Summarising the data using the COUNTIFS function](#)
 - [COUNTIFS: range and criteria](#)
 - [COUNTIFS: typing out the formula](#)
 - [Copying and pasting formula](#)
 - [Sense checking data](#)
- [Plotting a bar chart versus plotting a clustered bar chart](#)
- [Drawing conclusions and addressing limitations](#)

Summarising the data using the COUNTIFS function

Input data into spreadsheet so that it looks something like Figure 1.3.

Create a simple table to summarise your data into (Figure 1.4).

To complete the table you will be using the COUNTIFS function. Note that this function is slightly different to COUNTIF (without the S). When using COUNTIFS you select data from multiple columns, whereas only one column of data is selected when using COUNTIF.

	A	B	C	D	E	F
1	Week	Day	Time	Cage	Enrichment	Side
2		1 Mon	08:00	1 A		R
3		1 Mon	08:00	2 A		L
4		1 Mon	08:00	3 A		L
5		1 Mon	08:00	4 A		L
6		1 Mon	12:00	1 A		R
7		1 Mon	12:00	2 B		R
8		1 Mon	12:00	3 B		R
9		1 Mon	12:00	4 A		L
10		1 Mon	16:00	1 A		R
11		1 Mon	16:00	2 B		R
12		1 Mon	16:00	3 A		L
13		1 Mon	16:00	4 B		R
14		1 Tue	08:00	1 A		R
15		1 Tue	08:00	2 A		L
16		1 Tue	08:00	3 B		R
17		1 Tue	08:00	4 B		R
18		1 Tue	12:00	1 A		R
19		1 Tue	12:00	2 A		L
20		1 Tue	12:00	3 B		R
21		1 Tue	12:00	4 A		L
22		1 Tue	16:00	1 A		R
23		1 Tue	16:00	2 B		R
24		1 Tue	16:00	3 B		R
25		1 Tue	16:00	4 B		R
26		1 Wed	08:00	1 A		R
27		1 Wed	08:00	2 B		R
28		1 Wed	08:00	3 A		L
29		1 Wed	08:00	4 B		R

Figure 1.3. When entering the data that you have collected into a spreadsheet it will look something like this.

SUM ✕ ✓ fx =COUNTIFS(D2:D121, "4", E2:E121, "B")												
	A	B	C	D	E	F	G	H	I	J	K	L
1	Week	Day	Time	Cage	Enrichment	Side						
2		1 Mon	08:00	1 A		R	Cage	A	B			
3		1 Mon	08:00	2 A		L	1	24	6			
4		1 Mon	08:00	3 A		L	2	14	16			
5		1 Mon	08:00	4 A		L	3	16	14			
6		1 Mon	12:00	1 A		R	4	15	=COUNTIFS(D2:D121, "4", E2:E121, "B")			
7		1 Mon	12:00	2 B		R						
8		1 Mon	12:00	3 B		R		A	B			
9		1 Mon	12:00	4 B		R	TOTAL	69	51			
10		1 Mon	16:00	1 A		R						
11		1 Mon	16:00	2 B		R						
12		1 Mon	16:00	3 A		L						
13		1 Mon	16:00	4 B		R						
14		1 Tue	08:00	1 A		R						

Figure 1.4. Use the COUNTIFS function to summarise the data into a simple table.

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The COUNTIFS function will allow you to count data based on information from multiple columns (Figure 1.3). For the purpose of this investigation we want to know on how many occasions the focal mice were observed in the compartments of Enrichment A and Enrichment B.

We could just use the COUNTIF (without an S) function to give us the total of all the As and Bs in the Enrichment column. However, for reasons that will become clear further on in this example investigation, it will also be useful for us to look at this data for each individual cage. Therefore, we want to generate counts based on information from both the 'Cage' and 'Enrichment' columns (Figure 1.4).

COUNTIFS: range and criteria

The COUNTIFS function requires that you specify a 'range' and 'criteria', followed by at least one other set of 'range' and 'criteria'.

The range indicates which cells you want Excel to use in order to generate the counts you require. For our example, our 'ranges' are the cell numbers of the Cage column (Column D) and the Enrichment column (Column E) (Figure 1.4).

Both columns start at cell 2 and go on until cell 121 (there are 120 data points, plus an additional row for the column headers)

Therefore, the range of Column D is D2:D121. The range of Column E is E2:E121.

The criteria is what information within the cells you want Excel to count. This can be a number, a word, or symbols, but it must be contained within quotation marks (" "). For the purpose of our example, we want Excel to count how many As and Bs are within the Enrichment column (Column E) (Figure 1.4).

Therefore, our criteria are "A" and "B".

COUNTIFS: typing the formula

1. Type =COUNTIFS in the cell you want to fill with the data.
2. Highlight all the data in the Cage column (Column D) to generate the first range. Note that if you hold Ctrl+Shift and select the downwards arrow on your keypad the whole column of data will be automatically selected.
3. Now that you have the first range, insert a comma followed by your first criteria (e.g. "1" for cage number 1). Your criteria must match exactly what is written in the cell and must be contained within quotation marks. You now have the first part of your formula.
4. Insert a comma before moving onto the next part of the formula.
5. Highlight all the data in the Enrichment column (Column E) to generate the second range.
6. Insert a comma, followed by you second criteria (e.g. "A" for Enrichment A).

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Copying and pasting the formula

It is not necessary to keep typing the formula out from scratch, as you can use copy and paste and edit the criteria (in our example, the ranges will remain the same).

The formula has to be copied and pasted using the 'Excel formula bar', which is the long, white rectangular space where the formulae are displayed (Figure 1.4). The formula bar can be dragged down to make it larger, so on your version on Excel the formula bar may look thinner than in Figure 1.5.

	A	B	C	D	E	F	G	H	I	J
1	Week	Day	Time	Cage	Enrichment	Side				
2		1 Mon	08:00	1 A		R	Cage	A	B	
3		1 Mon	08:00	2 A		L	1	"A")		
4		1 Mon	08:00	3 A		L	2			
5		1 Mon	08:00	4 A		L	3			
6		1 Mon	12:00	1 A		R	4			
7		1 Mon	12:00	2 B		R				
8		1 Mon	12:00	3 B		R				
9		1 Mon	12:00	4 B		R				
10		1 Mon	16:00	1 A		R				
11		1 Mon	16:00	2 B		R				
12		1 Mon	16:00	3 A		L				
13		1 Mon	16:00	4 B		R				
14		1 Tue	08:00	1 A		R				

Figure 1.5. The COUNTIFS formula is highlighted in grey within the formula bar.

1. After typing out the first formula, highlight it within the formula bar as shown in the image.
2. Press Ctrl+C (or right click and select Copy).
3. Press the Escape key (Esc).
4. Select the empty cell that you would like to paste the formula into.
5. Click in the formula bar again. The empty cell will remain highlighted.
6. Press Ctrl+V (or right click and select Paste) to paste the formula into the formula bar.
7. You can now edit the criteria directly in the formula bar.
8. Press the Return key (Enter) to move into the cell directly below or use the cursor to navigate to a new cell.
9. Press Ctrl+V (or right click and select Paste) to paste the formula into the formula bar.
10. Edit the criteria directly in the formula bar (Figure 1.6).
11. Repeat the process (steps 8 – 10) to complete your summary table (Figure 1.7).

If anything unexpected happens during the process, press the Escape key (Esc) and, if necessary, use Ctrl+Z to Undo any unwanted changes to your spreadsheet.

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Note that if you drag the formula rather than copy and pasting, it will change the range, which we do not want to do in this example.

The screenshot shows the Excel formula bar with the formula `=COUNTIFS(D2:D121, "2", E2:E121, "A")`. Below the formula bar, a pivot table is displayed with the following data:

	Cage	A	B
1	1	24	
2	2	21, "2",	
3	3		
4	4		

Figure 1.6. Once you have pasted the formula you can edit the criteria in the formula bar.

The screenshot shows the Excel formula bar with the formula `=COUNTIFS(D2:D121, "4", E2:E121, "B")`. Below the formula bar, a pivot table is displayed with the following data:

	Cage	A	B
1	1	24	6
2	2	14	16
3	3	16	14
4	4	15	=COUNTIFS(D2:D121, "4", E2:E121, "B")

Figure 1.7. The completed table.

Sense checking data

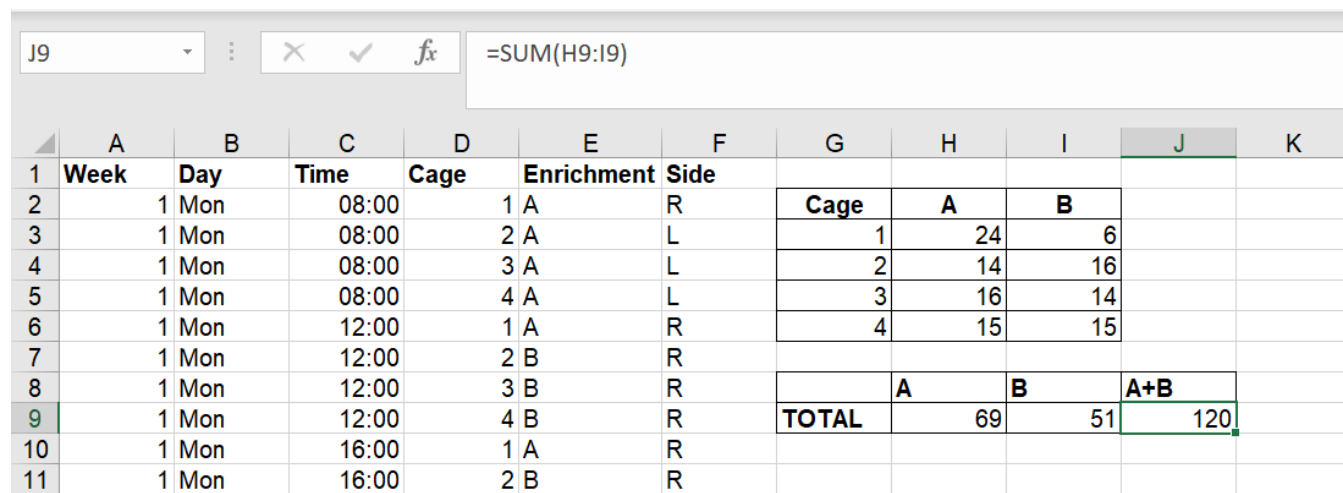
Even the most experienced researcher can make mistakes when handling data, especially when datasets are large. It is sensible to make checks to avoid mistakes; these are often referred to a 'sense checks'. Methods for sense checking data include:

Use Data Validation tools to highlight unexpected values (we will not cover this here, but Excel has this functionality).

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Picking a random sample from the data to check it closely (e.g. checking that the information on the spreadsheet matches the original data collection sheet).

Checking that totals in your summary table are what you would expect (e.g. in this example we have 120 data points, does the table we created in the previous step add up to 120? See Figure 1.8 for how to use the =SUM function to sense check data.)



	A	B	C	D	E	F	G	H	I	J	K
1	Week	Day	Time	Cage	Enrichment	Side					
2		1 Mon	08:00	1 A	A	R	Cage	A	B		
3		1 Mon	08:00	2 A	A	L	1	24	6		
4		1 Mon	08:00	3 A	A	L	2	14	16		
5		1 Mon	08:00	4 A	A	L	3	16	14		
6		1 Mon	12:00	1 A	A	R	4	15	15		
7		1 Mon	12:00	2 B	B	R					
8		1 Mon	12:00	3 B	B	R		A	B	A+B	
9		1 Mon	12:00	4 B	B	R	TOTAL	69	51	120	
10		1 Mon	16:00	1 A	A	R					
11		1 Mon	16:00	2 B	B	R					

Figure 1.8. Using the =SUM function to sense check the summary table.

Plotting a bar chart versus plotting a clustered bar chart

Now we have the data summarised, the next step is to plot it. At this point, we will remind ourselves our question: do the animals prefer Enrichment A or B?

To draw conclusions from our data we will plot a bar chart. For the purpose of this example, we will start by plotting a normal bar chart, comparing the total number of occasions the focal mice were observed on the side of Enrichment A against the side of Enrichment B.

The basic steps for creating a chart in Excel are:

- Arrange the data you want to visualise into a table.
- Highlight the table using the cursor.
- Select the 'Insert' menu.
- Choose the chart you wish to plot.
- Amend the chart as necessary (e.g. adding axis labels, changing the formatting or changing how the data is presented).

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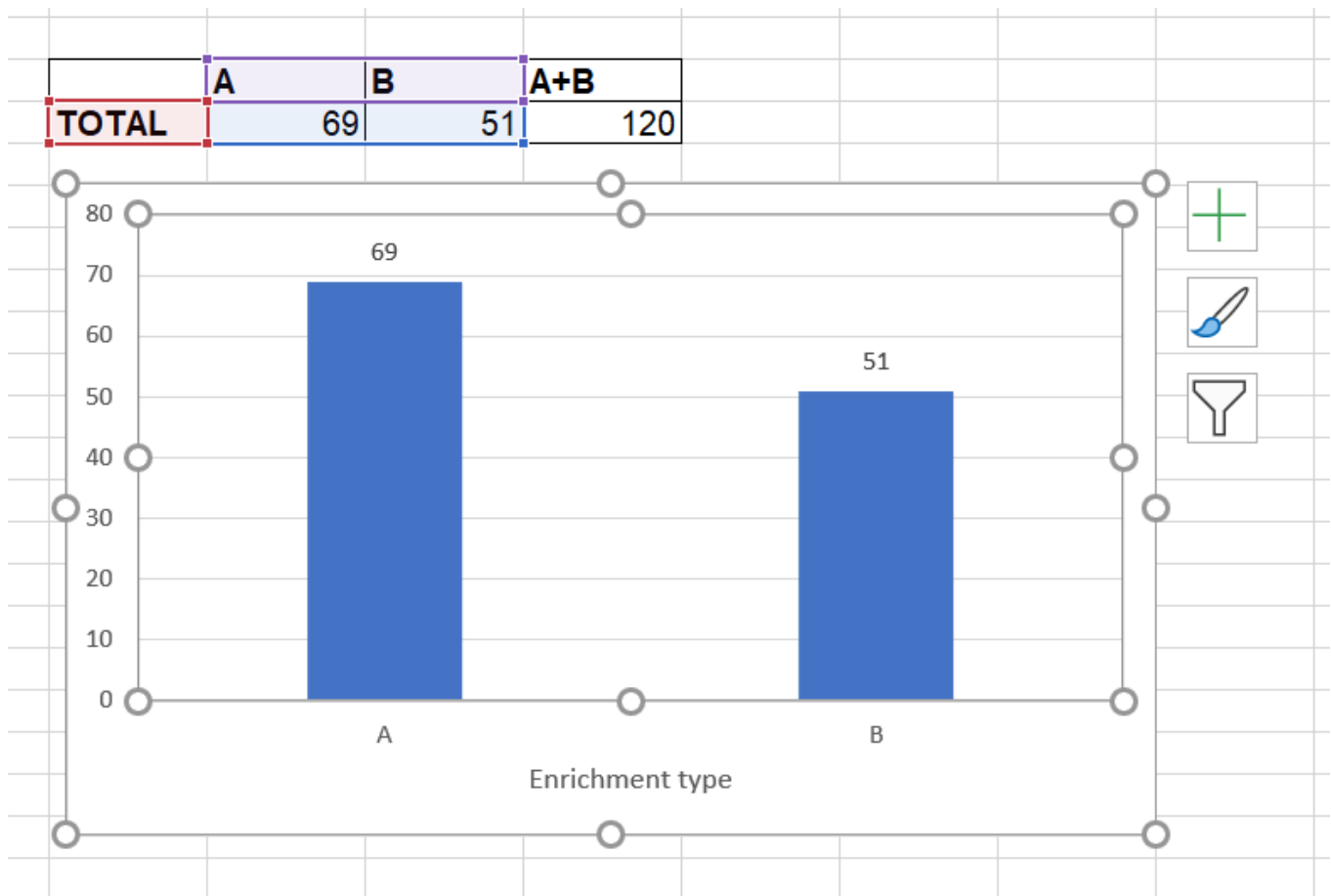


Figure 1.9 . Bar/column chart created using the highlighted data.

Looking at this basic bar chart (Figure 1.9) would lead us to conclude that enrichment A is preferred. It appears that the focal mice were observed on the Enrichment A side of the modified cage most often and therefore the mice must prefer using Enrichment A.

We will investigate this further by plotting a clustered bar chart, which breaks the data down by cage number (Figure 2.1).

When inserting the clustered bar chart from the table shown in the picture below Excel may include an extra bar for cage number (the blue bar, Figure 2.1).

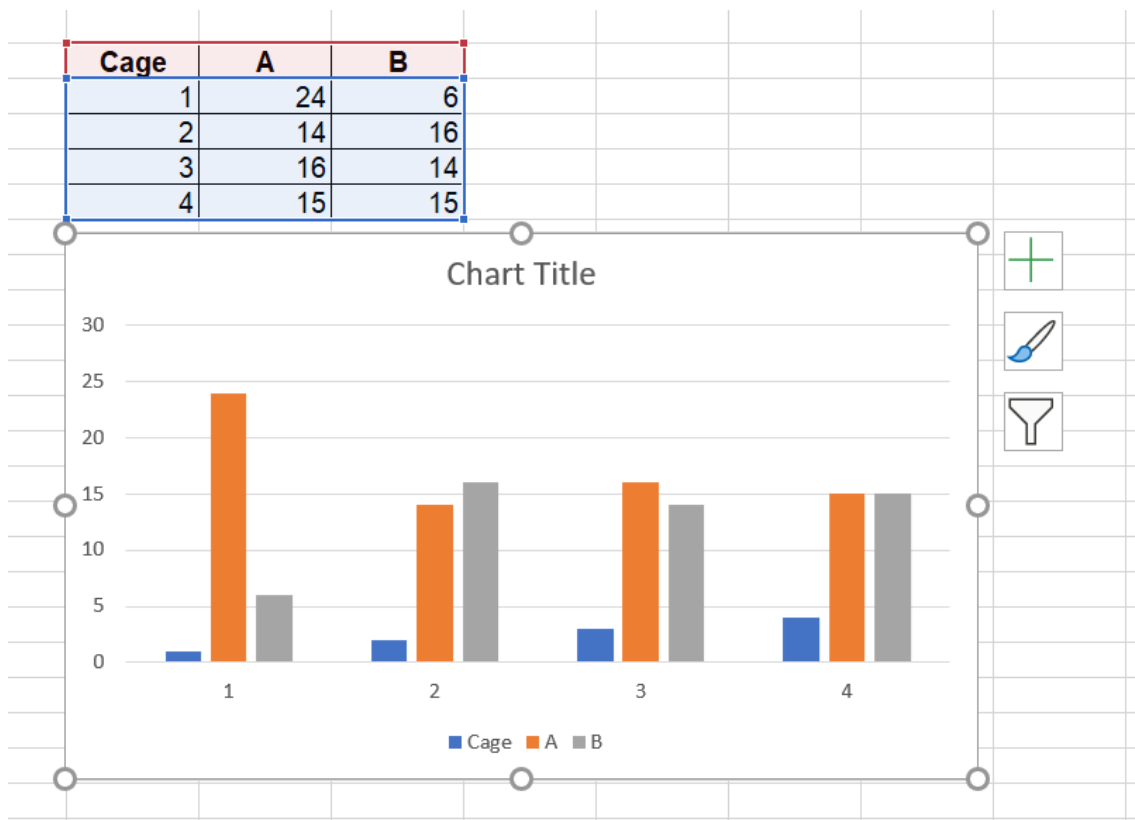


Figure 2.1. The blue bar represents cage number; this is not meaningful and will be removed from the plot.

To remove this extra bar, right click the chart area and select 'Select data'. On the left-hand side you will see 'Cage' (Figure 2.2). Unselect this box (as shown in the image below) to remove it from the plot.

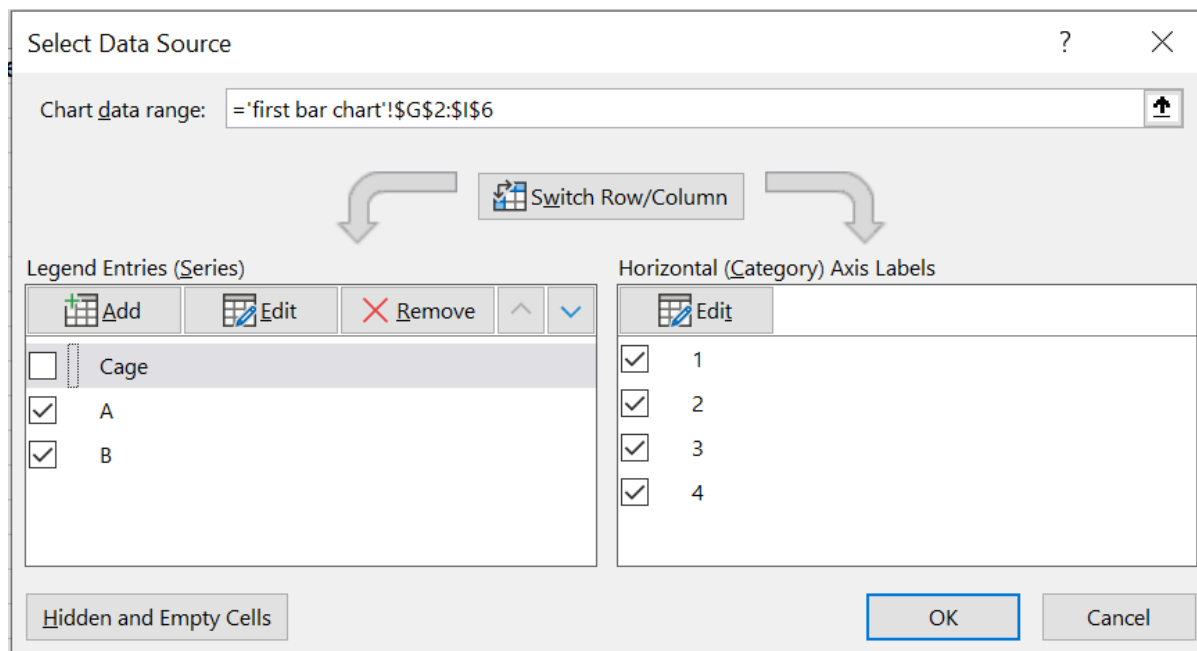


Figure 2.2. Use Select Data Source to remove the unnecessary blue bar from the plot.

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Plotting the data separated by cage number shows a clear preference for Enrichment A for cage 1, but an equal split between the two types of enrichment for the other cages (Figure 2.3). The basic bar chart (Figure 1.9) is not incorrect – it shows us the totals overall, but if we compare the two charts we can see that the clustered bar chart provides us with extra levels of information.

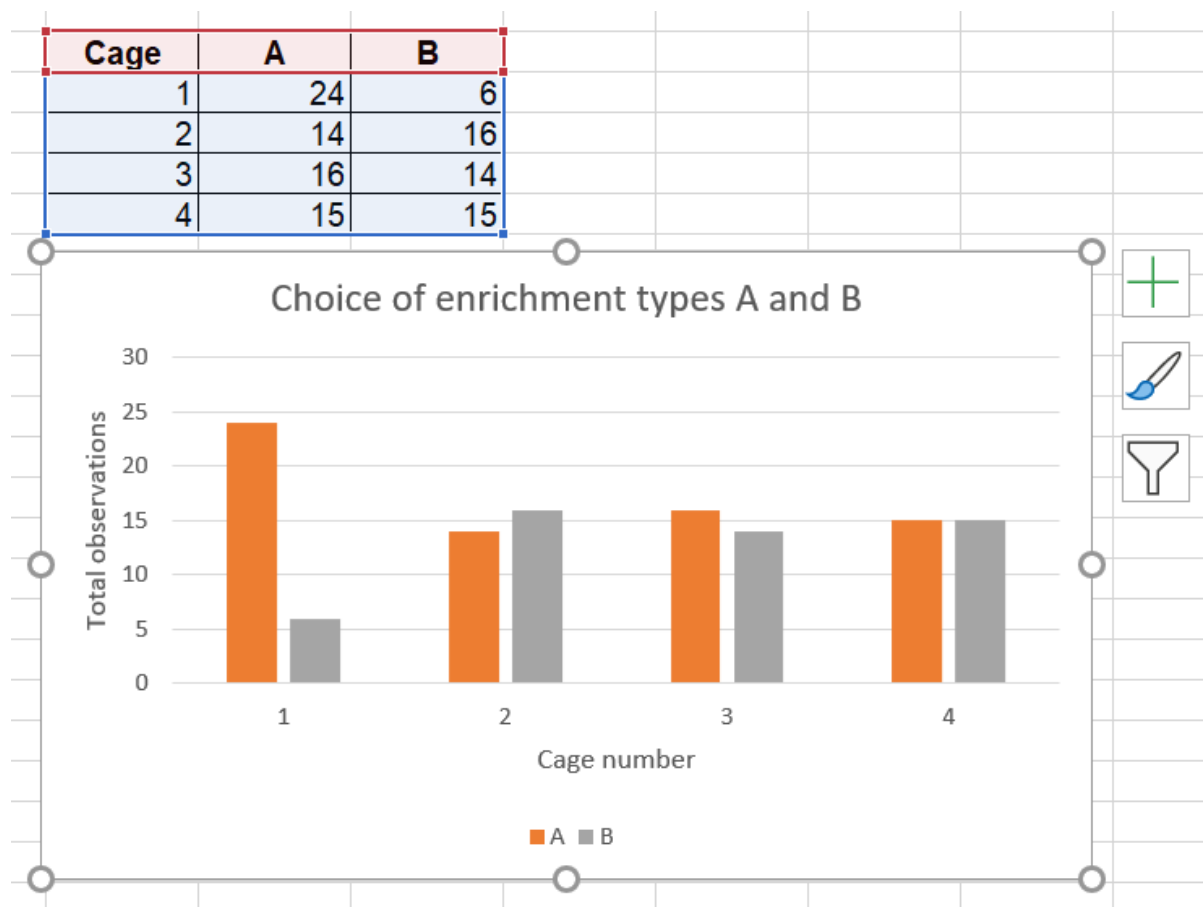


Figure 2.3. A clustered bar chart (or column chart) showing that cage 1 had a strong preference for Enrichment A, this preference is not seen for cages 2-4.

Drawing conclusions and addressing limitations

Could another factor, besides the enrichment, have influenced the behaviour of the mice in cage 1?

Revisiting the experimental set up reminds us that cage 1 is positioned near to the doorway of the room, and Enrichment B was positioned on the side closest to the door. You notice that although the animals use all of the modified cage, when the door opens they move to the side of cage furthest away from the disturbance – nearer to Enrichment A. The animals in the other cages, positioned away from the doorway, do not react in this way.

When the data was collected, the observer usually came through the doorway and recorded data for cage 1 first, as it was closest to them. This approach has introduced bias into the data collection, and unfortunately, we did not notice until the data was already collected. However, now we will know to be aware of this when

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[designing experiments and collecting data in the future](#). Introducing [randomisation](#) into the order that cages were observed would have meant that in some cases the animals in cage 1 would have had more time to settle down after being disturbed by the door. This would have reduced the bias during sampling.

One way to indicate whether there was a preference for one side of the modified cage, rather than one type of enrichment, is to swap the position of the enrichment items halfway through the observation period (e.g. on the Friday, so the animals have the weekend to adjust).

Excluding data should be done with caution, for example you should never exclude data just because it does not fit with your expectations. It is essential to have a well-justified reason. Before excluding data from a study, you should discuss your reasoning with others; and you should always be open about what data have been excluded when presenting your findings.

In this case, there is evidence that the data from cage 1 is unreliable. The three remaining cages all indicate that there is no strong preference for either enrichment type, leading us to (perhaps cautiously) conclude that the mice are equally as satisfied with both.

Other considerations for implementing new enrichment

This information can inform our next steps, with other factors leading the decision-making process. For example, whether to provide a new type of shelter or stick with an old type can be decided solely on factors relating to:

- Cost – is it cheaper to stick with the old shelter type or would the new shelter result in reduced costs in the long run?
- Time – is the new shelter quicker to clean or does it make the animals easier to observe? Would changing the shelter type be a time-consuming process?
- Other perceived benefits – what was the reason you wanted to evaluate this enrichment, were there some other potential benefits that may not have been reflected by this evaluation due to limitations in the study? It is important to remember that this approach has only provided a snapshot of behaviour over a short time frame.

This document forms part of the 'Evaluating environmental enrichment' resource, developed by the NC3Rs, IAT and RSPCA to support animal technicians to undertake robust evaluations of enrichment items and assess their impact on animal welfare. For more information, visit www.nc3rs.org.uk/evaluating-environmental-enrichment.