National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Protocol A: Use of an enrichment item

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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 answer the questions:

- Is an enrichment item used?
- How is an enrichment item used?

The example ethogram and data collection sheet that accompany this protocol are completed for evaluating a mouse mezzanine that also functions as a shelter. However, the protocol itself can easily be adapted to evaluate the use of any enrichment item.

Observations are made at multiple short intervals (< 10 min) across multiple days.

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).

Advice is given on summarising and visualising the data using MS Excel rather than carrying our formal statistical tests. The reliability of

Image credit: University College London.

statistical tests requires data to meet certain criteria (e.g. a sufficient amount of data has been collected). Therefore, 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 behaviour observations see the ['Monitoring behaviour using an ethogram' page](#) of the 'Evaluating environmental enrichment' resource. Published studies that *include behaviour observations can be found on the ['Example enrichment study protocols' page](#)*.

Protocol steps

1. Create an ethogram
2. Create a data collection sheet
3. Select the cages to be observed
4. Select your focal animals
5. Create a schedule: choose your time points
6. Create a schedule: order of observations
7. Introduce the enrichment item to the cages
8. Ensure you have everything you need
9. Carry out your observations: acclimatisation
10. Carry out your observations: record behaviour
11. Stay on top of data input
12. Complete your data collection

1. Create an ethogram

The behaviours of interest will vary depending on your study animals and the type of enrichment you are trialling. You can find [instructions and examples on how to construct an ethogram](#) on the NC3Rs website.

[An example ethogram for this protocol](#) is also available to download as a PDF.

2. Create a data collection sheet

It is useful to have data collection sheets that are specific to your evaluation, as they can help you to consistently record the correct information and follow the same procedures.

[An example data collection sheet for this protocol](#) can be downloaded in editable Word format from the NC3Rs website.

3. Select the cages to be observed

If you can easily observe animals within their usual cages, select***Rand** five***Flex** cages to observe. If you need to move some animals to separate cages for observations, select***Rand** your animals and assign***Rand** them to cages. If relocating animals is necessary, keep stable, established groups and allow them a week to acclimatise. Monitor the animals closely during this time.

4. Select your focal animals

Using the most refined method possible, clearly mark all your animals with numbers, then select***Rand** one from each cage to be the 'focal individual', this is the animal you will observe. If you have five separate cages, you will have five focal animals. Record the focal animal for each cage on your data collection sheet (Figure 1.1). Your focal animals will remain the same throughout the study.

Notes:	15/12/23 (observation day 1) – new mouse mezzanine with shelter. 48 hours after full cage clean; all females - C57BL/6.					
Cage #	Focal animal #	Obs. day	Time	Contact? (Y/N)	Type	Notes
3	1	1	09:00	N	OE	gnawing
4	1	1	09:00	N	U	eating
2	2	1	09:00	Y	EA	eating on top
1	6	1	09:00	Y	EC	
5	3	1	09:00	Y	ES	huddling
5	3	1	11:10	N	OE	
4	1	1	11:10	Y	ER	
2	2	1	11:10	Y	EA	Shredding nestlet inside shelter
1	6	1	11:10	N	OE	gnawing
3	1	1	11:10	Y	EC	

Figure 1.1. Example data collection sheet: mouse mezzanine with shelter. This example data collection sheet is completed for two time points on day 1 of observations. The columns completed in black (cage #, focal animal # and obs. day) can be filled in before the sheets are printed out. You can download a data collection template in editable Word format from the NC3Rs website.

5. Create a schedule: choose your time points

Create a schedule for your observations that is manageable for you***Flex**. Choose days where you will be able to stop and observe the animals at multiple time points (e.g. 08:00, 10:00, 12:00, 14:00 and 16:00). If you can only commit to observing a few times a day, make sure these occasions are spread out (e.g. 09:00, 12:00 and 17:00). Your observation time points will remain the same throughout the study.

6. Create a schedule: order of observations

Allocate a number to each cage***Rand**. You will use the assigned numbers to determine the order in which you will make your observations on each day. Fill out your ethogram sheets with this information ahead of time (**Figure 1.1**). You will observe the cages in a different order on each occasion.

7. Introduce the enrichment item to the cages

If the enrichment item is not already in the cages, then introduce it at least 24 hours before behavioural observations begin.

8. Ensure you have everything you need

Gather all the items you need for recording behaviour and keep them near to where you will make your observations; for example, your ethogram, data collection sheets (complete with cage numbers and focal animal numbers), a pen and information on your schedule. Having everything easily to hand will save you time and keep things running smoothly.

9. Carry out your observations: acclimatisation

Following the schedule that you set out previously, position yourself where you can see the cages while also being aware of your effect on the animals. For example, try not to loom over the cages and, if possible, allow the mice five-ten minutes to acclimatise to your presence. If an acclimatisation period is used, keep it the same throughout your study.

10. Carry out your observations: record behaviour

Note the behaviour of each focal animal on the ethogram sheet*Rand. Record: a) contact with the enrichment (Y/N); and b) type of contact, so you end up with something like Figure 1.1. Spend a few seconds observing an animal before moving onto the next cage and try to keep this period the same for each animal.

To increase observer consistency and reliability, carry out observations in pairs where possible.

11. Stay on top of data input

Enter the data from that day into a spreadsheet so that it looks something like Figure 1.2.

12. Complete your data collection

Following your schedule, repeat your observations until you have the data that you set out to collect.

	A	B	C	D	E
1	Day	Cage	Time	Contact	Type
2	Mon	1	09:00	Y	EC
3	Mon	2	09:00	Y	EA
4	Mon	3	09:00	N	OE
5	Mon	4	09:00	N	U
6	Mon	5	09:00	Y	ES
7	Mon	1	11:00	N	OE
8	Mon	2	11:00	Y	EA
9	Mon	3	11:00	Y	ES
10	Mon	4	11:00	Y	ER
11	Mon	5	11:00	N	OE

Figure 1.2. When the data is entered into a spreadsheet it will look something like this. The letters in the 'Type' column are the codes outlined in the ethogram.

Adapting the protocol

This protocol could be adapted to:

- Substitute in your own animals and different types of enrichment.
- Use video recording equipment to capture behaviour over 24-hours.
- Collect baseline data (i.e. make observations before you introduce the enrichment) to investigate if the presence of the enrichment item affects the number of incidents of desirable or undesirable behaviours.
- Include a control group with identical conditions, but without the new enrichment item you are evaluating. Use the control group to compare the number of incidents of desirable or undesirable behaviours with and without the enrichment item present.
- Compare two different types of similar enrichment within a cage or between cages (e.g. two types of shelter or two flavours of gnawing block – do the animals seem to use one variety more than the other?)
- Include supplementary information that is already recorded, or can be easily recorded, during daily checks (e.g. food and water intake, body weight, body condition, nest quality or breeding records).

Looking at the data

The guidance below focuses 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.

The 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.

When looking at data it is important to refer back to the question you are interested in – for Protocol A the questions of interest were:

- Is a new mouse mezzanine with a shelter used by the mice?
- How is the mouse mezzanine with a shelter used by the mice?

To approach these questions we will take the following steps:

Q1. Is a new mouse mezzanine with a shelter used by the mice?

- Summarising the data using the COUNTIFS function
    - COUNTIFS: range and criteria
    - COUNTIFS: typing out the formula
    - Copying and pasting the formula
    - Sense checking data
- Creating a chart and drawing conclusions

Q2. How is the mouse mezzanine with a shelter used by the mice?

- Filtering the data
- Summarising the data using the COUNTIF (without the S) function
- Creating a chart and drawing conclusions
- Using your notes for more insight and drawing conclusions
- A limitation of this approach

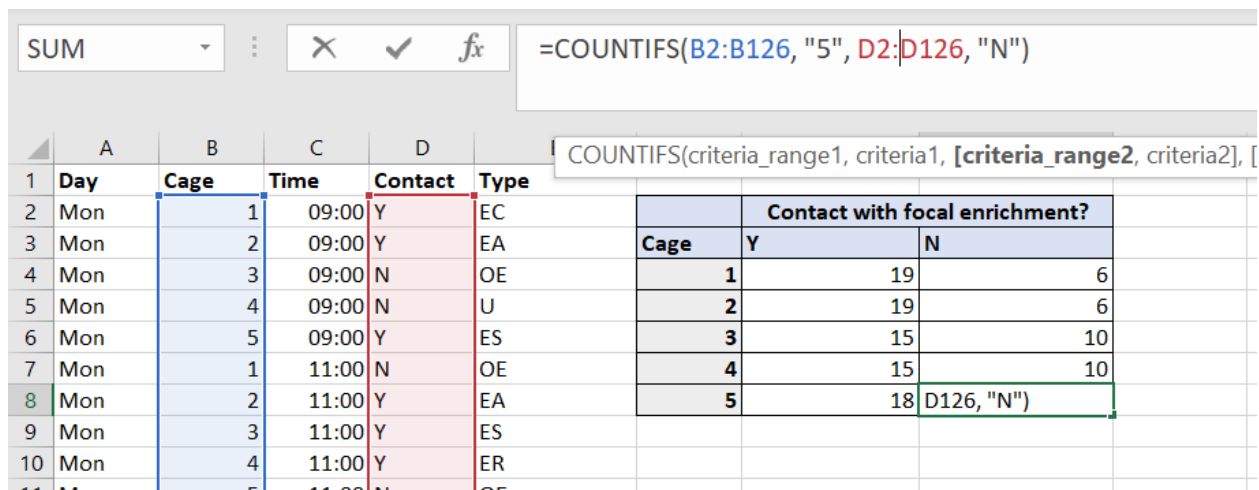**Q1. Is a new mouse mezzanine with a shelter used by the mice?**

Enter the data into a spreadsheet so that it looks something like Figure 1.2. The Type column in this image refers to 'type of contact' and the codes match those on the example ethogram for Protocol A.

Summarising the data using the COUNTIFS function

Create a table to summarise your data into (Figure 1.3).

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 the COUNTIFS you select data from multiple columns, whereas only one column is selected when using COUNTIF.

Figure 1.3. The data is summarised into the blue and grey table using the COUNTIFS function. The formula starts with =COUNTIF, the two ranges are shown in red and blue. The criteria are shown in quotation marks.

COUNTIFS will allow you to count data based on information from multiple columns. For the purpose of this investigation we want to know on how many occasions the focal mice were observed in contact with the focal enrichment (Y) or engaging in activities that did not involve the focal enrichment (N). We will break this down by cage number to check whether there were any obvious differences between the cages.

COUNTIFS: range and criteria

The COUNTIFS function requires a 'range' and 'criteria' followed by another 'range' and 'criteria' (Figure 1.3).

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 B) and the Contact column (column D).

Both columns start at cell 2 and go on until cell 126 (there are 125 data points, plus an extra row for the column headers).

Therefore, the range of Column B is B2:B126. The range of Column D is D2:D126.

The criteria is what information within the cells you want Excel to count. This can be numbers, words, or symbols but it must be contained within quotation marks (" "). For the purpose of our example, we want Excel to count how many Ys and Ns are within the Contact column (column D).
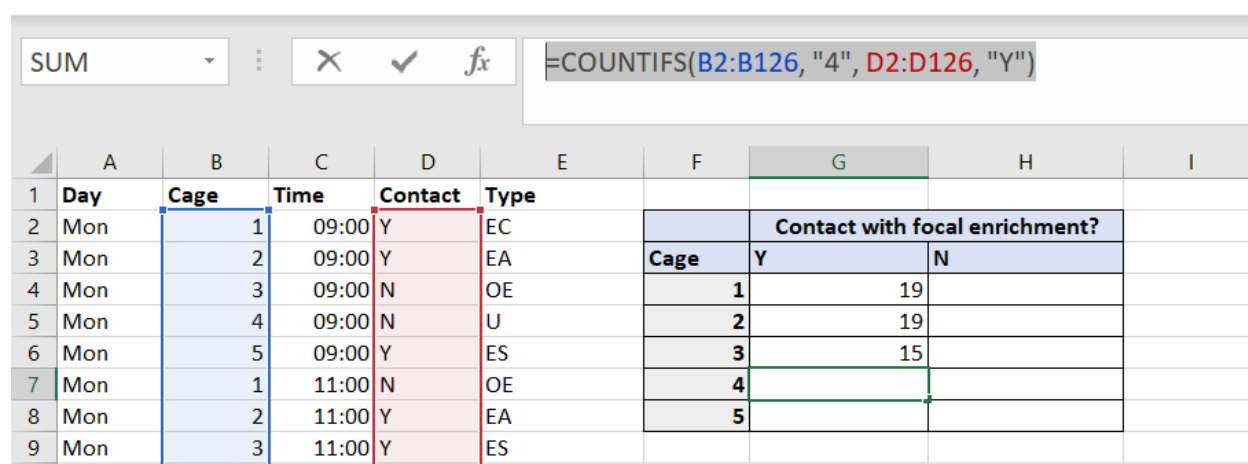
Therefore, our criteria are "Y" and "N".

COUNTIFS: typing out 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 B) 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 Contact column (column D) to generate the second range.
6. Insert a comma, followed by you second criteria (e.g. "Y").

Copying and pasting the formula

Figure 1.1. *The COUNTIF function is highlighted in the formula bar.*

1. After typing out the first formula, highlight it within the formula bar as shown in Figure 1.4.
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.

11. Repeat the process (steps 8 – 10) to complete your summary table.

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.

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.

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:
- Using Data Validation tools to highlight unexpected values (we do not cover this here, but Excel has this functionality).
- 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).
- Looking at the data to check that it is what you would expect: for example, check that totals in your summary table add up to (=SUM) the correct number (in this example we have 125 data points in total and we made 25 observations per cage – do the columns and rows of the table we created using the COUNTIFS function reflect that?)

Some of the values in the table are the same (Figure 1.5). Is this an error made when copying and pasting? In this case, clicking through the formulae shows that the repetition of numbers is just a coincidence, but this is an example of the sort of thing that should be double-checked.

Figure 1.5. *Confirm the data you have summarised are correct by double checking the formula bar for each cell in your summary table, and checking that the data adds up to what you would expect.*

Creating a chart and drawing conclusions

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).
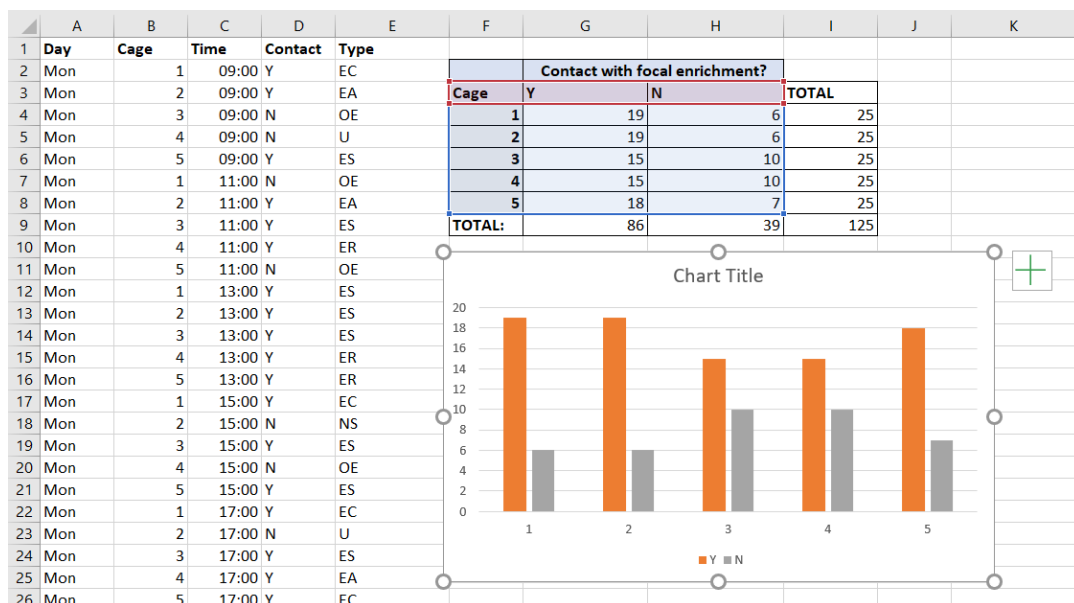

Figure 1.6. A clustered bar chart (or column chart) can be created by highlighting your summary table. The chart can then be edited for clarity.

Right clicking on the chart or selecting the Chart elements menu (the green plus sign to the top right of the chart, Figure 1.6) will allow you to customise your chart.
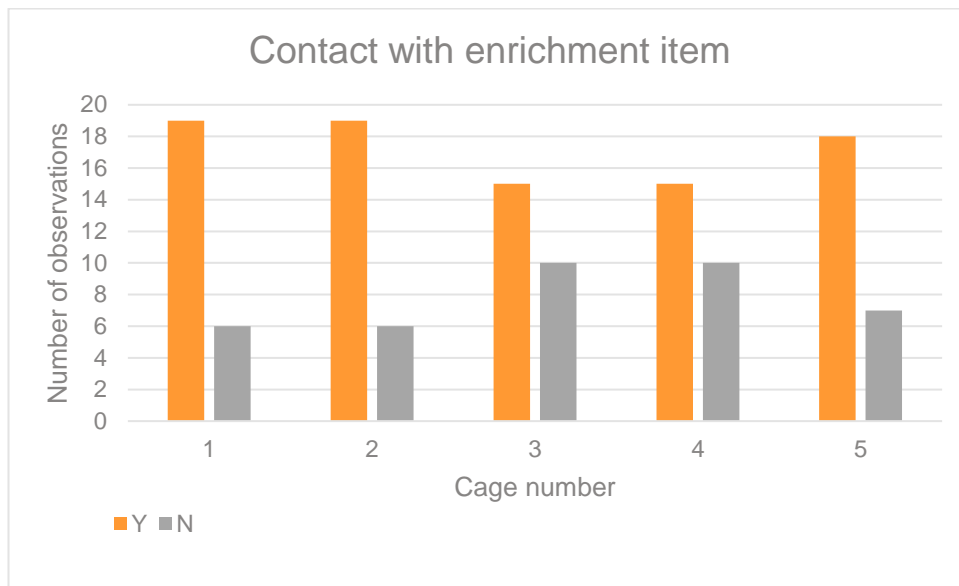
Figure 1.7. *Contact with the enrichment broken down by cage number.*

Looking at Figure 1.7 shows us that the focal animal in every cage was observed using the focal enrichment (Y, orange bars). The focal animals were using the enrichment item in some way more often that they were observed engaging in activities in other parts of the cage (N, grey bars). We can confidently say that the mice used their new mezzanine shelter.
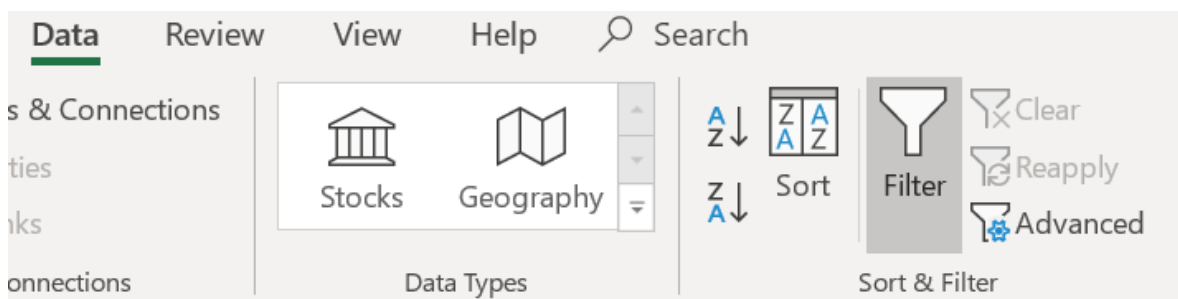
Q2. How is the mouse mezzanine with a shelter used by the mice?

Filtering the data

We have data for both Y (contact with focal enrichment) and N (no contact) within our data set. To answer our question on how the mice use the new enrichment we only need the Y data.

We will use the Filter tool, which is found via the Data menu and located next to the Sort tool (Figure 1.8a), to filter out the Ns from our data. Selecting Filter will create drop down menus for each column.

a)

b)

	A	B	C	D	E
1	**Day** ▾	**Cage** ▾	**Time** ▾	**Contact** ⛀	**Type** ▾
2	Mon	1	09:00	Y	EC
3	Mon	2	09:00	Y	EA
6	Mon	5	09:00	Y	ES
7	Tue	1	09:00	Y	EC

Figures 1.8a and 1.8b. Selecting the filter tool (Figure 1.8a) will allow you to filter the cell contents using the drop down arrows at the header of each column (Figure 1.8b).

Selecting the arrow and unchecking a box will hide that data from the dataset. You can see that the contact column has been filtered by the funnel symbol, the N has been unselected, and now only Y data is visible (Figure 1.8b).

We are going to summarise the Y data by the Type of contact column (column E, Figure 1.8b). Selecting the filter menu will remind us of the codes contained in column E so we are able to create a simple table for our data.

Summarising the data using the COUNTIF (without the S) function

As we have already filtered the data, we will use the COUNTIF function (note that we previously used COUNTIFS with an S). When using COUNTIF only one range and criteria are required.

H11   ⋮ ✕ ✓ *fx*   =COUNTIF(E2:E126, "ES")

	A	B	C	D	E	F	G	H	I
1	**Day** ▾	**Cage** ▾	**Time** ▾	**Contact** ⛀	**Type** ▾				
2	Mon	1	09:00	Y	EC				
3	Mon	2	09:00	Y	EA		**Type of contact**	**Total**	
6	Mon	5	09:00	Y	ES		**EA**	9	
7	Tue	1	09:00	Y	EC		**EC**	28	
8	Tue	2	09:00	Y	EC		**EG**	5	
11	Tue	5	09:00	Y	ES		**ER**	16	
12	Wed	1	09:00	Y	EG		**ES**	29	

Figure 1.9. Summarising the data into a simple summary table using the COUNTIF function.

Input the COUNTIF formula (Figure 1.9), refer to Steps 1 – 3 of 'COUNTIFS: typing the formula' if you are unsure how to do this. Complete the table, using Copy and Paste if this saves you time.

Creating a chart and drawing conclusions

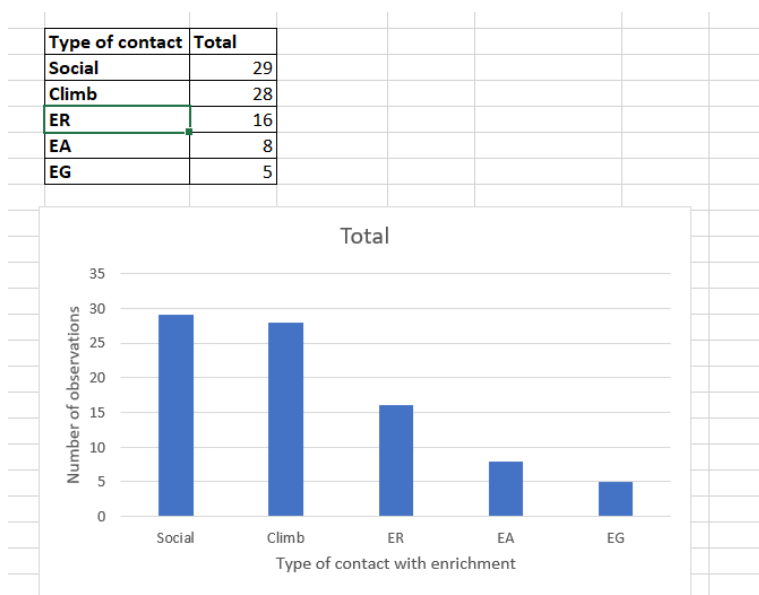| Type of contact	Total
Social	29
Climb	28
ER	16
EA	8
EG	5

Figure 2.1. When a table is used to create a figure, the presentation of the information within the table affects how it is presented on the chart.

Selecting the data in the table and inserting a 'column chart' will insert a bar chart showing the different ways the enrichment was used. Changing what is written in the table, and the order of the information, will change how it is presented on the chart (Figure 2.1). Note that in the Figure 2.1 the Total data has been sorted from largest to smallest (using Sort). Some of the codes indicating the different types of contact have been changed to words, making the chart easier to understand. Looking at the chart shows us that the mice have been mostly interacting with other mice in a positive way (social) and climbing on the new enrichment.

Using your notes for more insight and drawing conclusions

If you have taken additional notes on what was specifically observed when 'social' was recorded, you may be able to gain further insight into how the enrichment was used (if you find that this is necessary). However, even without this information it seems that the enrichment is mostly being used for the intended purpose – with the exception of gnawing (Figure 2.2).
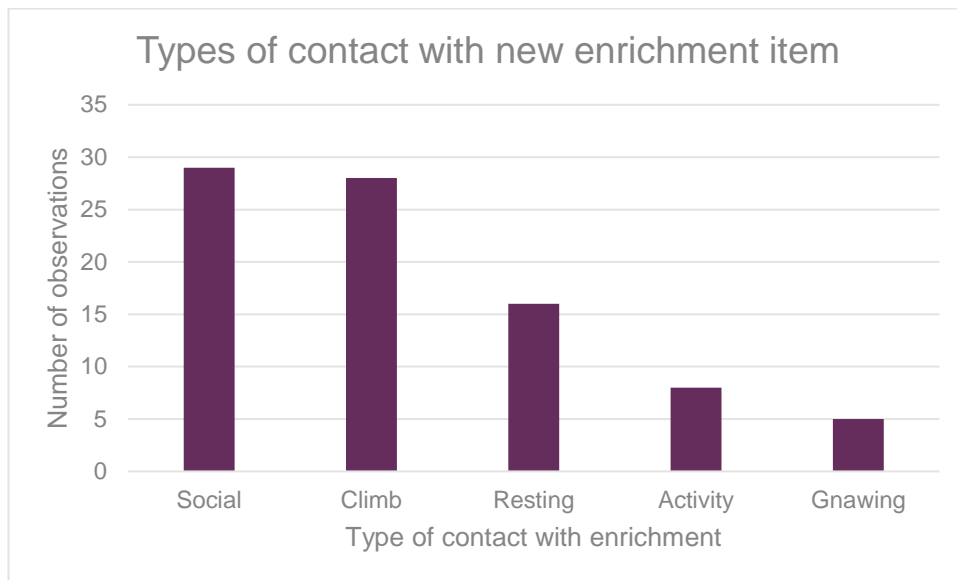
Figure 2.2. *A bar/column chart showing the different types of contact made with the focal enrichment item.*

The mezzanine is not intended to be chewed. This gnawing behaviour may highlight that there is a requirement for more materials specifically designed for gnawing within the cage. It may also highlight a potential issue with this enrichment item – will gnawing the mezzanine material harm the animals?

Plotting all the data (both Ys and Ns, Figure 2.3) shows us that when the animals were not using the focal enrichment they were most often using other enrichment (OE) within the cage. The data collection notes may provide further insight into this – could this behaviour also be related to gnawing?

Regardless of what the other enrichment behaviours were, this highlights the importance of variety and indicates that the new mezzanine should not replace any existing enrichment. There were also some instances of unclassified behaviour (U), again, it would be necessary to refer to the data collection notes to get more insight on what this behaviour entailed.
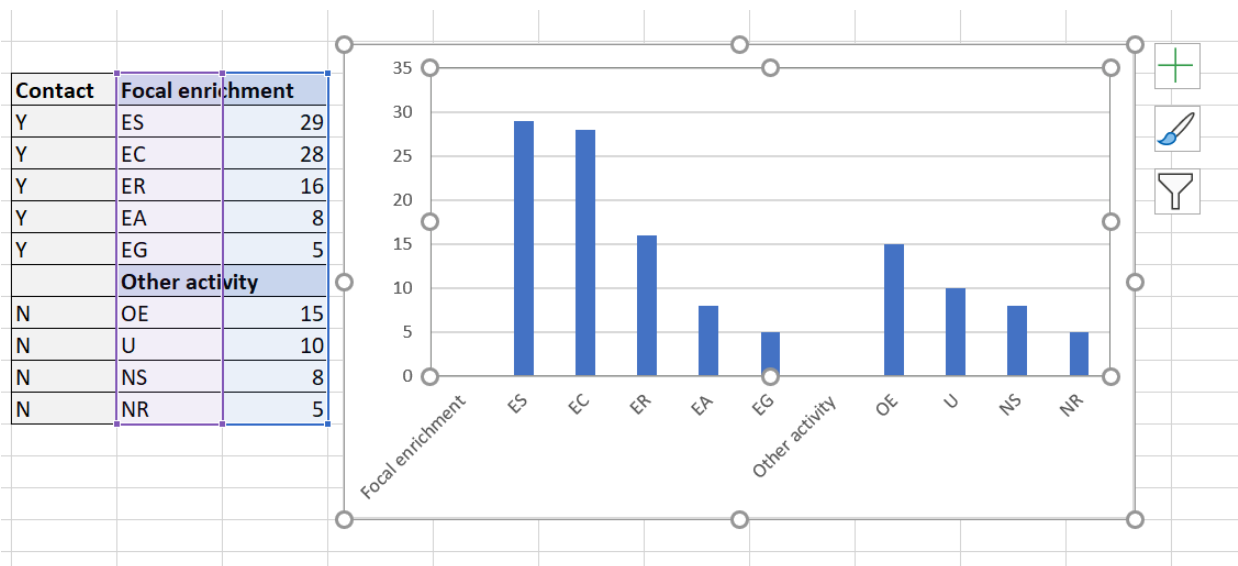
Contact	Focal enrichment	
Y	ES	29
Y	EC	28
Y	ER	16
Y	EA	8
Y	EG	5
	Other activity	
N	OE	15
N	U	10
N	NS	8
N	NR	5

Figure 2.3. *All the observed behaviours are plotted.*

From looking at all the charts we have plotted, we can conclude that the mezzanine with shelter is a suitable enrichment item because:

- The new enrichment is used by the mice.
- The enrichment is used as intended (however we have also identified an area to look into further – is enough gnawing enrichment provided within the cages?).
- No harmful effects of the enrichment are immediately obvious.

A limitation of this approach

No incidents of aggression were recorded during data collection, but does this mean that no aggressive behaviours took place?

A limitation of this approach is that the observations take place at brief intervals and so certain behaviours may be missed, especially if they are performed briefly or infrequently. It is also the case that only the behaviour of the focal mice was observed. If aggression were to occur involving other individuals, besides the focal mouse, then this information would not have been recorded.

For this reason, it is important to also use daily health checks, general monitoring and common sense when evaluating enrichment.