NC3Rs
National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Protocol B: Playrooms for rats

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Introduction

Providing access to a dedicated enriched space, or playroom, outside of the home cage has been validated as a refinement for many laboratory animal species, including rats. However, for strains prone to neophobia (fear of novelty) and anxiety-like behaviours, careful introduction and further evaluation may be necessary.

Image credit: Preclinical Research Facility, University of Leicester.

Playrooms for rats offer a greater amount of space, complexity and enrichment than is possible in the home cage, increasing the opportunities for exercise and performance of natural behaviours (e.g. climbing, burrowing).

Multiple kinds of enrichment item can be evaluated at once by observing which items the rats choose to interact with and the range of behaviours they perform, helping to ascertain if the animals' enrichment needs are currently being fulfilled.

In addition to the items within the designated enriched space, the playroom itself can be evaluated as a form of enrichment. The rats may be able to express a greater range of behaviours in the playroom in comparison to the home cage. They may also benefit psychologically from exposure to a varied space that they associate with playtime. Benefits of playroom exposure could last beyond the playroom itself, which can be investigated by observing home cage behaviour after the animals have used the designated enriched space.

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.

Use of a designated enriched area should not replace enrichment in the home cage, but it can inform it. For example, a preference for a particular type of shelter could be indicated in the playroom and these could then be given to the animals within their home cage.

About the protocol

This protocol below is a step-by-step guide on how you might prepare for and carry out basic behavioural observations to investigate the following questions:

- Does exposure to playroom increase or decrease the occurrence and/or duration of behaviours associated with positive or negative welfare?
- Do the animals indicate a preference for certain types of enrichment by interacting with them more often or for longer periods?
- Do any behavioural effects of using a playroom persist when the animals are back in their home cage?
- Do the animals perform a different range of behaviours in enriched versus standard cage environments?

The example ethogram and data collection sheet that accompany this protocol have been completed for the use of a playroom with rats. However, the materials could be adapted to evaluate, say, the use of enrichment items within the home cage, or other forms of environmental enrichment, such as exposure to music.

Behavioural observations are made over ten minutes in sessions spread across multiple days.

The instructions outline how to collect data at the cage side; however, video recording the animals is likely to be more convenient, generate more data, and be less disturbing for the animals. Using video recording equipment would also allow you to be blinded to whether the rats are 'before enrichment' or 'after enrichment'.

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. If you would like to carry out statistical tests on your data, we recommend consulting someone with statistical expertise before you begin data collection.

For more information on behaviour observations see 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. Decide what data to collect
3. Create a data collection sheet
4. Set up a rat playroom
5. Have a dry run
6. Select the cages to be observed
7. Select your focal animals
8. Create a schedule of observations
9. Decide how long the playroom session will last
10. Ensure that you have everything you need
11. Make your 'before' observations
12. Make your playroom observations
13. Make your 'after' observations

Image credit: University of Bristol

1. Create an ethogram

An example ethogram for this protocol is available to download. This can be adapted to meet the purpose of your study and the enrichment you include in your playroom. We also provide general advice on constructing an ethogram.

2. Decide what data to collect

This will depend on your behaviours of interest and what resources you have available to you. The options are outlined in Table 1.1.

Option	Data collection method	Data collected	Best option for
1	Record all behaviours continuously	Frequency of all behaviours Duration of all behaviours	When all behaviours are of interest.
2	Record only behaviours of interest	Frequency of specific behaviours Duration of specific behaviours	Only some key behaviours are of interest.
3	Record only behaviours of interest	Frequency of specific behaviours	Cage side observations may be possible without additional help.

Table 1.1. *Different approaches to data collection.*

Option 1 will result in all behaviours being recorded. This is a thorough approach and could lead to you noticing behaviours that you may otherwise have missed or did not initially consider as related to enrichment. A third helper may be required to assist with the timer.

Option 2 is less challenging than Option 1. Using this method, only data on specific behaviours is collected (e.g. positive and negative welfare indicators, such as play or stereotypies). This is a less thorough approach than Option 1 but may be all that is necessary for your evaluation.

Option 3 focuses on the number of occasions specific behaviours occur (e.g. the number of incidents of aggression). Recording the frequency of behaviours alone will provide limited insight. For example, you may record a single interaction with an enrichment item, but this does not distinguish between an interaction lasting one second or ten minutes.

3. 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.

Example data collection sheets for Option 1, Option 2 and Option 3 of this protocol can be downloaded in editable Word format. PDF instructions on filling out the data collection sheets are also available.

4. Set up a rat playroom

This can be an actual room in your facility or a larger-than-standard cage constructed from available materials (e.g. old rabbit cages).

Fill the playroom with a variety of suitable toys, treats and bedding. Try and make the environment as interesting to the rats as is feasible. Consider including a box for the rats to dig in (soil can be autoclaved, or shredded paper could be put inside a shoebox), ladders and platforms for the rats to climb on and explore upwards, and different flavoured or scented items to engage their senses.

If you are using items that have not been evaluated for these animals, initial monitoring will be required to ensure safety and suitability.

For further inspiration see:

- IAT Congress 2017 workshop summary: Playtime for rats
- Housing and husbandry of rats
- The importance of burrowing, climbing and standing upright for laboratory rats
- A good life for laboratory rodents?

5. Have a dry run

If you are unable to use video recording equipment, ensure that you are familiar with filling out the data collection sheet prior to your evaluation.

Observe rats in both the enriched (playroom) and unenriched (home cage) environments, for any duration of time. This is not the 'proper' evaluation and the purpose is to practice using the ethogram to fill out the data collection sheet. This is also a good opportunity to check the feasibility of your plan and make any adjustments. For example, this step will highlight whether you are trying to record too many behaviours at once and the adjustment may be to request help from a colleague or simplify your ethogram.

Recording both frequency of behaviour and duration of behaviour can take some practice. Ask colleagues to assist you with the timer, recording information on the sheet and checking observer consistency.

This is also an opportunity to check that you can observe the whole playroom with minimal disturbance to the animals.

6. Select the cages to be observed

Select *Rand four *Flex cages to observe. You will be observing animals in their home cages before and after a visit to the playroom.

Assign each cage a number, this will allow you to randomise the order in which you observe the rats.

7. Select your focal animals

Animals will need to be individually identifiable (e.g. from natural markings, tattoos, dye marks). Randomly select one *Rand from each cage to be the 'focal individual'; this is the animal you will observe. Record the focal animal ID for each cage on your data collection sheet. Your focal animals will remain the same throughout the study.

8. Create a schedule for observations

Set aside time for your observations and create a timetable. Try to avoid days and times where external influences are likely to influence the behaviour of the animals (e.g. feeding time or cage clean outs). If you cannot avoid these, note these disturbances on your data collection sheet.

For the purpose of this example, four cages are being observed. To account for changes in behaviour throughout the day, two cages will be observed in the morning and two in the afternoon. This requires the observations to be spread over two standard working weeks.

In Table 1.2, the schedule was created and then cage numbers were picked out of hat to create a random order of observation.

There can be some flexibility in when the 'before play' observations are carried out; however do not leave too long between the playroom visit and the 'after play' observations. Keep the time between the 'during play' and 'after play' observations the same for all cages.

Cage # (random order)	Week	Before play	During play	After play
3	1	Tues AM	Weds AM	Thurs AM
2	1	Tues AM	Weds AM	Thurs AM
4	2	Tues PM	Weds PM	Thurs PM
1	2	Tues PM	Weds PM	Thurs PM

Table 1.2. Schedule for observing rats before, during and after visiting an enriched playroom.

9. Decide how long the playroom session will last

All the playroom sessions should be a set length. Consider what is practical. For the purpose of this example, the rats will spend 30 minutes in the playroom [*Flex]. Note that you will only record behaviour for a subset of this time (e.g. 10 minutes [*Flex]), but a longer duration will allow the rats to settle into the playroom and have an opportunity to enjoy it.

Create a schedule to remind you when to move the rats to and from the playroom; and also when to begin your observations.

You will need to take acclimatisation into account. The rats will need time to adjust to being handled and placed in new surroundings. The rats will also need additional time to adjust to your presence near to the cage, as you prepare to observe them. Keep these periods the same throughout the study.

In Table 1.3, the rats are moved to the playroom and left for 5 minutes [*Flex]. The observer then returns and positions themselves where they can see the rats in the playroom and allows a further 5 minutes before beginning their observations at the times shown. You can use the acclimatisation period to add notes to your data collection sheet.

Cage	Day / Week	Time in playroom	Observations begin	Time out of playroom
3	Weds / Week 1	08:30	08:40	09:00
2	Weds / Week 1	10:00	10:10	10:30
4	Weds / Week 2	13:00	13:10	13:30
1	Weds / Week 2	16:00	16:10	16:30

Table 1.3. Rat playroom rota.

10. Ensure that you have everything you need

For example, the ethogram, data collection sheets (Option 1, Option 2 or Option 3), a pen, a stopwatch-style timer and information on your schedule, cage numbers and focal animals. Keep these items near to where

you will make your observations. Having everything easily to hand will save you time and keep things running smoothly.

11. Make your 'before' observations

Following your schedule, find an appropriate spot to observe your focal animal. You will need to be able to see the whole cage but be aware of your effect on the animals. For example, try not to loom over the cage and allow them some time (e.g. 5 – 10 minutes [*Flex]) to acclimatise to your presence before starting your observations. Keep the acclimatisation period the same for every observation.

If you have not already recorded any relevant information on your data collection sheet, you can use the acclimatisation period to do this.

Identify the focal individual and set the timer for 10 minutes [*Flex]. Follow the [instructions] to fill in the data collection sheet using the ethogram.

12. Make your playroom observations

Following your playroom rota, move the animals to the playroom and allow them to adjust to their surroundings and then to your presence.

Follow the same procedure as for the 'before' observations, recording behaviour on the data collection sheets.

Allow the animals to finish their playroom session.

13. Make your 'after' observations

Follow exactly the same procedure as when you made your 'before' observations.

Adapting the protocol

[A limitation] of Protocol B is that it does not use a separate control group, the animals act as their own controls (see [the 'Experimental designs' paragraph] of this paper for more information on within-subject ABA and between-subject approaches). If you find that it is necessary and feasible to do so, you can adapt this study protocol to include control animals:

Image credit: University College London.

- Introduce a control group into the evaluation by selecting two focal animals per cage instead of one. One focal animal will visit the playroom, while the other will act as a control (handled but with no access

to the playroom). Collect data cage-side with a colleague, each focusing on a different focal animal. Alternatively you can video record behaviour and play it back to observe each animal separately.

- Randomly assign cages to a control (no access to playroom) or playroom treatment (twice weekly access to playroom). Ensure your colleagues are blinded to which cages are in which group and ask them to score how calm and easy to handle the rats are during standard checks, or time how quickly the rats will approach their hands. Whatever approach you use be sure to keep scoring and data collection consistent with good planning and communication.

If you have access to the correct equipment you can adapt this protocol to:

- Playback video recordings and look at which types of enrichment the rats spend the most time interacting with in the playroom.
- Use a bat detector to gain more insight into the affective state (mood) of the rats when they are exposed to different conditions. Ultrasonic vocalisations can indicate whether rats are having a positive (50-kHz) or negative (22-kHz) experience.

If you have a longer period of time you can adapt this protocol to:

- Make observations over a longer timeframe to capture any longer-term changes in behaviour.

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.

Image credit: Preclinical Research Facility, University of Leicester.

Approaches to interpreting Protocol B data

Remind yourself of the aims of the study. The questions of interest are shown below with suggestions on how to approach them.

Does exposure to a designated enriched space increase or decrease the occurrence and/or duration of behaviours associated with positive or negative welfare?

Compare the frequency and duration of positive and negative welfare indicators before and after playroom visits, or between the home cage and the playroom.

Do the animals indicate a preference for certain types of enrichment by interacting with them more often or for longer periods?

Image credit: University College London.

Compare the frequency and duration of contact or interaction with different enrichment items.

Do any behavioural effects of using a playroom persist when animals are back in the home cage?

Compare the behaviours observed in the 'after enrichment' condition with the two other conditions. Focus on positive and negative welfare indicators.

Do the animals perform a different range of behaviours in enriched versus standard cage environments?

Compare the behaviours observed in the 'during enrichment' condition with the two other conditions. Are there any behaviours that the animals perform in the playroom that are not observed in the home cage or vice versa? For example, the rats stretch horizontally in their home cages and perform this behaviour less often in the playroom. However, the rats rear (stretch vertically) in the playroom, but do not perform this behaviour in their cages. This could indicate that stretching is important and if given the opportunity (i.e. enough vertical space) rearing upwards is preferred.

Inputting and sorting data using Excel

Regardless of your approach to data collection you will have collected frequency data. You may also have collected data on the duration of behaviours. The following guidance on summarising your data refers to both but can be followed for frequency-only data by ignoring the references to duration.

1. Enter the frequency and duration data into a spreadsheet and convert the time to seconds.

You can convert the time to seconds (s) before inputting it onto a spreadsheet, but if you decide to input the times as they are shown on the stopwatch, then ensure the Excel cells are formatted correctly (right click → format cells → custom → mm:ss.0).

You can then use Excel to convert the times from stopwatch format (mm:ss:0) to seconds by using the formula shown in column D (Figure 1.1).

In Figure 1.1, the formulae shown in Column D were entered into Column C before pressing enter. This should result in the time in Column B being duplicated in Column C presented in a different time format (seconds).

If an unexpected value is shown when you press enter, it is likely that you need to change the cell formatting from 'time' to 'general' or 'number' (right click → format cells) for Excel to correctly display the time in seconds (Column C).

	A	B	C	D
1	**Behaviour**	**Duration (mm:ss.0)**	**Duration (s)**	**Conversion formula**
2	Abnormal	03:53.0	233	=MINUTE(B2)*60+SECOND(B2)
3	Eat	01:59.0	119	=MINUTE(B3)*60+SECOND(B3)
4	Gnaw	00:15.7	16	=MINUTE(B4)*60+SECOND(B4)
5	Groom	00:25.0	25	=MINUTE(B5)*60+SECOND(B5)
6	Immobile	07:46.9	467	=MINUTE(B6)*60+SECOND(B6)

Figure 1.1. *Converting stopwatch time into seconds using an Excel formula. The formula can be typed in the first box and then populated in the below rows by dragging the top box downwards. Always check that formulas have populated correctly and that the numbers seem sensible.*

If a colleague is summarising the data for you, the home cage data can be blinded. In Figure 1.2 the condition column shows the letter 'A' rather than stating whether the data is from before or after visiting the playroom.

	A	B	C	D	E	F	G	H
1	**Condition**	**Cage**	**Behaviour**	**Duration (mm:ss.0)**		**Condition A**	**Frequency**	**Duration (s)**
2	A	1	Abnormal	01:00.0		Abnormal	3	233.00
3	A	4	Abnormal	01:30.4		Eat	2	119.00
4	A	4	Abnormal	01:21.1		Gnaw	1	16.00
5	A	2	Eat	00:50.4		Groom	4	25.00
6	A	4	Eat	01:05.3		Immobile	4	467.00
7	A	2	Gnaw	00:11.2		Move	0	144.00
8	A	3	Groom	00:02.5		Neg soc	1	2.00
9	A	4	Groom	00:09.3		Other enrich	1	158.00
10	A	4	Groom	00:01.0		Pos soc	1	5.00
11	A	4	Groom	00:10.0		Sleep	4	1154.00
12	A	1	Immobile	02:04.4		Sniff	1	47.00
13	A	2	Immobile	05:06.3		Stretch	4	30
14	A	4	Immobile	00:16.1				

Figure 1.2. The data from the data collection sheets can be transferred to spreadsheet to look something like this. Excel functions can help you to create tables, like the one shown in blue, to summarise your data. Note that the stopwatch durations (Column D) have been converted into whole seconds in Column H.

You may want to put the data for each condition (e.g. before, during, after) on a different tab within the same spreadsheet.

2. Sort the data by the behaviour to group all the behaviours together.

Once the data is sorted in this way you can use functions (e.g. =COUNTIF or =SUM) to create summary tables for the different conditions (the blue box in Figure 1.2).

Detailed guidance on using the COUNTIF and SUM functions is included in 'Looking at your data' for Protocol A and Protocol D.

Use the =COUNTIF function in the behaviour column (Column C in Figure 1.2) to the give the total number of occasions that each behaviour occurred.

Use the =SUM function in the duration column (Column D in Figure 1.2) to give the total number of time spent on each behaviour.

3. Look for patterns, similarities and differences in your data.

Arranging your data into tables will allow you to look for patterns and pick out behaviours of interest, that may indicate an effect of exposure to enrichment.

By looking at Table 1.4 you can see that negative social interactions did not occur during or after exposure to enrichment, whereas positive social interactions were more frequent and lasted longer. Stereotypies only occurred before exposure to enrichment. Rats in the home cage did stretch horizontally, but they did not stretch upright – does the height of the home cage allow standing in an upright position? This is fictional data, but it illustrates how you can begin to answer your questions of interest.

Behaviour	Before	After	During
Positive social	1 (5)	4 (101)	3 (273)
Negative social	1 (2)	0	0
Abnormal	3 (233)	0	0
Horizontal stretch	4 (30)	2 (4)	0
Vertical stretch	0	0	4 (9)

Table 1.4. Frequency of behaviours for 40 minutes of observation time before access to a playroom, after access to a playroom and during access to a playroom. The total duration (seconds) of the observed behaviours is given in brackets. Observations were made of four rats for 10 minutes per rat per condition. This is fictional data for illustrational purposes only.

4. Sort the 'during enrichment' data by frequency or duration to get insight into which enrichment items were used (as an indication of preference).

Table 1.2 shows us that the rats spent the most time in the soil box, closely followed by climbing up ladders. It might not be possible to incorporate these enrichment items into the home cage, which could highlight the importance of providing access to a designated enriched space.

Playroom	
Enrichment type	**Frequency (duration)**
Soil	2 (390)
Climb	2 (380)
Tunnel	2 (219)
Ball pit	2 (64)
Other	1 (3)
Gnaw stick	1 (2)

Table 1.5. Frequency and duration (seconds) of contact or interaction with different enrichment items in the designated enriched space (playroom). Observations were made of four rats for 10 minutes per rat.

Creating charts in Excel

Creating charts to visualise your data is also useful for seeing what the data shows 'at a glance'. You can do this for all the behaviours, or focus on behaviours of interest. This section will cover the following chart types:

- Bar charts
- Pie and doughnut charts
- Line graphs
- Scatter plots

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More details are provided below, however the basic steps for plotting a chart in Excel are as follows:

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

Another approach is to Insert the chart and then select the data you want to plot by right clicking the chart area and choosing Select Data. However, as you will already be creating a summary table of your data the above method should be the quickest and easiest approach.

Bar charts

Bar charts can be used to visualise the frequency data for each condition (before enrichment, during enrichment and after enrichment), separately or on the same figure. Bar charts are easy to create in Excel and are commonly used to present categorical data (data that is divided into groups/categories, such as before, during and after).

Figure 1.3a shows the bar chart that was automatically created from the highlighted table shown within the same image. The Y-axis shows the total number of seconds that each category of behaviour was performed, however this is not clear as the chart needs labels adding to the axes. The chart can be formatted using the three tiles shown to the right of it. The green plus symbol is the 'Chart elements' menu, which can be used to add various labels.
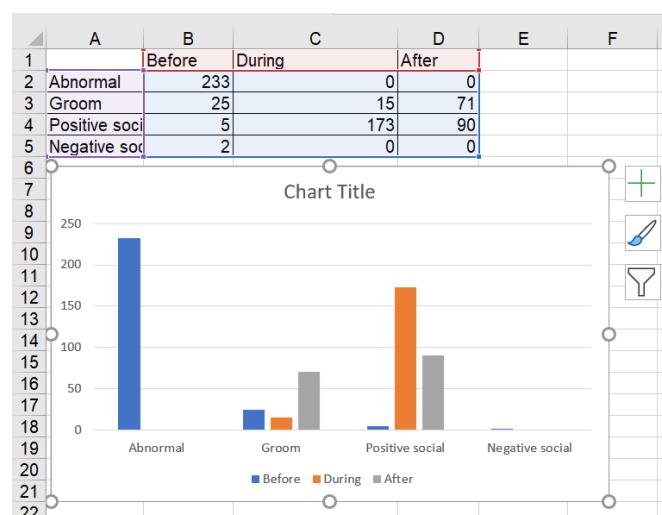
Figure 1.3a. The clustered bar chart that is automatically generated using the highlighted table.

Figure 1.3b. The same chart with data and axis labels added.

Figure 1.3b has labels, which make it easier to understand at a glance. The chart also has a title which can aid clarity on a poster or presentation slide.

You can use a bar chart when:

- You want to use a chart that is relatively quick and simple to plot within Excel.
- You are plotting data that is separated into groups or categories (categorical data).

You should avoid a bar chart when:

- You want to maximise how informative a figure is. The data plotted on the bar charts is the combined total for all the focal animals, whereas a figure that shows individual data points for each animal (e.g. a scatter plot) will show the range of responses.
- Bar charts may not be considered appropriate if you are trying to publish your findings.

Pie or doughnut charts

Pie or doughnut charts (Figure 1.4) can be used to visualise the duration data for each different condition. The duration data can be plotted without being converted from seconds. Inserting these charts follows the same procedure as for other charts.

A pie or doughnut chart is better when there are fewer 'slices', otherwise they become cluttered and difficult to read. These charts can look nice in a presentation, however it can be difficult to accurately compare the size of the 'slices' to each other. For an example of this, look at the doughnut chart compared to the table of numbers that it was created from.
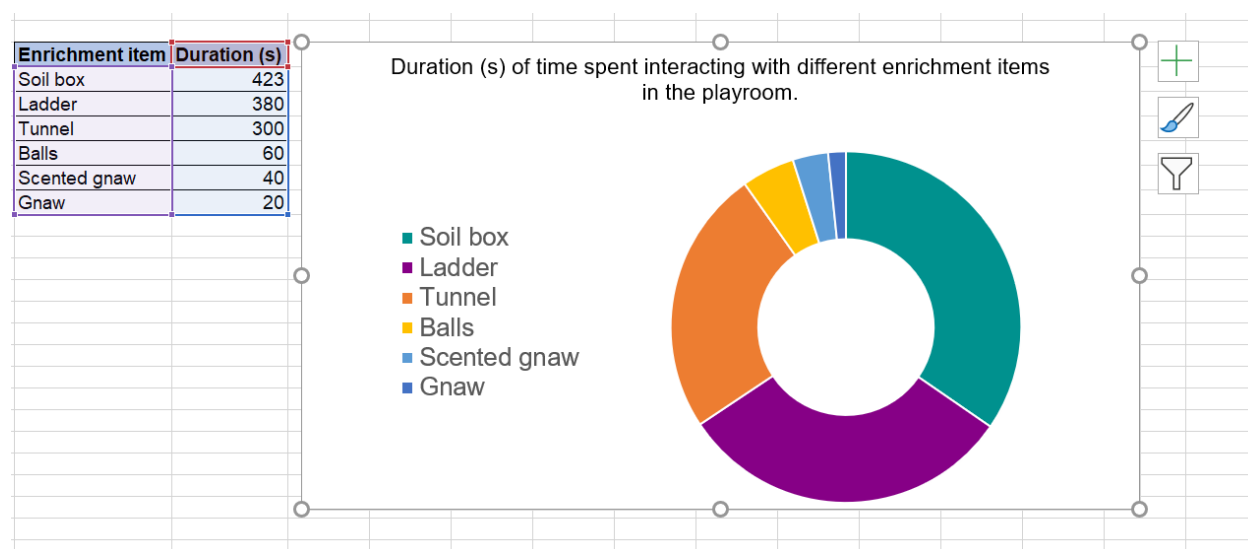
Figure 1.4. *Doughnut charts can be a nice visual for a presentation, but it can be difficult to estimate the size of the 'slices' at a glance.*

You can use a pie or doughnut chart when:

- You are plotting categorical data (data grouped by categories, e.g. enrichment item).
- You have large and obvious differences in size between the values (data labels can be added using the 'Chart elements' menu to improve clarity.
- You want to present a colourful figure to discuss with others (e.g. in a presentation).

You should avoid a pie or doughnut chart when:

- You have many groups or categories to present on one figure.
- You are intending to submit your findings for publication.
- You want to maximise how informative your figure is (as for bar charts).

Line graphs

Line graphs can be used to illustrate behaviours that may have increased or decreased over time (e.g. before and after exposure to enrichment). Inserting a line graph follows the same procedure as for other charts.

For example, Figure 1.5 shows an increase in positive social interactions, and a decrease in negative social interactions after exposure to a designated enriched space.
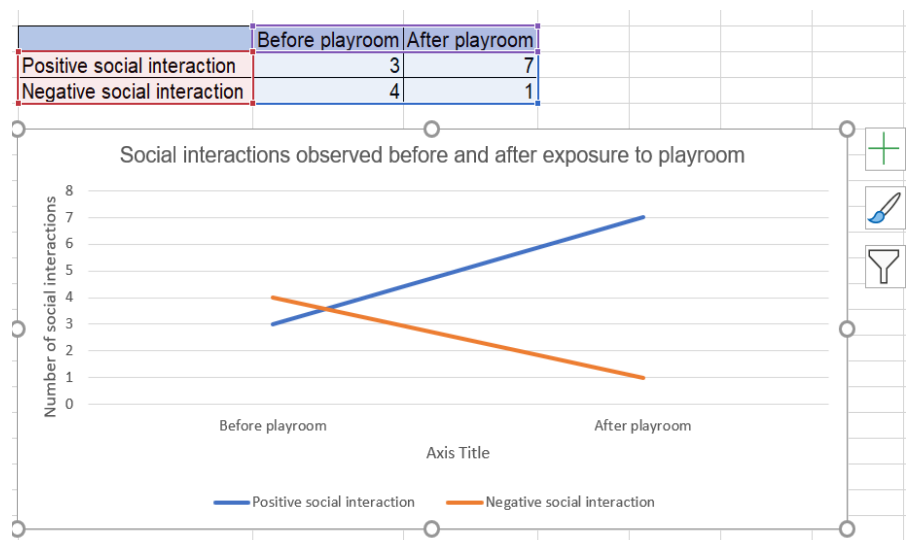

Figure 1.5. Line graphs can be used to highlight changes over time, or to summarise differences between groups.

You can use a line chart when:

- Your main aim is to illustrate whether change has occurred over time (e.g. over the hours, days or months since enrichment was introduced).

You should avoid a line chart when:

- You have many time points to compare. Too many lines will make the figure difficult to read.

Scatter plots

A scatter plot can represent each focal animal as an individual data point, allowing the reader the observe the range of responses shown by each animal. Plotting data in this way increases how informative your figure is.

If you are unsure of why it can be useful to represent each focal animal as an individual data point, consider the following example:

Focal rats 1-4 all consistently spent time using tunnels. However, their interactions with the tunnels was often brief as they would quickly run through them.

Rat 2 played in the tunnels for a short time but then spent the majority of the observation period eating treats inside a shelter. Only rat 2 used this shelter, but the overall duration of time spent in the shelter was greater than the time that all the rats spent playing in the tunnels.

A plot that does not represent each animal as an individual data point could lead the reader to believe that the shelter was 'favourite' enrichment.

A drawback of creating a scatter plot is that they are slightly more difficult to create using data grouped by categories in Excel. There is a way to work around this in Excel, which is shown below. However, if you are looking to create a publication-quality scatter plot you may need to create it using different software.

1. Create the basic plot using the same method as the charts above.

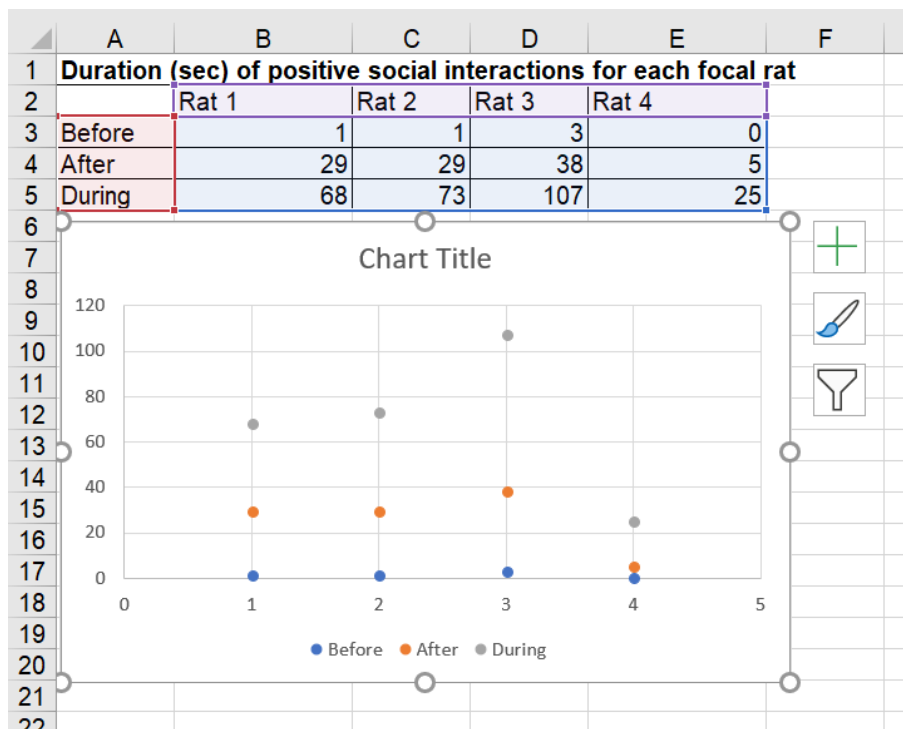This will automatically generate something like Figure 1.6.

Figure 1.6. *The chart plotted automatically doesn't look how we want it to.*

2. Right click the chart, choose 'Select data' and switch the data so it appears as shown in Figure 1.7.
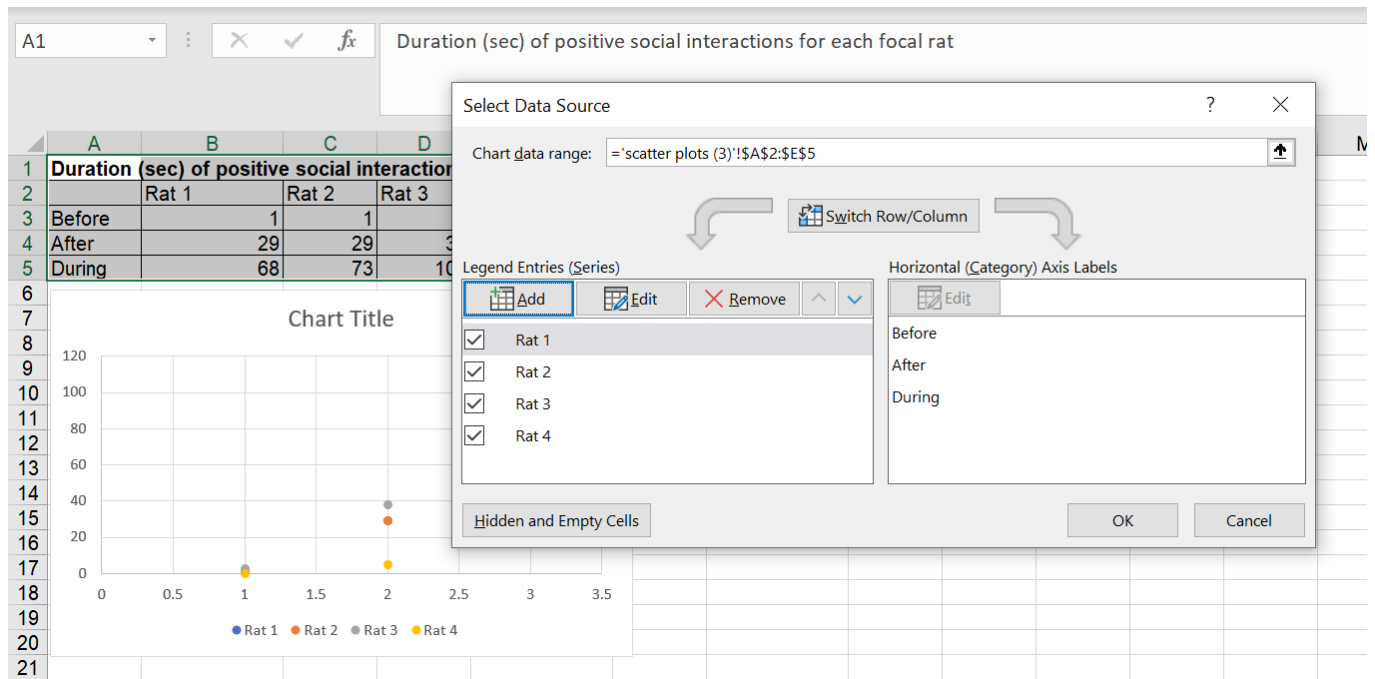

Figure 1.7. *Using the Select Data menu to switch rows/columns.*

The scatterplot still looks confusing. The horizontal X-axis shows numbers rather than the 'before, after and during' categories and the data points are overlapping. Firstly, we will address the X-axis.

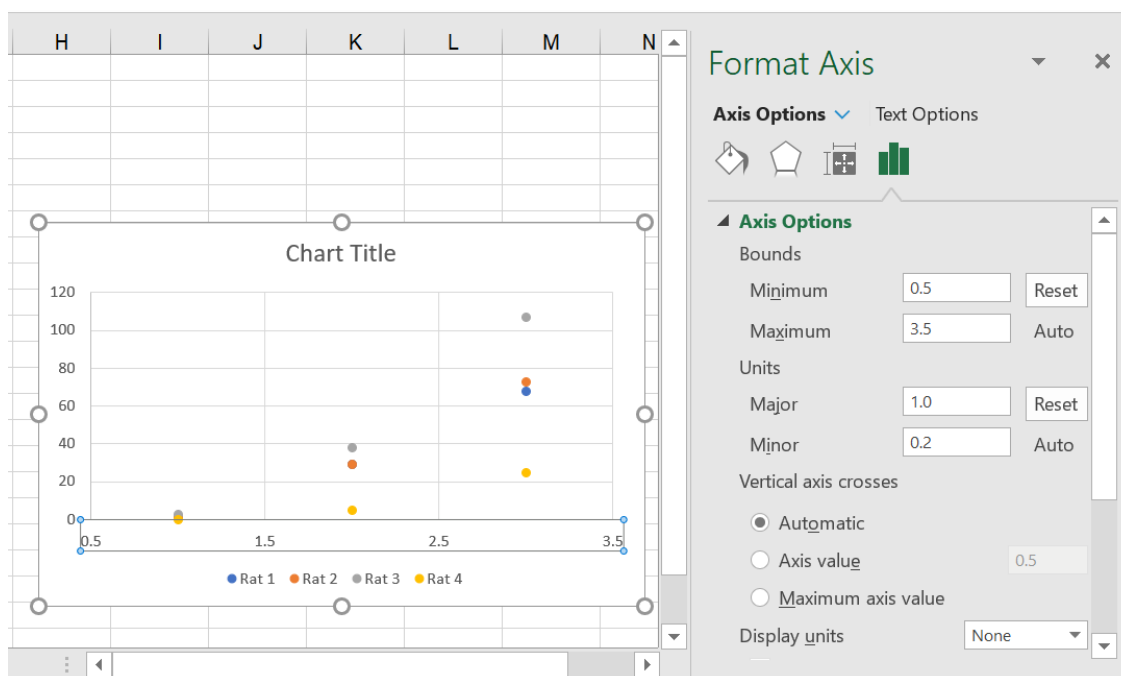*3. Right click on these X-axis values. This should give you the option to 'Format axis' (Figure 1.8).*

Figure 1.8. *The Format Axis menu allows you to format how the numbers on the x-axis are displayed.*

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4. In 'Bounds' change the minimum to 0.5 and the maximum to 3.5 (Figure 1.8).

This will change the highest value on the X-axis to 3.5, which will leave the three groups we want to plot, plus some extra space so group 3 (during) is not at the edge of the scatterplot. The lowest value will become 0.5 rather than 0.0, which will shift the data to a central position on the plot.

5. In 'Units' change the major to 1.0 and the minor to 0.2 (Figure 1.8). Changing the major units to 1 will result in only whole numbers (i.e. not 1.5, 2.5 etc.) being shown on the X-axis. Now the data is positioned centrally on the chart area.

6. Right click the horizontal X-axis again and select 'Delete' to remove numbers along the bottom of the figure (Figure 1.8).

7. Select 'Axis titles' from the 'Chart elements' menu (the green plus symbol shown the left of the plot) (Figure 1.9).

8. Select the text on the X-axis label and replace it with the text you want it to show (e.g. before, after and during) (Figure 1.9).

9. Type 'Before After During' and use the tab key or space bar to create spaces between each word so that they align with the corresponding data points.
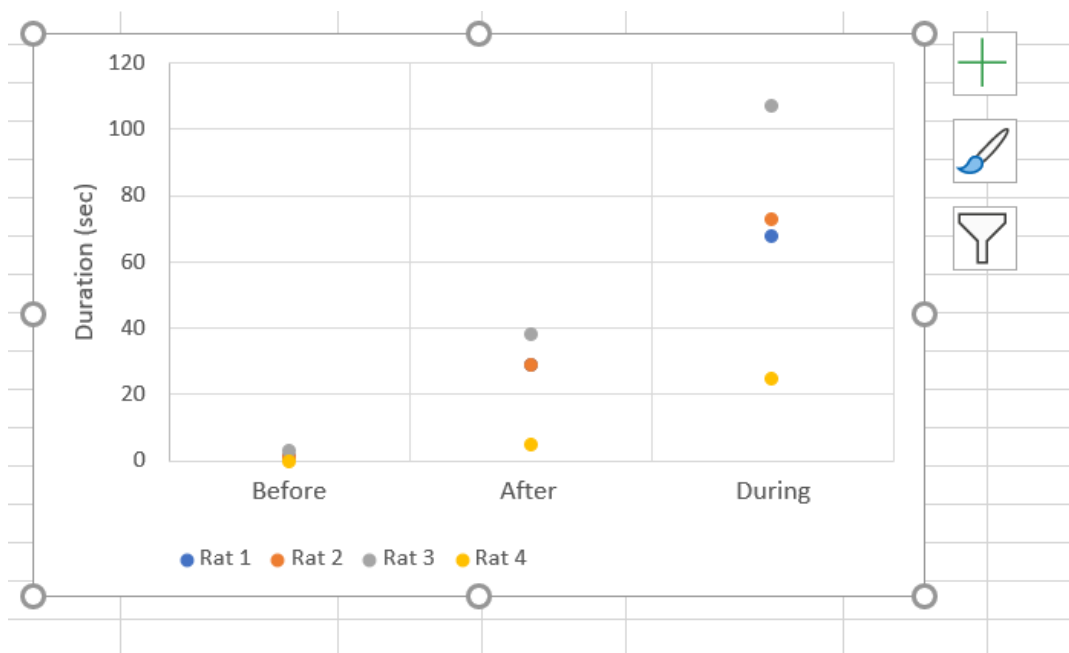

Figure 1.9. *Your scatter plot may look OK at this step, but if the data points are grouped very closely together the graph may be difficult to read.*

Now the scatter plot has the labels we want it to show, however the data points for 'Before' are overlapping (Figure 1.9). You may not have this issue with your own data but if you do you can edit the data points so that they are easier to see when they are grouped closely together.

10. Click (only once!) on any of the data points. This will select all the data points for that rat (Figure 2.1).
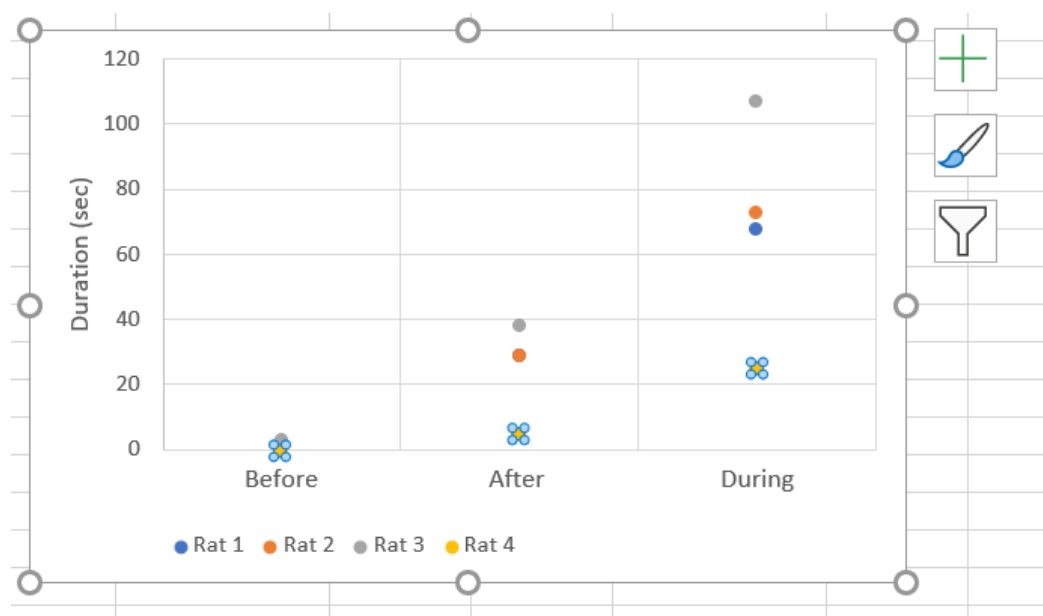

Figure 2.1. *Selecting a data point will highlight the data points in same category.*

11. If the 'Format data series' menu does not automatically open, right click on the chart area while the data points are selected and open the menu from there.
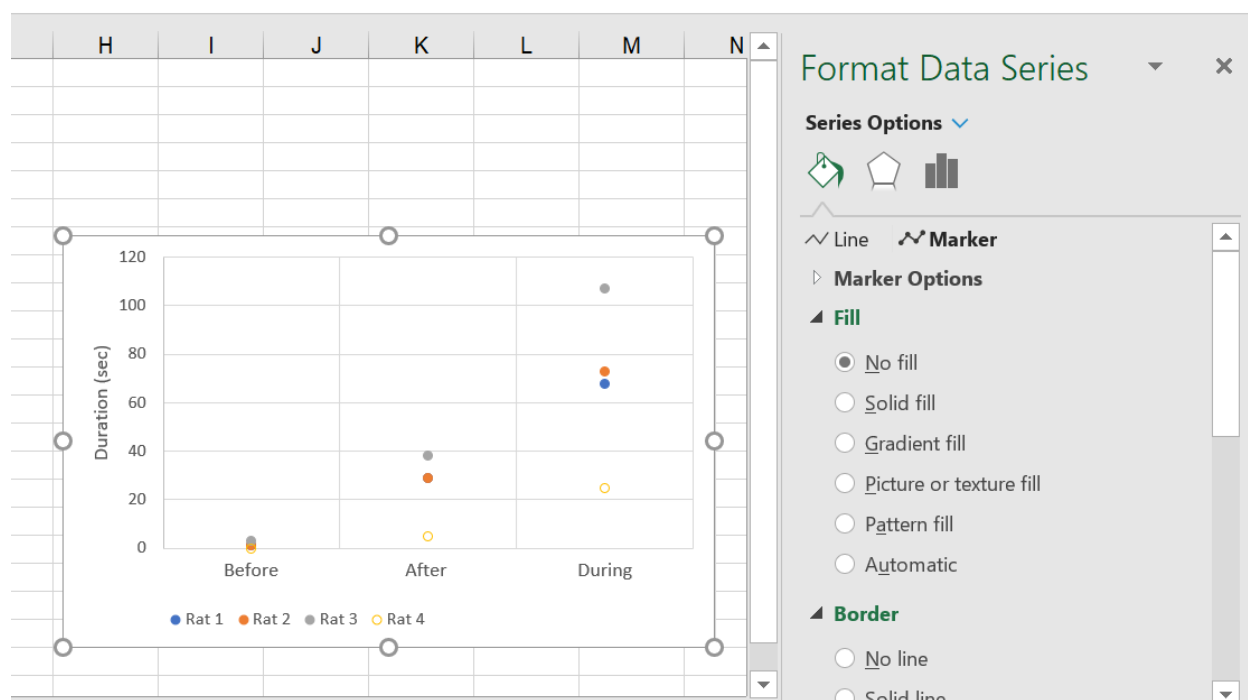

Figure 2.2. *The appearance of the data points can be changed to make the graph easier to read.*

12. Navigate to the paint pot icon and then to 'Marker'. Select 'No fill' to leave only the coloured outlined of the data points (Figure 2.2).

13. Repeat these steps for the other rats/data points.

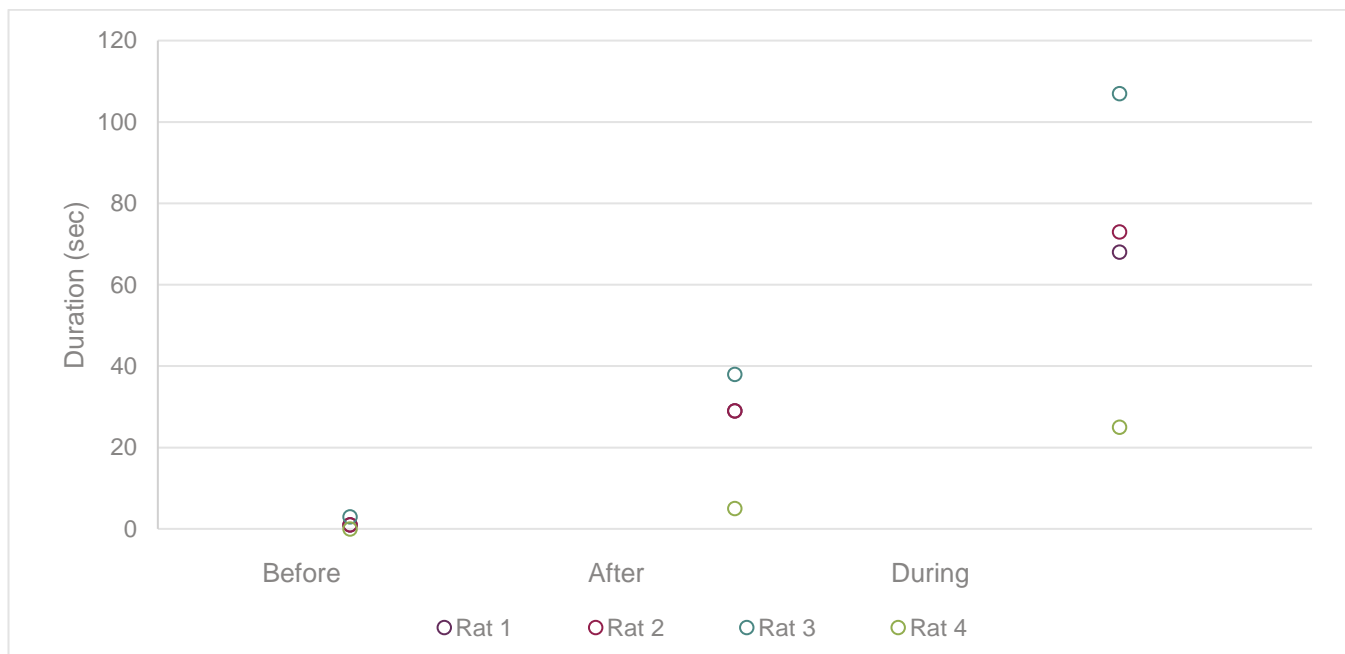*14. Give your plot an informative figure legend or title (Figure 2.3).*

Figure 2.3. *The duration of positive interactions performed by rats observed within the home cage 1h before (Before) and 1hr after (After) playroom access, and during a playroom session (During).*