How to Create a Univariate Scatterplots for Paired Data
(2 groups, 2 conditions/group) in GraphPad PRISM 6

These instructions will allow you to make graphs like this:

Use these instructions to create scatterplots for paired data (2 conditions) in two groups of subjects. Paired data are when you measure the variable of interest more than one time in each participant. If your data are independent, please see the instructions for Independent data.
1. Under “New Table & Graph”, select “Column”
2. Select “Enter paired or repeated measures data – each subject on a separate row”. Click “Create”.

*Note that these graphs can also be made using the “Grouped” option, however data entry is more complex.
3. Enter the name for Condition 1 just below “Group A” and “Group “C”. Enter the name for Condition 2 just below “Group B” and “Group D”. The condition names that you enter will appear as labels on your graph.

Enter you data as follows (one row per subject or specimen):
- First column (before “Group A” column): Subject or specimen ID
- “Group A” column: Data from Group 1, Condition 1
- “Group B” column: Data from Group 1, Condition 2
- “Group C” column: Data from Group 2, Condition 1
- “Group D” column: Data from Group 2, Condition 2

Subjects from Group 1 will only have data in the “Group A” and Group B” columns, whereas subjects from Group 2 will only have data in the “Group C” and “Group D” columns.
4. From the insert menu at the top of the screen, select “New graph of existing data”
5. Ensure that the “Symbols & lines” plot is selected. Click “OK” to create the graph.
6. Your graph will look something like this. Click on “YTitle” to replace this label with the names and units for your axis. Click on “XTitle” and replace this with the names for Group 1 and Group 2, using the spaces in between to center both labels. Delete the chart title (Data1 in this example) or click on the chart title to replace it with the desired text.
7. Your new axis labels should appear on the graph.

8. Left click on one of the x-axis labels; then right click and select "Format axis".
9. In the pop-up menu, under the x-axis tab change “Below, angled” to “Below, horizontal”.

10. Select the “Titles & Fonts” tab. Change "Distance from axis" for the x-axis title to the desired value. Click “Apply” to view the change. Click “OK” when you are happy with the result.
11. Your graph will appear. Click on the right end of the x-axis and drag the blue box to extend the length of the axis as needed.

Paired Data
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12. Click on the Group labels on the x-axis and adjust the spacing as needed to center the Group 1 and Group 2 labels.
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13. Select “File”, then “Export” to save the graph.

14. Select the file format, enter a file name and chose the location where you want to save the file. Click “OK”. Your graph should be saved in the format and location that you selected.
15. The next step is to graph the differences for both groups. Enter the name for Group 1 just below “Group E”. Enter the name for Group 2 just below “Group F”. The condition names that you enter will appear as labels on your graph. Enter your data as follows (one row per subject or specimen):

- “Group E” column: Difference scores for Group 1 (Condition 2 – Condition 1)
- “Group F” column: Difference scores for Group 2 (Condition 2 – Condition 1)

Subjects from Group 1 will only have a difference score in the “Group E” column, whereas subjects from Group 2 will only have a difference score in the “Group F” column.

16. From the insert menu at the top of the screen, select “New graph of existing data”
17. Select “scatterplot” in the pop-up menu. Under the plot menu, select any summary statistics that you would like the graph to show. You should show the mean if you are using parametric statistics for your data analysis, and the median if you are using non-parametric statistics for your data analysis. Click “OK”.

![GraphPad PRISM 6 interface and scatterplot creation dialog box]

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18. The graph will look something like this. You will need to delete the groups that are not difference scores. In the “Change” menu at the top of the screen, click on the “Format Graph” symbol.
19. Go to the “Data Sets on Graph” tab in the pop-up menu. Highlight the groups that contain raw data for each condition. Click “Remove”, then click “OK”.

20. The graph will look something like this. Click on “YTitle” to replace this label with the names and units for your axis. Either delete the chart title (Data1 in this example) and “XTitle” or click on these titles to replace them with the desired text.
21. Your new axis labels should now appear on the scatterplot. Left click on one of the x-axis labels (Group 1, Group 2, etc.); then right click and select “Format axis”.

22. In the pop-up menu, under the x-axis tab change "Below, angled" to "Below, horizontal".
23. If you are using an x-axis title, select the “Titles & Fonts” tab. Change “Distance from axis” for the x-axis title to the desired value. Click “Apply” to view the change. Click “OK” when you are happy with the result.

24. Your univariate scatterplot will look something like this. Select “File”, then “Export” to save the scatterplot.
25. Select the file format, enter a file name and chose the location where you want to save the graph. Click “OK”. Your scatterplot will be saved in the format and location that you selected.