GraphPad PRISM®

Version 4.0

Step-by-Step Examples

Two-Way ANOVA with Post Tests[1]

Two-way analysis of variance may be used to examine the effects of two variables (factors), both individually and together, on an experimental response. Suppose you've studied the effects on heart rate of three experimental treatments (factor 1) before and during exercise (factor 2). Two-way ANOVA, in combination with post testing, can answer the following questions:

- Is there an effect of treatment on heart rate?
- Is there an effect of exercise on heart rate?
- Is there interaction between the factors? That is, does the affect of treatment differ between exercise states, or equivalently, does the effect of exercise differ among treatments?
- At which treatments is there a significant difference between exercise levels?

To learn more about two-way analysis of variance, consult the companion *Statistics Guide*. If your data are grouped by only one factor, see the Step-by-Step Example "One-Way ANOVA and Nonparametric Analyses".

Entering and Graphing the Data

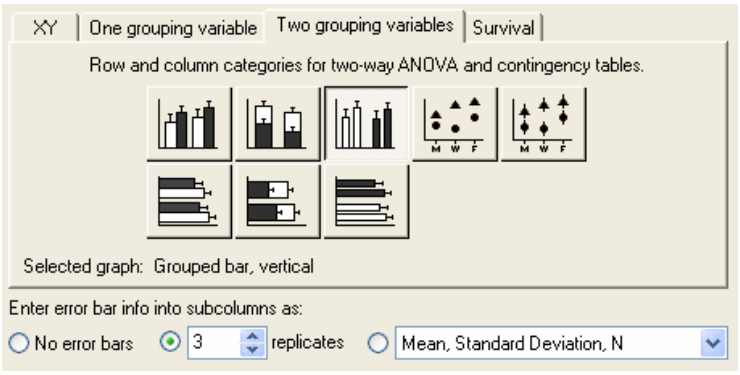
When you launch Prism, the Welcome dialog appears. Choose to **Create a new project** and indicate that you will create the initial data table and linked graph by choosing the **Type of graph**.

Our data are organized by two grouping variables, or factors:

- *Exercise*, which has two levels—Before exercise and During exercise
- *Treatment*, which has three levels—None, Lesion, and Lesion+Drug

Therefore, select the tab for **Two grouping variables**.

[1] Adapted from: Miller, J.R., *GraphPad Prism Version 4.0 Step-by-Step Examples*, GraphPad Software Inc., San Diego CA, 2003. *Step-by-Step Examples* is one of four manuals included with Prism 4. All are available for download as PDF files at www.graphpad.com. While the directions and figures match the Windows version of Prism 4, all examples can be applied to Apple Macintosh systems with little adaptation. We encourage you to print this article and read it at your computer, trying each step as you go. Before you start, use Prism's **View** menu to make sure that the Navigator and all optional toolbars are displayed on your computer.



The Welcome dialog displays eight choices for the graph type. You can switch freely between the buttons, reading the "Selected graph" descriptions if you don't find the thumbnail illustrations clear. Choose the top-center button—for grouped vertical bars.

With the graph type selected, indicate that you will enter data into subcolumns as **3 replicates**. That means that you will enter your replicate measurements, and Prism will compute the means and standard deviations or standard errors automatically. Click **OK** to exit the Welcome dialog. Prism creates and displays the new table.

Data entry for two-way ANOVA in Prism differs from that in most other statistics programs. Other programs require you to enter data in a three-column indexed format—all measurements in one column and the levels of the two factors in two parallel columns. With Prism, you enter your data onto a table as you'd normally illustrate the results, with the row-and-column position of each datum indicating the factor level. The data table contains an X column, which holds the levels of one grouping variable. Since we indicated in the previous dialog that we will enter data as 3 replicates, Prism subdivides each data set (A, B, C, ...) to make room for our replicates (A:Y1, A:Y2, A:Y3; B:Y1, B:Y2, B:Y3; etc.). Each data set represents a level of the other grouping variable.

Enter the values show below into your table, including column headings.

	X Labels	A			B		
	Treatment	Before exercise			During exercise		
	X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	None	75	70	73	131	125	140
2	Lesion	69	80	81	175	174	169
3	Lesion+Drug	78	75	76	134	126	137

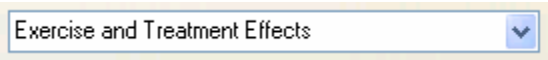
Although we instructed Prism to format the table for "3 replicates", in this example, the values in subcolumns Y1, Y2, and Y3 are matched measurements, not simple replicates (we'll indicate that when we enter the two-way ANOVA analysis parameters later). This affects Prism's tolerance for missing values:

- If you request ordinary two-way ANOVA (no matching), Prism will work even if some values are missing. You must have at least one value for each condition in each row. For example, if your data table is formatted for triplicate values (as in the example), you can't leave all three values empty.
- If you specify repeated-measures two-way ANOVA (row or column matching), Prism will allow you to have a different number of subjects in one data set than you do in another, but within each data set you must have the same number of subjects on each row.

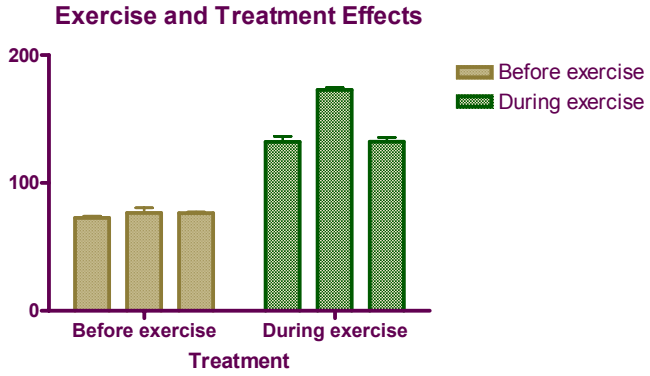
> In the Welcome dialog, we could have specified a data entry format of mean; standard deviation or standard error; and N (unless you want to do repeated-measures analysis, in which case you must provide individual replicate measurements).

	X Labels	A			B		
	Treatment	Before exercise			During exercise		
	X	Mean	SD	N	Mean	SD	N
1	None	72.7	2.5	3	132.0	7.5	3
2	Lesion	76.7	6.7	3	172.7	3.2	3
3	Lesion+Drug	76.3	1.5	3	132.3	5.7	3

Click on the default table name (drop-down list on the toolbar). Type a new name for the table. That name will be used as the title for your graph, although you are free to name the graph independently.



Click on the yellow **Graphs** tab on the toolbar to view the bar chart that Prism has produced automatically.



Note that the error bars on this graph will show standard error (*SEM*) rather than standard deviation (*SD*) if that is the default setting in Prism (to check this, choose **Edit... Preferences...**, select the **Graph** tab, and check the **Error Bar** settings). If you don't want to change the default setting to *SD* error bars but you do want the error bars to show *SD* on *this* graph, double-click on one of the bars to open the **Format Bars** dialog. Choose the **Appearance** tab, use the button to change all data sets at once,

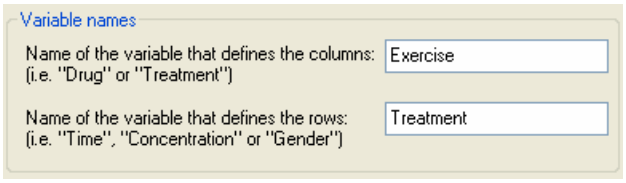
and set **Error values** to **Mean & SD**.

Performing the Analysis

Click **Analyze**. In the **Analyze Data** dialog, select **Statistical analyses** and **Two-way ANOVA**. Accept the default setting to analyze **All data sets**.

> If you have three or more data sets, you can choose to limit the analysis to only some of the data sets by choosing **Selected data sets** and clicking **Select** to open the **Select Data Sets** dialog and tell Prism which sets to analyze. If you later change your mind about which data sets to analyze, click the **Change** button and choose **Data Analyzed...**.

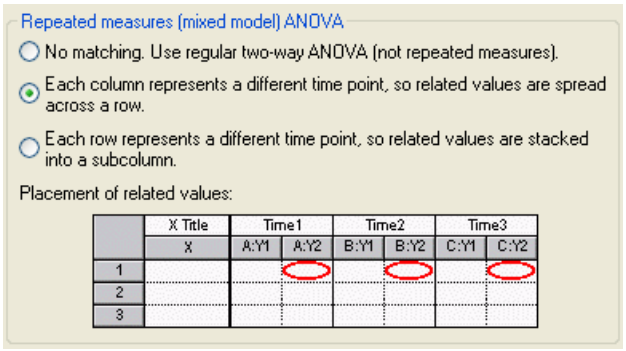
In the **Parameters: Two-Way ANOVA** dialog box, enter names for the factors (variable names) defining the columns and the rows. This step is optional. It doesn't affect the computations; it just makes the output easier to follow.



Now tell Prism whether or not you have repeated measures, and if so, whether the measurements are repeated row-wise or column-wise. We'll assume that subjects were assigned to only one of the three treatment groups (None, Lesioned, Lesioned + drug treated) but that measurements were made on each subject both before and during exercise. This is matching by row—the value in *row 1*, subcolumn A:Y1 was obtained from the same subject as the value in *row 1*, subcolumn B:Y1.

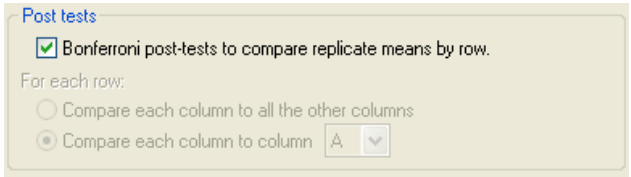
	X Labels	A			B		
	Treatment	Before exercise			During exercise		
	X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	None	75.0	70.0	73.0	131.0	125.0	140.0
2	Lesion	69.0	80.0	81.0	175.0	174.0	169.0
3	Lesion+Drug	78.0	75.0	76.0	134.0	126.0	137.0

The dialog setting is...



Note that the **Repeated measures** section will be greyed-out if you do not provide individual replicate values.

Finally, select the Bonferroni post-test option.



> You can get more help with these settings by clicking **Help Me Decide** at the bottom of the Parameters dialog box.

Viewing and Understanding the Results

When you leave the Parameters dialog, Prism runs the analysis. The Results page for this example consists of two views. You can switch between the views (**Tabular results** and **Narrative results**) in the drop-down box on the toolbar or in the Navigator. Prism displays the **Tabular results** view first, indicating significant effects of both exercise and treatment, and interaction between the two factors (the effect of one factor is influenced by the level

of the other factor, e.g., the difference in heart rate before exercise and heart rate during exercise is not the same with all treatments).

Source of Variation	% of total variation	P value
Interaction	5.70	0.0008
Exercise	7.06	0.0004
Treatment	86.07	P<0.0001
Subjects (matching)	0.5858	0.5041
Source of Variation	P value summary	Significant?
Interaction	***	Yes
Exercise	***	Yes
Treatment	***	Yes
Subjects (matching)	ns	No

The results show that, in this case, matching of subjects was not effective in controlling for variability between subjects. If you click the "Analysis Check List" button

and request information on subject matching—choose **How to think about results from two-way ANOVA…Subject (matching)**—Prism advises you to reconsider using repeated-measures ANOVA.

Further down the **Tabular results** view, the ANOVA table is presented

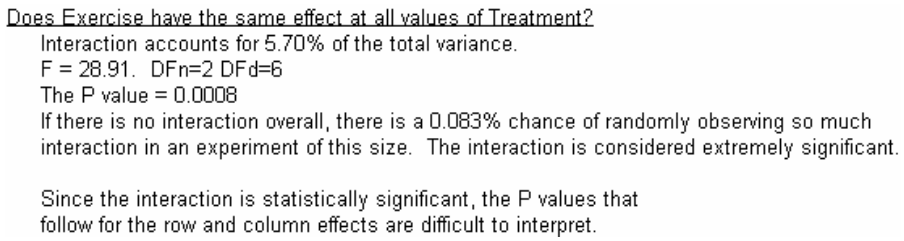
Source of Variation	Sum-of-squares	Mean square	F
Exercise	1832	916.2	36.17
Treatment	22330	22330	873.8
Subjects (matching)	152.0	25.33	0.9913
Residual	153.3	25.56	

as well as the post test results.

Bonferroni posttests			
Exercise	During exercise	Difference	95% CI of diff.
None	132.0	59.33	45.76 to 72.90
Lesion	172.7	96.00	82.43 to 109.6
Lesion+Drug	132.3	56.00	42.43 to 69.57
Exercise	t	P value	Summary
None	14.37	P<0.001	***
Lesion	23.26	P<0.001	***
Lesion+Drug	13.57	P<0.001	***

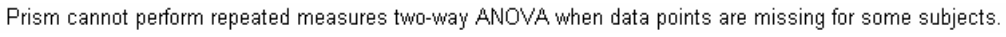
For an easy-to-understand synopsis of the results, switch to the **Narrative results** view.

Here's a partial illustration of the narrative:



The narrative points out the "extremely significant" interaction (very small interaction P value), which makes the P values for factor (row and column) effects hard to interpret. It is particularly worthwhile in that case to investigate the results of the post tests. If the interaction P value had been high, suggesting a consistent difference from before to during exercise among all treatments, the post tests would not have been very helpful.

If Prism cannot do the analysis (e.g., you request a repeated-measures analysis, but data are missing from one or more cells), the **Tabular results** view is empty, and you can read an explanation in the **Narrative results** view.



While Prism makes two-way ANOVA very simple, it does not offer all the options of a high-end statistics program. Specifically, Prism cannot do the following:

- Two-way nonparametric ANOVA
- Post testing between row means
- Post testing between column means

Reconfiguring the Analysis

You can easily change the parameters for, and then re-run, your ANOVA. In the Navigator, choose any of the sheets containing the analysis results. Click the **Change** button and then choose **Analysis Parameters...**. The **Parameters: Two-Way ANOVA** dialog reappears. Make your changes, then click **OK** to recompute the analysis and display the new results.