

One-Way ANOVA and Nonparametric Analyses

Prism offers four ways to compare three or more sets of data grouped by a single factor or category: regular ANOVA, repeated-measures ANOVA, Kruskal-Wallis test, and Friedman test. Following ANOVA, Prism can perform the Bonferroni, Tukey, [Student] Newman-Keuls, or Dunnett's post test. Following nonparametric analysis, Prism can perform Dunn's post test.

In this step-by-step example, we'll demonstrate a simple repeated-measures ANOVA followed by the Tukey post test, but we'll point out other options as we go. To learn more about analysis of variance, consult the ANOVA sections of the companion *Statistics Guide*. If you have only two data groups to compare, see the Step-by-Step Example "Performing a *t* Test". If you have more than two groups, but the data are grouped by *two* factors, refer to "Two-Way ANOVA with Post Tests".

This article includes the following techniques:

- Performing a one-way analysis of variance or nonparametric analysis with post testing, using Prism to help you choose the correct test
- Computing and displaying column (descriptive) statistics

Entering and Graphing the Data

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

Suppose that you measure the mean blood pressures of six animals during a pre-treatment control period, then while administering a neural stimulus alone, in the presence of a placebo drug, and in the presence of a putative antagonist designed to inhibit the effect of neural stimulation. The data are matched, since each treatment is repeated in succession with each subject. Since the data are organized by one grouping variable, *Treatment*, which has four levels—Control, Stimulus alone, Stimulus plus placebo, and Stimulus plus antagonist, select the tab for **One grouping variable**.

The Welcome dialog now displays 10 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. Note that you are not limited to using bars on a column (one-grouping-variable) graph; you may also choose dot plots, box-and-

©2003 GraphPad Software, Inc. All rights reserved. GraphPad Prism is a registered trademark of GraphPad Software, Inc. Use of the software is subject to the restrictions contained in the software license agreement.

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 <u>www.graphpad.com</u>. 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.

whiskers configurations, etc. The top-middle button—for vertical bars—is the correct choice for our intended graph. Click **OK** to exit the Welcome dialog. Prism creates and displays the new table.

The table contains no X column, and for each data set (A, B, C, ...; corresponding to a level of the grouping variable), there is a single Y column. With no X values, there is no categorical distinction from row to row, so replicate values can now be stacked vertically within single columns.

Click the default table name in the drop-down list on the toolbar. Type a new name for the table. This name will be used as the title for your graph and in the names of other linked sheets (you may, however, name linked sheets independently).

Effect of Neural Stimulation on Blood Pressure

Enter the values show below into your table. Be sure to include column headings. The name of the grouping variable is not included on the table, but the levels are identified in the Y-column headings "Control", "Stimulus", "+Placebo", and "+Antagonist".

	Α	В	С	D
	Control	Stimulus	+Placebo	+Antagonist
	Y	Y	Y	Y
1	90	117	120	98
2	88	116	118	90
3	96	116	109	105
4	94	131	127	97
5	91	123	127	99
6	97	132	129	108

If you want to change the number format, select the columns you want to change, then choose **Change... Number Format...**

When you choose a graph type under the **One grouping variable** tab, Prism omits the X column from the table, but you may find it useful to add an X column simply to keep track of subject identities, as shown here:

	X Labels	Α	В	С	D
	Animal No.	Control	Stimulus	+Placebo	+Antagonist
	Х	Y	Y	Y	Y
1	W-1078	90	117	120	98
2	W-1079	88	116	118	90
3	W-1080	96	116	109	105
4	N-562	94	131	127	97
5	W-1129	91	123	127	99
6	N-588	97	132	129	108

To include an X column without causing Prism to graph the data as if it had two grouping variables, start with a graph type under the **One grouping variable** tab, then from the data table, select **Change... Format Data Table ...** and change the X-column format to **Text (bar graph)**.

As soon as you have entered your data, Prism creates a graph automatically. In the Navigator, choose the sheet **Effect of Neural Stimulation on Blood Pressure graph** (or the name you gave to the data table earlier).

You could also format your table for entry of mean, standard deviation or standard error, and N. Choose a graph type from the **One grouping variable** tab, then from the data table, select **Change.. Format Data Table ...** and change the Y Columns setting to **Mean, Standard Deviation, N** (or other desired format).

	A			В			
	Control		Stimulus				
	Mean	SD	N	Mean	SD	N	N
1	92.7	3.6	6	122.5	7.5	6	

With this format, you can't perform repeated-measures or nonparametric analyses, which require raw data.

Click on the yellow **Graphs** on the toolbar to view the default graph.



Effect of Neural Stimulation on Blood Pressure

If the baseline labels overlap, you can either select the baseline and reduce the font size

or select and drag to elongate the baseline.



A

We won't discuss the graph further; for more information about working with one-grouping-variable graphs, see the Step-by-Step Example "Bar Graphs with One Grouping Variable".

Performing the Analysis

Click on the yellow **Data** tab to return to the data table. Click **Analyze**. In the **Analyze Data** dialog, select **Statistical analyses** and **One-way ANOVA (and nonparametric)**.

Prism compares three or more sets of data grouped by one factor in four ways. The correct approach depends on whether observations are matched by subject at each factor level and whether you assume that measurements are drawn from a population that follows a normal (Gaussian) distribution. Your options are outlined below.

		Assume Gaussian distribution?		
		Yes – Parametric test	No – Nonparametric test	
No – data NOT Repeated matched by subject Regi		Regular ANOVA	Kruskal-Wallis test	
measures?	Yes – data matched by subject	Repeated-measures ANOVA	Friedman test	

You don't have to memorize this table; Prism can help you with your choice.

In the **Parameters: One-way ANOVA (and Nonparametric)** dialog box, you have two options for selecting the appropriate test.

One option is to set the checkboxes according to (a) whether or not you have repeated measures and (b) whether or not you're assuming that measurements are drawn from a population that follows a Gaussian distribution. Prism provides the test name automatically:

Choose Test
You may either choose a test by checking the two option boxes, or you may choose a test by name below.
Repeated measures test. Values in each row represent matched observations.
Nonparametric test. Don't assume Gaussian distributions.
Test Name: Repeated Measures ANOVA

The other option is to select the desired test from the **Test Name** drop-down box. In this case, Prism sets the checkboxes for you:

- Choose Test				
You may either choose a test by checking the two option boxes, or you may choose a test by name below.				
Repeated measures test. Values in each row represent matched observations.				
Nonparam	etric test. Don't assume Gaussian distributions.			
Test Name:	Repeated Measures ANOVA			
- Post Test	Kruskal-Wallis test			
FUSITESI	One-way analysis of variance			
Test Name:	Repeated Measures ANOVA No Post Test			

Next, choose the post test, if any. Set the checkbox to tell Prism whether or not to do a post test when, on the basis of the *F* statistic computed in the course of the ANOVA or nonparametric analysis, it does not find an overall difference in group means. Then choose the post test from the **Test Name** drop-down list.

Post Test	✓ Only compute post test if overall P < 0.05	
Test Name:	Tukey: Compare all pairs of columns.	
	No Post Test	
	Tukey: Compare all pairs of columns.	
L	Newman-Keuls:Compare all pairs of columns 🛵	
Options	Bonferroni: Compare all pairs of columns.	
Confidence In	Bonferroni: Compare selected pairs of columns.	
Dunnett: Compare all columns vs. control column		
L	Test for linear trend between mean & col. number	

You can get help choosing both the basic analysis and the post test when you click **Help me decide** at the bottom of the Parameters dialog box.

Before leaving the Parameters dialog, check the box for the optional table of descriptive statistics.

Viewing and Understanding the Results

When you leave the Parameters dialog, Prism runs the analysis. The Results page for this example consists of three views (you can switch among the views (**Tabular results**, **Commentary**, **Column statistics**) either in the drop-down box on the toolbar or in the Navigator.

Prism displays the Tabular results view first, indicating whether there is an overall difference in means

Effect of Neural Stimulation on Blood Pressure	
Repeated Measures ANOVA	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	58.42
R squared	0.9212

and whether subject matching was effective (and hence increased the power to detect differences among means).

Was the pairing significantly effective?	
R squared	0.09355
F	3.927
P value	0.0178
P value summary	*
Is there significant matching? (P < 0.05)	Yes

The ANOVA table is presented

ANOVA Table	SS	df	MS
Treatment (between columns)	4198	3	1399
Individual (between rows)	470.3	5	94.07
Residual (random)	359.3	15	23.96
Total	5028	23	

as well as the post test results.

Tukey's Multiple Comparison Test	Mean Diff.	q	P value
Control vs Stimulus	-29.83	14.93	P < 0.001
Control vs +Placebo	-29.00	14.51	P < 0.001
Control vs +Antagonist	-6.833	3.420	P > 0.05
Stimulus vs +Placebo	0.8333	0.4171	P > 0.05
Stimulus vs +Antagonist	23.00	11.51	P < 0.001
+Placebo vs +Antagonist	22.17	11.09	P < 0.001

You can get help understanding the results by clicking the "Analysis Check List" button on the toolbar:

Ŷ

If Prism cannot do the analysis (e.g., you request a repeated-measures analysis, but data are missing from one or more cells), this is indicated on the **Tabular results** view also, and you are referred to the **Commentary** view.

Table Analyzed
Effect of Neural Stimulation on Blood Pressure
Error:See Commentary
Results F, Tabular results F, Commentary F, Column statistics

ANOVA: Overview

Table analyzed:Effect of Neural Stimulation on Blood Pressure This test cannot be performed with missing values.

Finally, choose the **Column statistics** view.

	Control	Stimulus	+Placebo	+Antagonist
	Y	Y	Y	Y
Number of values	6	6	6	6
Minimum	88.00	116.0	109.0	90.00
25% Percentile	89.00	116.0	113.5	93.50
Median	92.50	120.0	123.5	98.50
75% Percentile	96.50	131.5	128.0	106.5
Maximum	97.00	132.0	129.0	108.0
Mean	92.67	122.5	121.7	99.50
Std. Deviation	3.559	7.450	7.581	6.348
Std. Error	1.453	3.041	3.095	2.592
Lower 95% Cl	88.93	114.7	113.7	92.84
Upper 95% Cl	96.40	130.3	129.6	106.2

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: One-way ANOVA (and Nonparametric)** dialog reappears. Make your changes, then click **OK** to recompute the analysis and display the new results.