

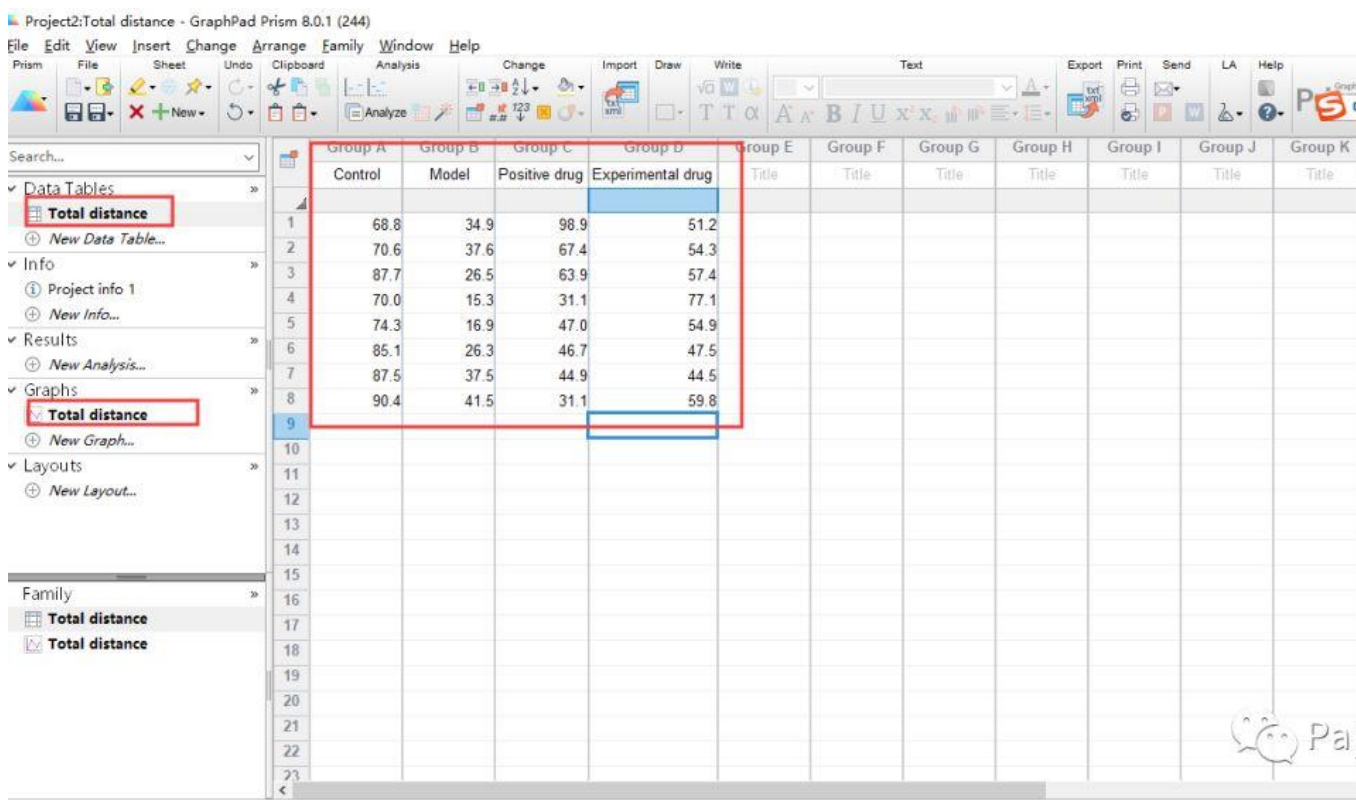
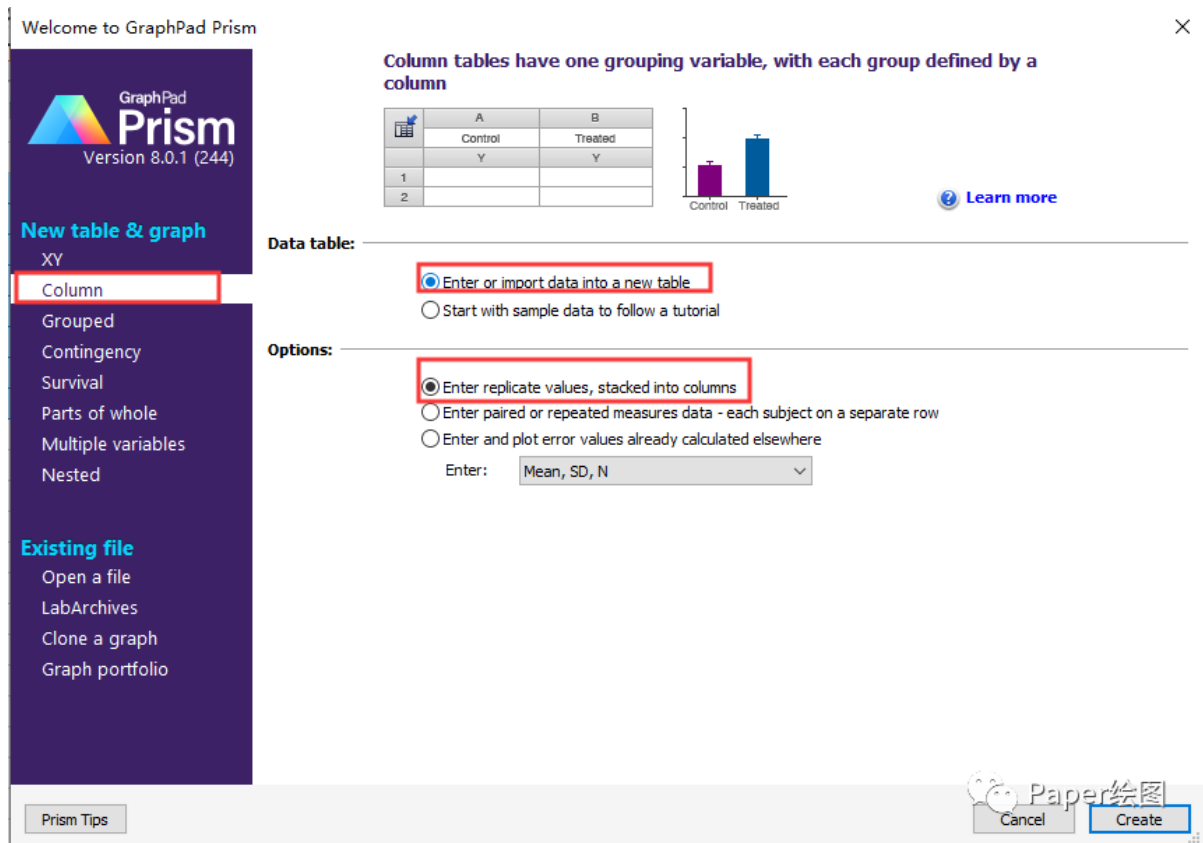
如何使用 Graphpad 做单因素方差分析

方差分析就是对试验数据进行分析，检验方差相等的多个正态总体均值是否相等，进而判断各因素对试验指标的影响是否显著，根据影响试验指标条件的个数可以区分为单因素方差分析、双因素方差分析和多因素方差分析。试验中要考察的指标称为试验指标，影响试验指标的条件称为因素，因素所处的状态称为水平，若试验中只有一个因素改变则称为单因素试验，若有两个因素改变则称为双因素试验，若有多个因素改变则称为多因素试验。——《百度百科》今天，我们就以动物行为学数据进行一下示范，因为影响因素只有药物，所以我们应该使用单因素方差进行分析的方法。

我们回顾一下进行单因素方差的基本步骤：**a.** 对各个组数据进行正态性检验 **b.** 对各个组数据进行方差齐性检验 **c.** 进行各个组之间均值的比较（单因素方差分析）1. 下面是我们的数据，我们首先进行数据的统计描述

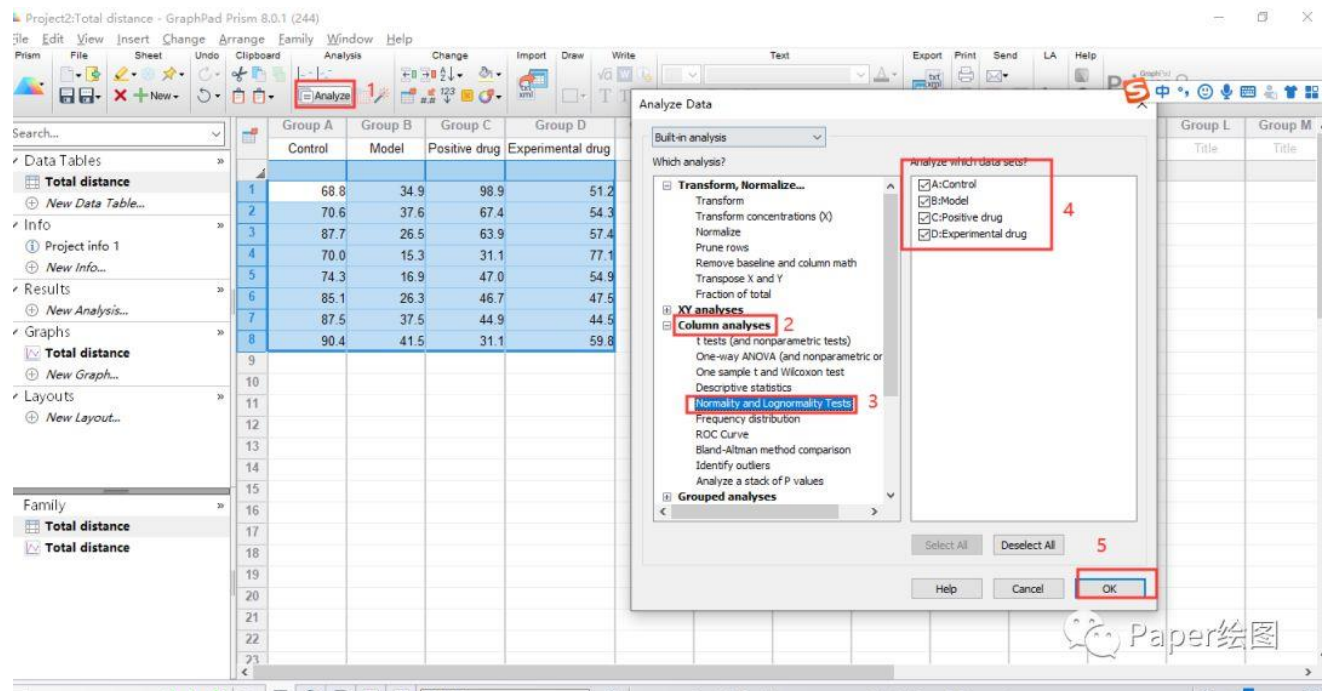
Control	Model	Positive drug	Experimental drug
68.8	34.9	98.9	51.2
70.6	37.6	67.4	54.3
87.7	26.5	63.9	57.4
70	15.3	31.1	77.1
74.3	16.9	47	54.9
85.1	26.3	46.7	47.5
87.5	37.5	44.9	44.5
90.4	41.5	31.1	59.8

2. 首先打开 Graphpad 软件，输入数据，更改组名，更改 Data1 为 Total distance



3. 进行数据正态性检验：选中数据 – Analyze - Column analyses - Normality and Log normality Tests -选中组别

-OK



4. 进行正态性检验软件参数设置

Parameters: Normality and Lognormality Tests

Which distribution(s) to test?

☒ Normal (Gaussian) distribution

☐ Lognormal distribution

☐ Compute the relative likelihood of sampling from a Gaussian (normal) vs. a lognormal distribution (assuming no other possibilities)

Methods to test distribution(s)

☒ Anderson-Darling test

☒ D'Agostino-Pearson omnibus normality test

☒ Shapiro-Wilk normality test

☒ Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefors P value

Graphing options

☒ Create a QQ plot

Subcolumns

☒ Average the replicates in each row, and then perform the calculation for each column

☐ Perform calculations on each subcolumn separately

☐ Treat all the values in all subcolumns as single set of data

Calculations

Significance level (alpha)

Output

Show this many significant digits (for everything except P values):

P value style: GP: 0.1234 (ns), 0.0332 (*), 0.0021 (**), N =

☐ Make these choices the default for future analyses.

Learn Cancel OK

5. 查看结果，因为选中了四种统计方法，所以出现了四种统计结果来进行高斯分布的描述，我们只看一种就可以了。四种方法均显示, Passed normality test (alpha=0.05), P value summary 为 ns。因此，可以进行单因素方差分析了。

Parameters: One-Way ANOVA (and Nonparametric or Mixed) ✕

Experimental Design Repeated Measures Multiple Comparisons Options Residuals

Experimental design

☒ No matching or pairing

☐ Each row represents matched, or repeated measures, data

	Group A	Group B	Group C	Group D
	Data Set-A	Data Set-B	Data Set-C	Title
	Y	Y	Y	Y
1				
2				

Assume Gaussian distribution of residuals?

☒ Yes. Use ANOVA.

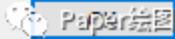
☐ No. Use nonparametric test.

Assume equal SDs?

☒ Yes. Use ordinary ANOVA test.

☐ No. Use Brown-Forsythe and Welch ANOVA tests.

Based on your choices (on all tabs), Prism will perform:
- Ordinary one-way ANOVA.

Learn Cancel 

8. 设定需要比较的组，或者两两比较也行

Parameters: One-Way ANOVA (and Nonparametric or Mixed) ✕

Experimental Design Repeated Measures Multiple Comparisons Options Residuals

Followup tests

☐ None.

☐ Compare the mean of each column with the mean of every other column. 两两比较

☒ Compare the mean of each column with the mean of a control column. 与特定的组比较

Control column: Column B : Model

☐ Compare the means of preselected pairs of columns.

Selected pairs:

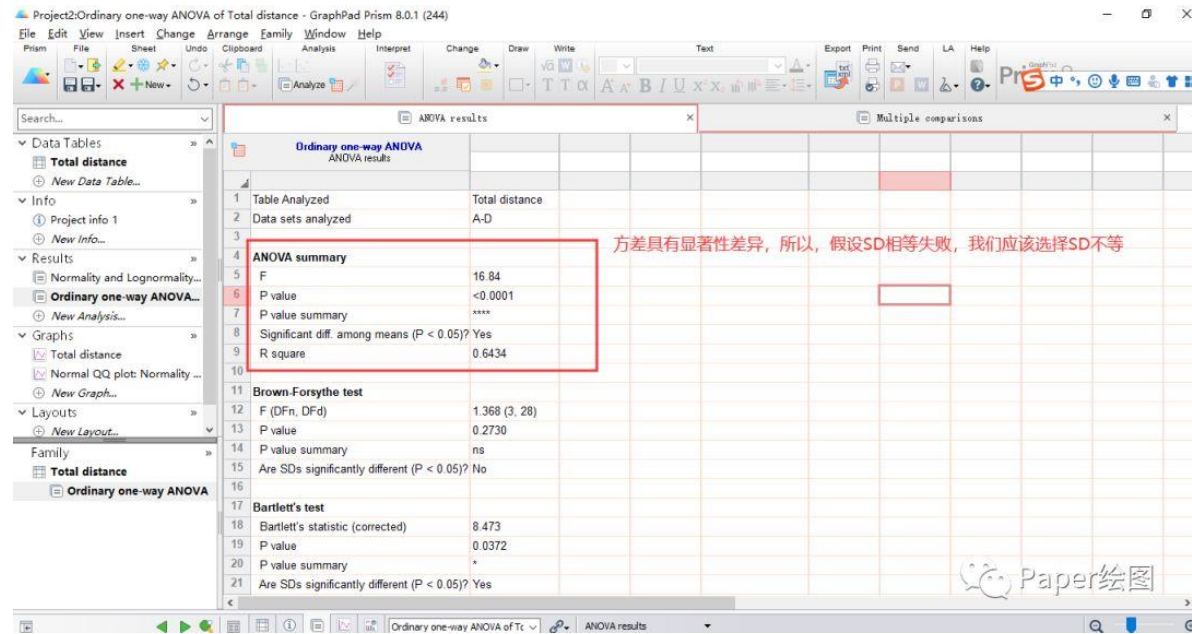
☐ Test for linear trend between column mean and left-to-right column order.

Which test?

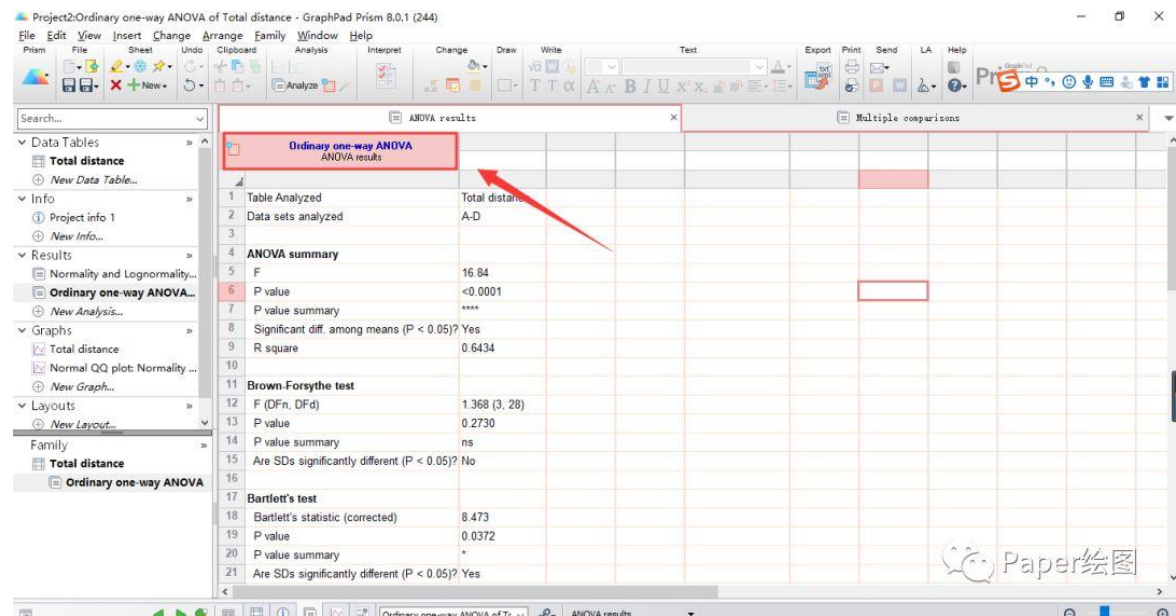
Use choices on the Options tab to choose the test, and to set the defaults for future ANOVAs.

 Paper 绘图

9. 方差如果相等的话就可以直接看结果了，但是方差不等，如下所示，那么我们应该假设方差不等。



10. 我们应该选择方差不等, 使用 Brown-Forsythe and Welch ANOVA tests。



Parameters: One-Way ANOVA (and Nonparametric or Mixed)

Experimental Design Repeated Measures Multiple Comparisons Options Residuals

Experimental design

☒ No matching or pairing
☐ Each row represents matched, or repeated measures, data

	Group A	Group B	Group C	Group D
	Data Set-A	Data Set-B	Data Set-C	Title
	Y	Y	Y	Y
1				
2				

Assume Gaussian distribution of residuals?

☒ Yes. Use ANOVA.
☐ No. Use nonparametric test.

Assume equal SDs?

☐ Yes. Use ordinary ANOVA test.
☒ No. Use Brown-Forsythe and Welch ANOVA tests.

Based on your choices (on all tabs), Prism will perform:

- Brown-Forsythe and Welch ANOVA tests.
- Dunnett's T3 multiple comparisons test, with individual variances computed for each comparison.

Learn Cancel OK

11. 然后查看结果，看看之间的比较是否具有显著性差异。这里显示出了 Significant，星号，P 值等。

Project2: Brown-Forsythe and Welch ANOVA tests of Total distance - GraphPad Prism 8.0.1 (244)

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Search...

Data Tables

- Total distance
- New Data Table...

Info

- Project info 1
- New Info...

Results

- Normality and Lognormality...
- Brown-Forsythe and Welch ANOVA tests**
- New Analysis...

Graphs

- Total distance
- Normal QQ plot: Normality...
- New Graph...

Layouts

- New Layout...

Family

tal distance

Brown-Forsythe and Welch ANOVA tests

ANOVA results

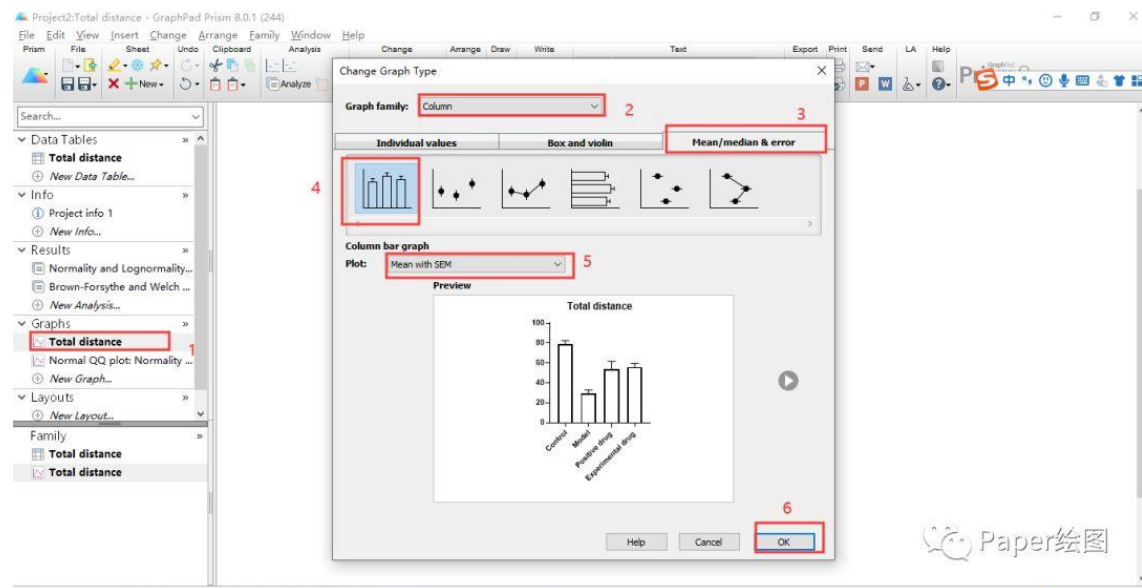
Brown-Forsythe and Welch ANOVA tests

Multiple comparisons

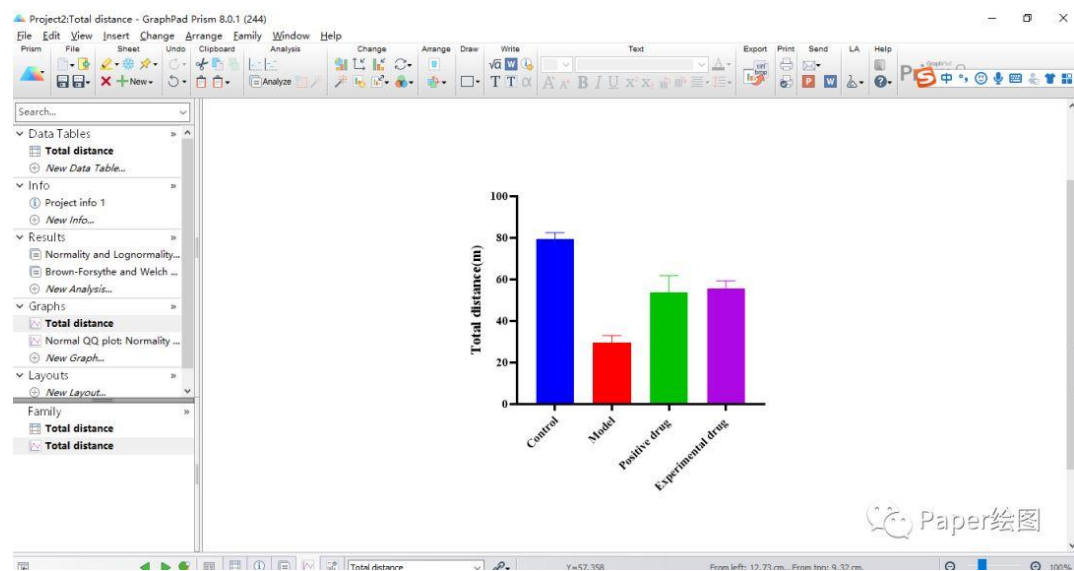
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	B?
1 Number of families	1					
2 Number of comparisons per family	3					
3 Alpha	0.05					
4						
5 Dunnett's T3 multiple comparisons test						
6 Model vs. Control	-49.74	-62.30 to -37.18	Yes	****	<0.0001	A Control
7 Model vs. Positive drug	-24.31	-48.40 to -0.2257	Yes	*	0.0479	C Positive drug
8 Model vs. Experimental drug	-26.28	-39.31 to -13.24	Yes	***	0.0003	D Experimental drug
9						
10 Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2 t
11 Model vs. Control	29.56	79.30	-49.74	4.771	8	8 10.43
12 Model vs. Positive drug	29.56	53.88	-24.31	8.670	8	8 2.804
13 Model vs. Experimental drug	29.56	55.84	-26.28	4.956	8	8 5.302
14						
15						
16						
17						
18						
19						
20						
21						

Paper绘图

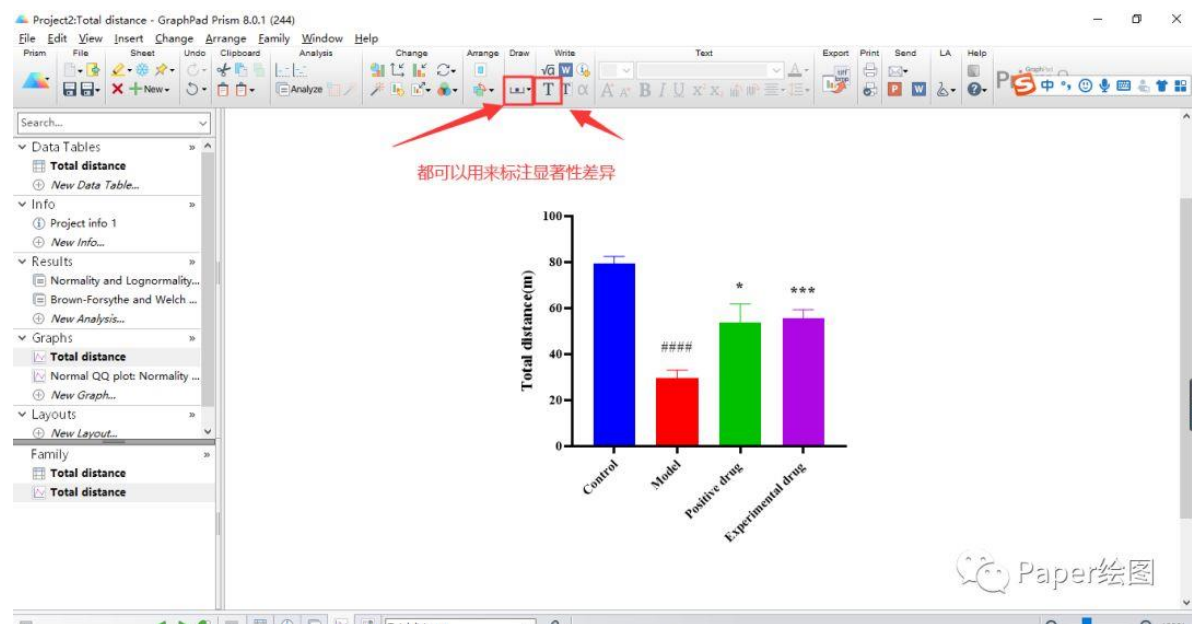
12. 作图，选择图形，Column，Mean/median&error，我们来做个基本的柱状图



13. 调色，改变标签。（不会的请看 Graphpad 做小提琴图和箱线图图等）。



14. 进行显著性标记，模型组和空白组我们使用#表示，模型组和给药组我们用*表示，进行标记。



15. 保存 Graphpad 文件，保存 TIFF 图片，300dpi。

