Supplementary File 2

Figure 2 Statistical Analysis

Statistical analysis of changes in total microglial area among LPS groups (generated by SigmaPlot)

**Two Way Analysis of Variance** Wednesday, August 08, 2018, 2:39:11 PM

**Data source:** Data 1 in Hippo LPS & Microglia activation time course.JNB

General Linear Model

Dependent Variable: Col 11

**Normality Test (Shapiro-Wilk):**  Passed (P = 0.233)

**Equal Variance Test (Brown-Forsythe):** Passed (P = 0.310)

**Source of Variation DF SS MS F P**

Time of Sacrifice 4 878065991.987 219516497.997 29.098 <0.001

Brain Region 1 12422693.929 12422693.929 1.647 0.206

Time of Sacri x Brain Region 4 15253052.944 3813263.236 0.505 0.732

Residual 48 362108709.006 7543931.438

Total 57 1268577294.681 22255742.012

The difference in the mean values among the different levels of Time of Sacrifice is greater than would be expected by chance after allowing for effects of differences in Brain Region. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Brain Region is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time of Sacrifice. There is not a statistically significant difference (P = 0.206).

The effect of different levels of Time of Sacrifice does not depend on what level of Brain Region is present. There is not a statistically significant interaction between Time of Sacrifice and Brain Region. (P = 0.732)

Power of performed test with alpha = 0.0500: for Time of Sacrifice : 1.000

Power of performed test with alpha = 0.0500: for Brain Region : 0.124

Power of performed test with alpha = 0.0500: for Time of Sacri x Brain Region : --

Least square means for Time of Sacrifice :

**Group Mean SEM**

6 h 25876.202 792.881

12 h 28809.566 792.881

24 h 37401.763 792.881

72 h 31607.900 734.066

2 wks 32068.222 971.077

Least square means for Brain Region :

**Group Mean**

PC 30681.738

HIPPO 31623.723

Std Err of LS Mean = 519.063

Least square means for Time of Sacri x Brain Region :

**Group Mean SEM**

6 h x PC 25412.930 1121.304

6 h x HIPPO 26339.474 1121.304

12 h x PC 28902.087 1121.304

12 h x HIPPO 28717.045 1121.304

24 h x PC 35988.436 1121.304

24 h x HIPPO 38815.090 1121.304

72 h x PC 31334.477 1038.125

72 h x HIPPO 31881.322 1038.125

2 wks x PC 31770.761 1373.311

2 wks x HIPPO 32365.684 1373.311

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: **Time of Sacrifice**

**Comparison Diff of Means t P P<0.050**

24 h vs. 6 h 11525.562 10.279 <0.001 Yes

24 h vs. 12 h 8592.197 7.663 <0.001 Yes

24 h vs. 72 h 5793.864 5.362 <0.001 Yes

72 h vs. 6 h 5731.698 5.305 <0.001 Yes

2 wks vs. 6 h 6192.021 4.939 <0.001 Yes

24 h vs. 2 wks 5333.541 4.254 <0.001 Yes

12 h vs. 6 h 2933.365 2.616 0.047 Yes

2 wks vs. 12 h 3258.656 2.599 0.037 Yes

72 h vs. 12 h 2798.333 2.590 0.025 Yes

2 wks vs. 72 h 460.323 0.378 0.707 No

Statistical analysis of changes in number of microglial with an area > 300 µm among LPS groups (generated by SigmaPlot)

**Two Way Analysis of Variance** Wednesday, August 08, 2018, 3:07:36 PM

**Data source:** Data 1 in Hippo LPS & Microglia activation time course.JNB

General Linear Model

Dependent Variable: > 300 µm

**Normality Test (Shapiro-Wilk):**  Passed (P = 0.411)

**Equal Variance Test (Brown-Forsythe):** Passed (P = 0.333)

**Source of Variation DF SS MS F P**

Time of Sacrifice 4 7763.524 1940.881 19.242 <0.001

Brain Region 1 288.532 288.532 2.861 0.097

Time of Sacri x Brain Region 4 98.668 24.667 0.245 0.912

Residual 48 4841.566 100.866

Total 57 12997.234 228.022

The difference in the mean values among the different levels of Time of Sacrifice is greater than would be expected by chance after allowing for effects of differences in Brain Region. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Brain Region is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time of Sacrifice. There is not a statistically significant difference (P = 0.097).

The effect of different levels of Time of Sacrifice does not depend on what level of Brain Region is present. There is not a statistically significant interaction between Time of Sacrifice and Brain Region. (P = 0.912)

Power of performed test with alpha = 0.0500: for Time of Sacrifice : 1.000

Power of performed test with alpha = 0.0500: for Brain Region : 0.267

Power of performed test with alpha = 0.0500: for Time of Sacri x Brain Region : --

Least square means for Time of Sacrifice :

**Group Mean SEM**

6 h 27.097 2.899

12 h 33.353 2.899

24 h 59.872 2.899

72 h 46.274 2.684

2 wks 45.896 3.551

Least square means for Brain Region :

**Group Mean**

PC 40.228

HIPPO 44.768

Std Err of LS Mean = 1.898

Least square means for Time of Sacri x Brain Region :

**Group Mean SEM**

6 h x PC 24.278 4.100

6 h x HIPPO 29.917 4.100

12 h x PC 30.844 4.100

12 h x HIPPO 35.861 4.100

24 h x PC 55.800 4.100

24 h x HIPPO 63.944 4.100

72 h x PC 45.929 3.796

72 h x HIPPO 46.619 3.796

2 wks x PC 44.292 5.022

2 wks x HIPPO 47.500 5.022

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: **Time of Sacrifice**

**Comparison Diff of Means t P P<0.050**

24 h vs. 6 h 32.775 7.994 <0.001 Yes

24 h vs. 12 h 26.519 6.468 <0.001 Yes

72 h vs. 6 h 19.177 4.854 <0.001 Yes

2 wks vs. 6 h 18.799 4.101 0.001 Yes

24 h vs. 72 h 13.598 3.442 0.007 Yes

72 h vs. 12 h 12.921 3.270 0.010 Yes

24 h vs. 2 wks 13.976 3.049 0.015 Yes

2 wks vs. 12 h 12.543 2.736 0.026 Yes

12 h vs. 6 h 6.256 1.526 0.249 No

72 h vs. 2 wks 0.378 0.0849 0.933 No