**Project Report 2: Change in Fluorescence in Mice Injuries**

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**Background**

The client wants to measure the change in fluorescence over time in mice with 3 different types of injuries (conditions): sham surgery (control), crush injury (more severe), cut injury (most severe). Measurements are taken in 3 time points: 2 days (acute stage), 10 days (regenerative stage), 60 days (healed stage). Change in fluorescence is measured with an examination of neurons and obtaining five different measurements: Rise Time, Peak Amplitude, Slope, DT1, DT2. Analysis for rise time is included in this report. Other measurements can be analyzed in the same manner.

**Data**

There are 45 animals in total. Observation 468 (Cut, Healed) was removed due to missing data. Each animal can only be used for one time point of each condition, and therefore each animal is assigned a Condition and a Time Point. For each animal, at the assigned time point, multiple measurements are taken. Number of measurements can vary from 7 to 20 and are considered pseudo replications since they are done on the same animal. Condition, Time Point, and Sex are considered independent variables. Random effect is included for Animal ID to account for the multiple observations per experimental unit at each time point.

The data appears to follow a non-linear trend going from Acute to Regeneration to Healed state in all three conditions. (Fig. 1) Because of the difficulty in producing a robust non-linear model with 3 categorical time points, all Sham time points are combined into a single group. This is possible because according to the client, there is no time effect in the Sham category. Time Point and Condition are also combined for Crush and Cut conditions to make one variable. In summary, Condition and Time Point are grouped together into a new independent variable called Group, with 7 levels:

Sham

Crush Acute

Crush Regeneration

Crush Healed

Cut Acute

Cut Regeneration

Cut Healed

Figure 1: Non-linear Relationship

**Method**

Overall alpha level of 0.05 was used for five models (from 5 variables) and Bonferroni correction was applied to the five models and an alpha level of 0.01 was used for each analysis. Proc Mixed procedure in SAS was utilized to carry out analysis to allow for correlation in data and non-constant variance. Random scatter of data points in the residual plot and the approximate linear relationship in the normal probability plot for Rise Time (Fig. 2) allow for the approximation of normality in data.



Fig 2: Residual plots for Rise Time.

Bonferroni stepdown procedure in Proc Glimmix was utilized to make pairwise comparisons on mean rise time for the following Group combinations:

Crush Acute - Sham

Crush Regeneration - Sham

Crush Healed - Sham

Cut Acute - Sham

Cut Regeneration - Sham

Cut Healed - Sham

Cut Acute - Crush Acute

Cut Regeneration - Crush Regeneration

Cut Healed - Crush Healed

**Results**

Descriptive statistics for Rise Time sorted by Group are provided in Table 1.

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Table 1: Descriptive Statistics

Initial analysis with Proc Mixed revealed that interaction between Group and Sex are statistically insignificant at significance level 0.01. (Table 2)



Table 2

The model was reanalyzed without the interaction and the results are shown in Table 3. Group is still statistically significant in determining Rise Time with a p-value of 0.0002. Sex is still statistically insignificant at significance level 0.01.



Table 3

Parameter estimates for Group and Sex are shown in Table 4. It is important to note here that estimates for Group 7(Cut Healed) and Sex Male are set to zero. There is only one statistically significant parameter estimate at alpha level 0.01: The intercept (1.4652) estimates the mean Rise Time for Males in Group 7(Cut Healed). The p-value (<0.0001) is from a test that this mean equals zero. Since p-value is <0.01, mean Rise Time for Males in Cut Healed group is significant.



Table 4: Parameter Estimates

Pairwise comparisons between specified group combinations are provided in Table 5. From results, only the difference in mean Rise Time for Sham and Cut Regeneration is significant at significance level 0.01.

Table 5: Pairwise Comparisons