BLOOD-BRAIN BARRIER EXPERIMENT

10. Analysis of Variance Model

The effects of design variables and covariates on the response can also be investigated by general factorial analysis of variance available in SPSS. This very useful statistical tool encompasses both analysis of variance and regression. It will be discussed in class after multiple regression techniques are covered. Thus you can skip this section now to return to the material later after the analysis has been covered in your lectures.

10.1 The Blood-Brain Barrier Experiment as a Factorial Experiment

The blood-brain barrier experiment is an example of a factorial experiment. A factorial experiment consists of several factors (sacrifice time, treatment) which are set at different levels, and a response variable (concentration ratio). The purpose of the experiment is to assess the impact of different combinations of the levels of sacrifice time and treatment type on the concentration ratio. Analysis of variance allows us to test the null hypothesis that the design variables (sacrifice time, treatment) and covariates have no impact on the response.

The General Factorial Procedure available in SPSS 8.0 provides regression analysis and analysis of variance for one dependent variable by one or more factors or variables. The SPSS data file used for this study is available in the SPSS file *brain.sav* located on the FTP server in the Stat252 directory. The design variables in this experiment, sacrifice time and treatment type can be both treated as categorical variables, which means they should be entered as factors in the GLM General Factorial procedure.

10.2 The ANOVA Output for the Blood-Brain Barrier Experiment

Analysis of variance allows us to test the null hypothesis that the design variables and covariates have no impact on the response. There are four sources of variation in the experiment: the main effects of design variables and covariates, the interaction effects, and the error variation. Corresponding to these four sources, there are several null hypotheses that may be tested. In particular, we test the following hypotheses:

1. H_0: No main effect of *Sacrifice Time*,
2. H_0: No main effect of *Treatment*,
3. H_0: No interaction effect between *Sacrifice Time* and *Treatment.*

To produce the output for this model, from the menus choose:

Statistics

General Linear Model

GLM- General Factorial…

- Dependent: *LNRATIO*
- Fixed Factor(s): *TIME, TREAT*
- Covariate(s): *DAYS, SEX, WEIGHT, LOSS, TUMOR*

Observe that the dependent variable is log-transformed concentration ratio, not ratio itself to make the assumption of equal variances satisfied. As we observed in Section 4.2, the variance of RATIO increases as TIME increases. The log transformation helps to compress the RATIO values uniformly over the range of TIME.

Model

♦ Full

The following output will be displayed:

Tests of Between-Subjects Effects

Dependent Variable: LNRATIO

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	160.121[a]	12	13.343	41.918	.000
Intercept	1.816E-02	1	1.816E-02	.057	.814
DAYS	4.982E-03	1	4.982E-03	.016	.902
SEX	3.830E-06	1	3.830E-06	.000	.997
WEIGHT	3.492E-03	1	3.492E-03	.011	.918
LOSS	1.219	1	1.219	3.831	.064
TUMOR	.509	1	.509	1.600	.220
TIME	70.570	3	23.523	73.898	.000
TREAT	5.356	1	5.356	16.826	.001
TIME * TREAT	.498	3	.166	.522	.672
Error	6.685	21	.318		
Total	232.373	34			
Corrected Total	166.806	33			

a. R Squared = .960 (Adjusted R Squared = .937)

The table contains rows for the components of the model that contribute to the variation in the dependent variable. The row labeled *Corrected Model* contains values that can be attributed to the regression model, aside from the intercept. The sources of variation are identified as *Days*, *Sex*, *Weight*, *Loss*, *Tumor*, *Time*, *Treat*, *Time*Treat* (interaction), and *Error*. *Error* displays the component attributable to the residuals, or the unexplained variation. *Total* shows the sum of squares of all values of the dependent variable. *Corrected Total* (sum of squared deviations from the mean) is the sum of the component due to the model and the component due to the error.

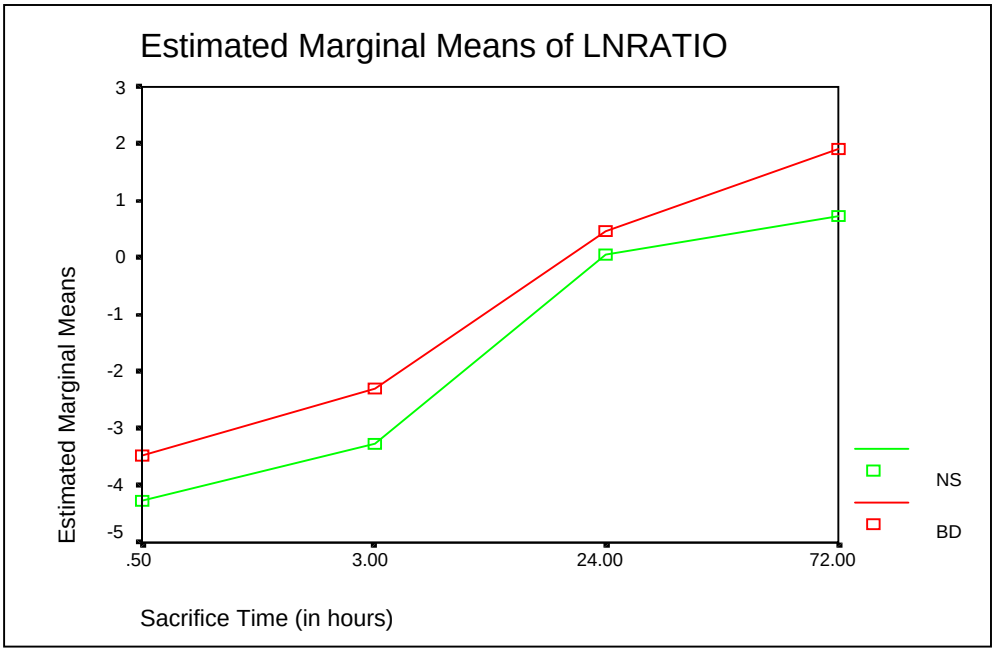
According to the output, the model sum of squares is 160.121 and the error sum of squares is 6.685. The total sum of squares (corrected total) is 167.806. Notice a very small contribution of error in the total sum of squares. The p-value of the F-test for the model is reported as 0.000 indicating convincing evidence of an effect of at least one of the factors on the response.

The sum of squares for the treatment factor is estimated to be 5.356. The value of the F-statistic equal to 5.356 and p-value of the F-test reported as 0.000 indicate very strong evidence of effect of treatment on the response. Although *Treatment* main effects are also statistically significant, they are not that strong as the main effects due to brand factor.

The p-value of the interaction term *Time*Treatment* is equal to 0.672, indicating no evidence of an interaction between the two factors. The table also shows that the covariates are not significant when the design variables are also included in the model.

10.3 Exploring the Interaction Effects

We have found above that there is no evidence of an interaction between sacrifice time and treatment. The same conclusion can be reached by examining the interaction effects with a profile plot. In general, profile plots (interaction plots) are useful for comparing marginal means in your model. A profile plot is a line plot in which each point indicates the estimated marginal mean of a dependent variable at one level of a factor. The plot for our data is displayed below.



The plot indicates that the rats subjected to the BD treatment had higher concentration ratios than those subjected to the control treatment for all four sacrifice time levels. The lines corresponding to the two levels of treatment are almost parallel. The parallelism in this chart indicates that there is little or no interaction between the two factors indicating no interaction between treatment type and sacrifice time. This conclusion reinforces the previous statements about the F test. In other words, the effect of the BD treatment is approximately the same for the four sacrifice time levels.

10.4 Estimating the Treatment Effect

In this section we will estimate the effect of blood-brain diffusion treatment (BD) on the effectiveness of the disruption method measured by *LNRATIO*. We have found before that the treatment effects are consistent across all sacrifice time levels. As we want to compare the BD to NS effects on the response variable *LNRATIO*, simple contrast will be used.

The SPSS output for the simple contrast applied to the *TREAT* factor is

Contrast Results (K Matrix)

TREAT Simple Contrast[a]			Dependent Variable LNRATIO
Level 1 vs. Level 2	Contrast Estimate		-.870
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		-.870
	Std. Error		.394
	Sig.		.036
	95% Confidence Interval for Difference	Lower Bound	-1.681
		Upper Bound	-6.03E-02

a. Reference category = 2

Test Results

Dependent Variable: LNRATIO

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1.515	1	1.515	4.878	.036
Error	8.077	26	.311		

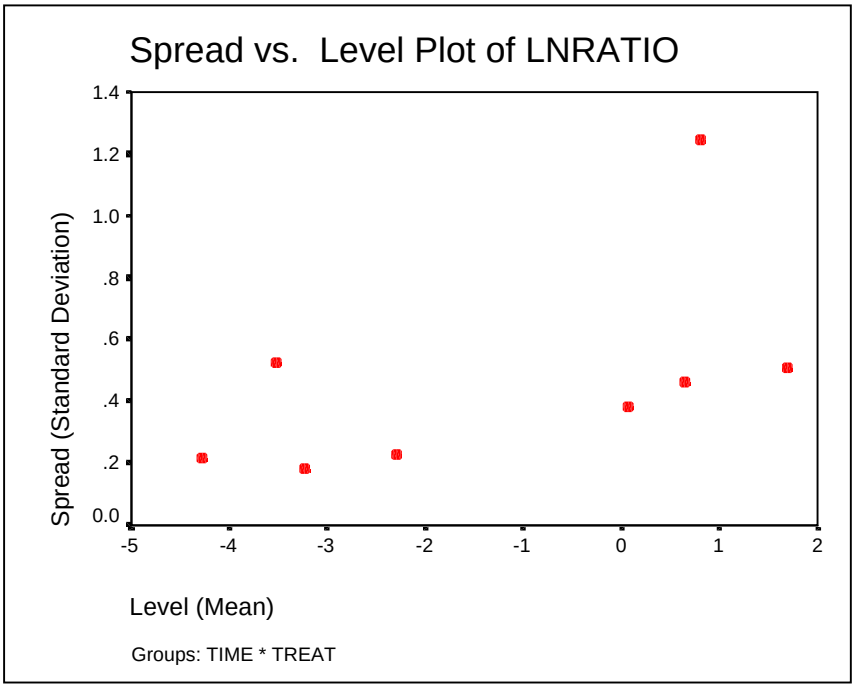
The p-value of two-sided test about the contrast is 0.036. Thus the p-value for the one-sided test is 0.036/2 = 0.018. This provides strong evidence of the effectiveness of the blood-brain diffusion treatment.

The point estimate of the contrast is -0.870 (NS versus BD). So, expressed in accordance with the interpretation for log-transformed responses, the median ratio of antibody concentration in the brain tumor to antibody concentration in the liver is estimated to be exp(.870) = 2.3869 times greater for the blood-brain diffusion treatment than for the saline control.

10.5 Diagnostics for the ANOVA Model

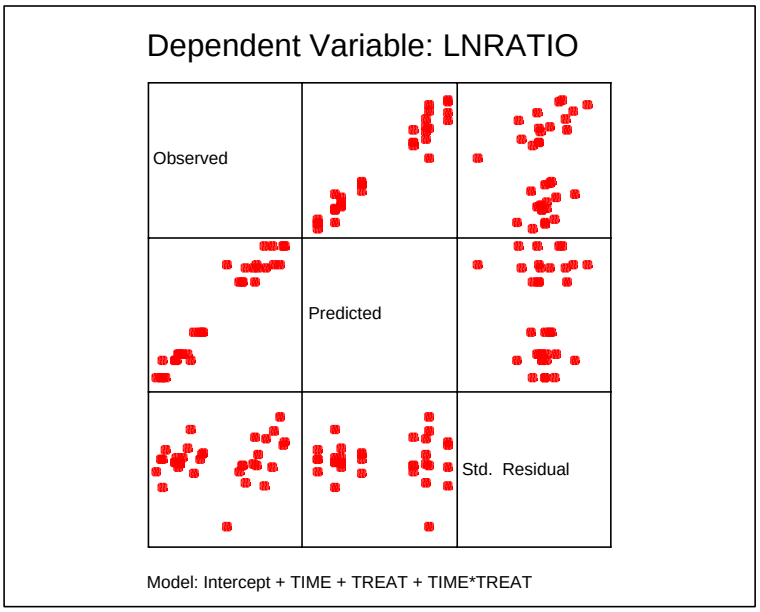
Now we examine the feasibility of the ANOVA model for the brain-barrier data. The GLM General Factorial procedure works under the assumptions that the data are a random sample from a normal population, and in the population all cell variances are the same.

The spread-versus-level plot shows that the assumption of equal variance of the error across all groups may be violated. The plot shows the spread for each of the eight combinations of the four levels of time and the two levels of treatment. The standard deviation for the group corresponding to time 72 and treatment NS is over 1.2, and is significantly higher than the standard deviation for the remaining groups. It is easy to check that the case 34 makes the standard deviation that large compared to the other groups. You can easily verify that without the case, the standard deviation for the group would be similar to the standard deviations in other groups.



The case 34 should be examined carefully in order to determine why the case produced the ratio that different from the values obtained for the other cases.

The scatterplot of observed values versus predicted values in the following matrix scatterplot shows that the ANOVA model provides a very good fit.



The Cook's distance for the case 34 is equal to 0.52, and it is significantly higher than the distance for the other observations.