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Analyzing the Behavior of Rats by Repeated Measurements

Kenita A. Hall

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by

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Under the direction of Yichuan Zhao

ABSTRACT

Longitudinal data, which is also known as repeated measures, has grown increasingly within the past years because of its ability to monitor change both within and between subjects. Statisticians in many fields of study have chosen this way of collecting data because it is cost effective and it minimizes the number of subjects required to produce a meaningful outcome. This thesis will explore the world of longitudinal studies to gain a thorough understanding of why this type of collecting data has grown so rapidly. This study will also describe several methods to analyze repeated measures using data collected on the behavior of both adolescent and adult rats. The question of interest is to see if there is a change in the mean response over time and if the covariates (age, bodyweight, gender, and time) influence those changes. After much testing, our data set has a positive nonlinear change in the mean response over time within the age and gender groups. Using a model that included random effects proved to be a better method than models that did not use any random effects. Taking the log of the response variable and using day as the random effect was overall a better fit for our dataset. The transformed model also showed all covariates except for age as being significant.

INDEX WORDS: Longitudinal Data, Repeated Measures, Mixed Models, Non-Linear Models

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Chapter One: REPEATED MEASURES

1.1 Introduction

Longitudinal studies sometimes known as repeated measures are used in many fields of study and the need to analyze this unique data is growing increasingly. Sometimes a distinction is drawn between longitudinal designs (where subjects are followed for extended periods of time) and repeated measures designs (where the measurements are collected over a relatively short period) (Ware, 1985). This thesis is focused on explaining longitudinal studies and finding the best model to analyze the data. Longitudinal data is the union of cross-sectional and time series data. As with many regression data sets longitudinal data measures a cross section of subjects but unlike most regression data sets longitudinal data observes the subjects repeatedly over time. Unlike time-series data, many subjects are observed and the number of measurements per subject is usually not large in longitudinal studies, (Frees, 2004). Studies that contain data on individuals who were measured repeatedly over time are defined as longitudinal studies.

(Fitzmaurice, Laird, and Ware, 2004) and (Lindsey, 1999) provide excellent overviews as well as general theoretical developments and examples of longitudinal data. Longitudinal data is used to study the changing patterns of the response variable and the factors that influence those changes both within and between individuals. Within subject effects are values that differ from measurement to measurement such as time and can only be achieved within a longitudinal study. Between subject effects are those values that change only from subject to subject and remain the same for all observations on a single subject such as treatment, gender or age.

One unique feature of longitudinal data is that they are clustered. The clusters contain repeated measurements obtained from a single individual at different occasions. The observations within a cluster will usually display a positive correlation and must be accounted for in the analysis, which means models used to analyze clustered data must account for and describe their correlation. Measurements on subjects within a cluster are more alike than measurements on subjects in different clusters. This assumption eliminates the assumption of independence that plagues the statistical world.

Repeated measures are a subset of longitudinal designs. The example used in this thesis consists of repeated measurements that will be analyzed using models that are appropriate for repeated observations.

1.2 Example

The data used in this thesis is from Mahin Shahbazi's paper "Age and Sex Differences in the Acquisition and Maintenance of Intravenous Amphetamine Self-Administration in Rats".

> The purpose of the study was to investigate differences in vulnerability to psycho-stimulant drugs such as amphetamine, cocaine or nicotine in adolescent vs. adult animal (this paper used amphetamine). An operant conditioning paradigm in which lever pressing behavior is maintained by i.v. drug delivery is used to create an animal model of human intake.
>
> Operant conditioning is a procedure in which a specific behavior is enhanced through the process of reinforcement. The subject's behavior determines whether or not a reinforcer will be given. A reinforcer or reward is only given when the subject produces the targeted response. It is assumed that a

subject will repeat a behavior if its consequences are rewarding. There are two parts to operant conditioning: the behavior (something the subject does) and the consequence (something that happens as a result of the behavior). In the i.v. drug self-administration example, lever pressing is the behavior and drug infusion is the consequence. If the behavior (lever pressing) increases when followed by the consequence (drug infusion) then drug infusion is considered a reinforcer.

Different schedules of reinforcement determines how much lever pressing behavior is required to receive a reinforcer and under what timetable. There are two common reinforcement schedules: fixed ratio (FR) and progressive ratio. Shahbazi's paper uses both but for the longitudinal study we will only focus on the fixed ratio. A reinforcement schedule is a rule that states under what conditions a reinforcer will be delivered. When a reinforcer follows every targeted response, the schedule is called a continuous reinforcement of fixed ratio 1 (FR1). The rule for a FR schedule is that a reinforcer is delivered after every *n* response, where *n* is the size of the ratio. Therefore, on a FR10 schedule there is a reinforcer after every 10 responses.

The rate of acquisition of intravenous amphetamine through self-administration was compared between periadolescents (ages 35-52 days) and adults (ages 89-106 days) male and female Spraque Dawley rats. The rats were housed in groups of 2-3 and placed in chambers and allowed to press between two levers, an active and an inactive that extended into the chambers at the start of each session. Pressing the active lever allowed the drug to be pumped from a syringe into the rat's jugular vein. Pressing the inactive lever resulted in no consequence but was used to determine whether or not the rats were able to discriminate between the two levers. Sessions were two hours in duration and repeated daily for 14 days. Sessions began when the two levers were extended into the chambers. Lever pressing was reinforced by i.v. injection of .05mg/kg/0.1ml amphetamine under a FR1, time-out (TO20) schedule. A time-out (TO20) schedule is where there was a 20 second pause after each infusion (an infusion was not allowed even if the rat pressed on the active lever). The concentration of the amphetamine solution was titrated daily to adjust for weight change.

Behavior (lever pressing) was measured over 14 days to determine if age, sex, and bodyweight (measured in grams) were factors in the changes of behavior over time. The random samples consisted of 39 rats (n=8 periadolescent male, n=7 adult male, n=12 periadolescent female, and n=12 adult female) with 14 observations each, for a total of 546 observations. The mean behaviors were 92.46 (male periadolescent), 58.24 (male adult), 73.84 (female periadolescent), and 62.78 (female adult).The mean body weights were 210.6 (male periadolescent), 406.9 (male adult), 166.7 (female periadolescent), and 257.9 (female adult). A graphical display of the behaviors at each occasion for each rat is shown in Figure 1.1

From the graph it can be seen that there is substantial within subject (the jagged appearance of the line segments) and between subject variability (some rats remain high throughout the study while others remain low). The graph also shows a slight increase in the responses over time along with increasing variance.

A simple t-test was done to determine if there was any difference between the age and gender groups. For the age group day 3 was chosen to be the time period for the first t-test. On day 3 $n_A = 19$, $\bar{x} = 46.08$, $\sigma = 21.11$, and $\sigma^2 = 445.63$ for the adults and for the periadolescents $n_P = 20$, $\bar{x} = 47.75$, $\sigma = 36.91$, and $\sigma^2 = 1362.35$ which gave a t value of .172 which is very small and shows that there is probably no difference in age groups. Day 8 was also chosen to test for any differences within the age groups. On day 8 $n_A = 19$, $\bar{x} = 72.32$, $\sigma = 37.02$, and $\sigma^2 = 1370.48$ for the adults and $n_P = 20$, $\bar{x} = 104.7$, $\sigma = 57.87$, $\sigma^2 = 3348.93$ for the periadolescents which resulted in a t value of 2.07. This value is much larger than day 3 and indicates that there maybe some differences in the age groups as the study progress. For the gender groups' day 4, day 7, and day 12 were chosen. On day 4 $n_m = 15$, $\bar{x} = 48.5$, $\sigma = 30.08$, and $\sigma^2 = 904.81$ for the males and $n_F = 24$, $\bar{x} = 54$, $\sigma = 28.8$, and $\sigma^2 = 829.44$ for the females which gave a t value of .188. The hypothesis that the genders are similar would not be rejected because of this small t value. Day 7 has $n_m = 15$, $\bar{x} = 103.9$, $\sigma = 80.5$, and $\sigma^2 = 6480.3$ for the males and $n_F = 24$, $\bar{x} = 64$, $\sigma = 31.5$, and $\sigma^2 992.3$ for the females with a t value of 2.12. The larger t value indicates that there is a difference in the genders response on day 7.

On day 12 the mean response for the males was 90.9 with a standard deviation of 54.2. The females had a mean response of 80.02 and a standard deviation of 28.1. The t test resulted in a value of .8 which is an indication that there is probably no difference in

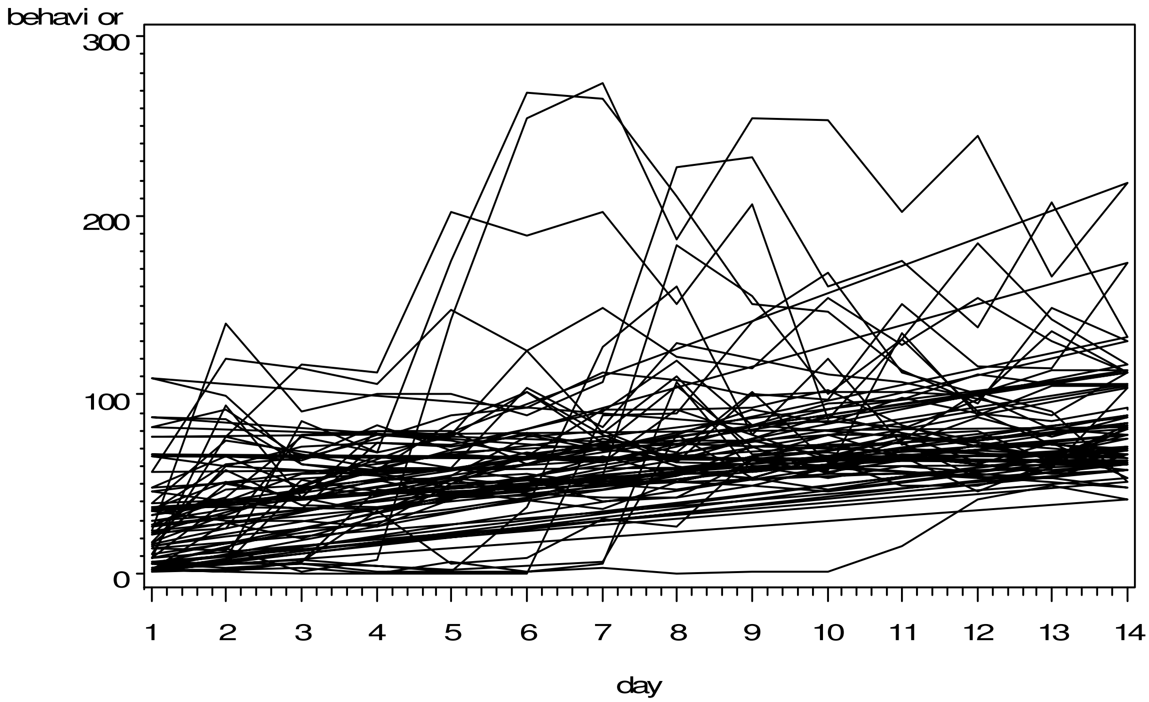

Figure 1.1 Time plot of behavior against day

the genders' response as the study progressed. Overall there is probably no difference in the mean response over time within the gender groups.

Time plots for repeated measures on the same subject can be very enlightening. Time plots are able to show if there are any extreme outliers in the data set and if the variability in the data changes over time. A plot of the mean response can be very useful and provides a good basis to selecting a suitable model for the data set. Figure 1.2 displays a plot of the mean behavior at each day for each age group.

Measuring differences in the mean response over time is like measuring the within individual change (Fitzmaurice, Laird, and Ware, 2004). From Figure 1.2, it can be seen that the periadolescents mean behavior grew much faster than the adults. The trend in the mean response for the adults grew at a slower pace and is relatively flat after day 8. The graph also shows some within individual effects. Figure 1.2 agrees with the t-test that was done earlier. Figure 1.3 shows a plot of the mean behavior at each day for each gender. From the graph, at the beginning of the study it can be seen that both the males and females are very similar up to day 3 and then after day 3 the males' behavior grew at a much faster rate (the males' behavior increased by 54% from day 4 to day 5) than the females until day 11 where their behaviors were almost equal.

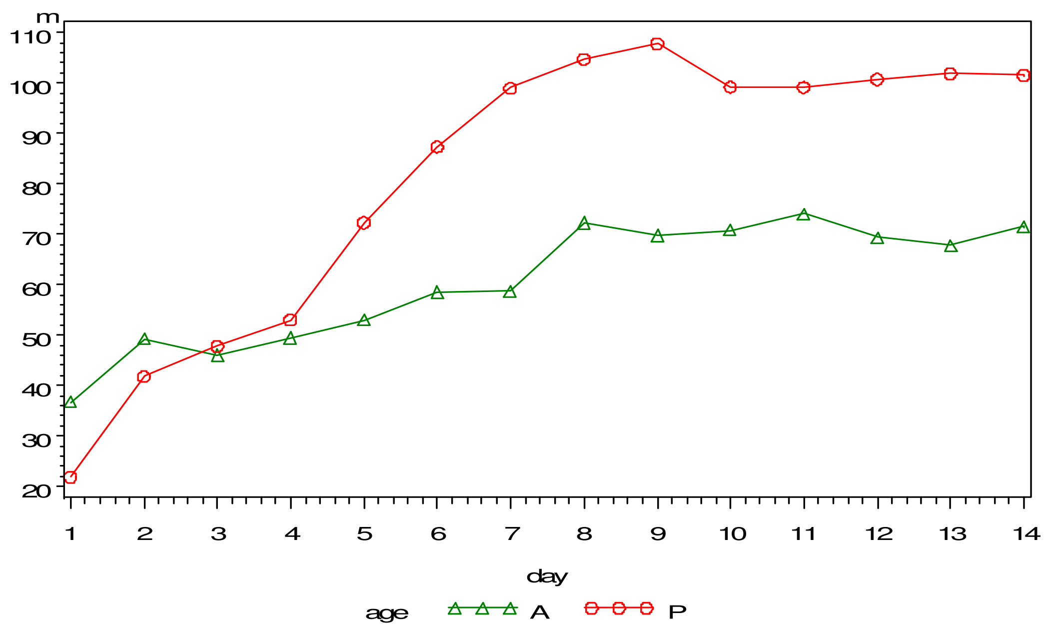

Figure 1.2 Mean behaviors for age groups at each occasion

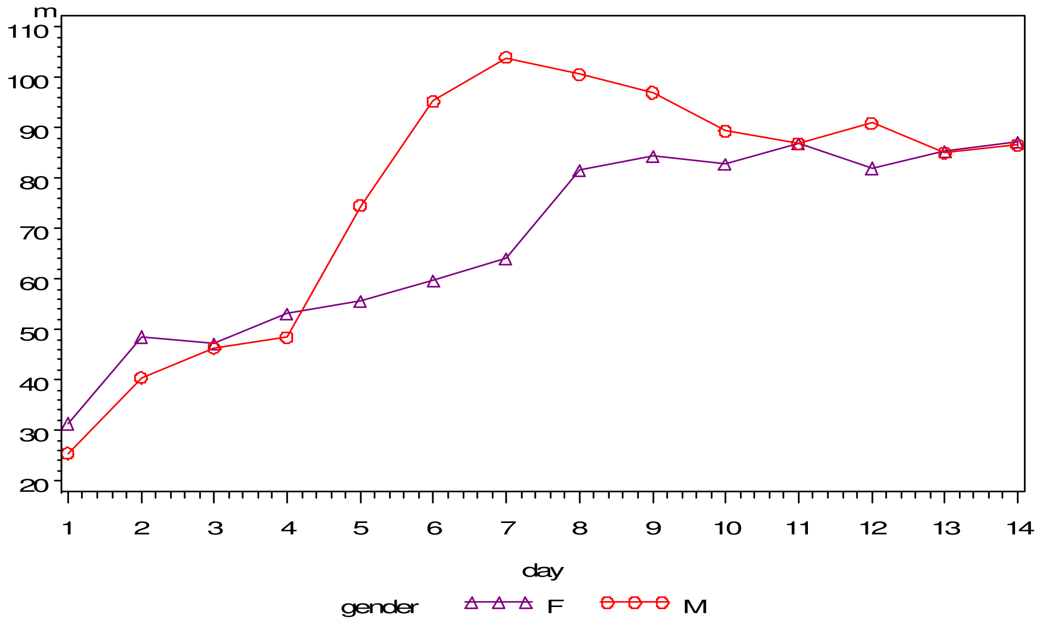

Figure 1.3 Mean behaviors for gender groups at each occasion

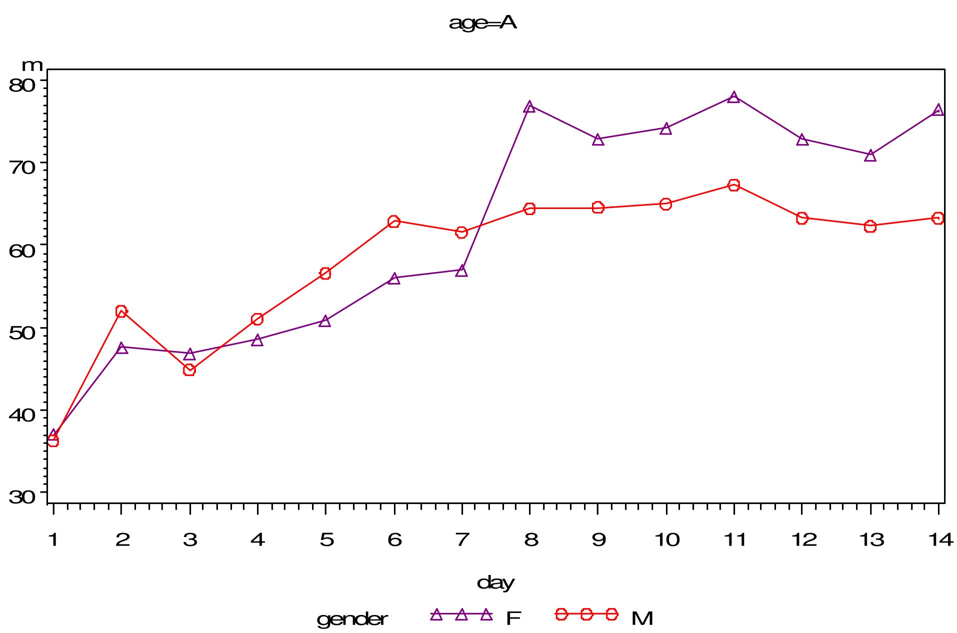

Figure 1.4 Mean behaviors for adults at each occasion

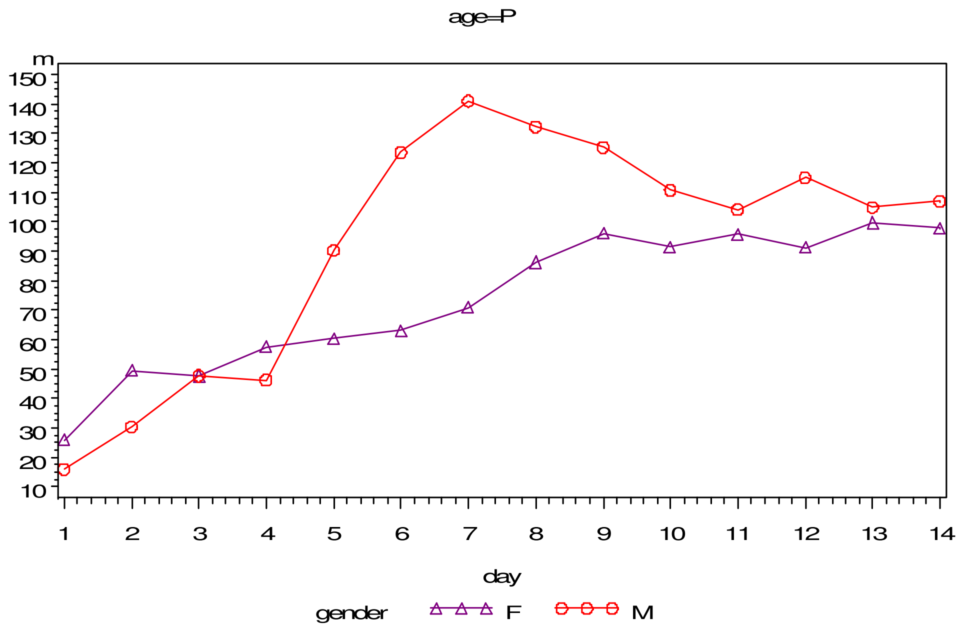

Figure 1.5 Mean behaviors for periadolescents at each occasion

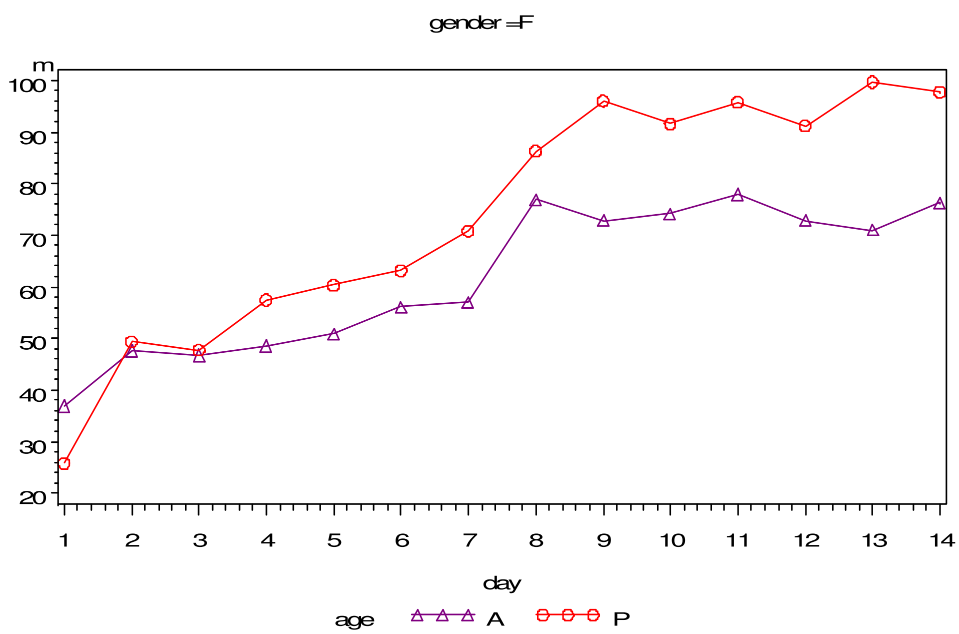

Figure 1.6 Mean behaviors for females at each occasion

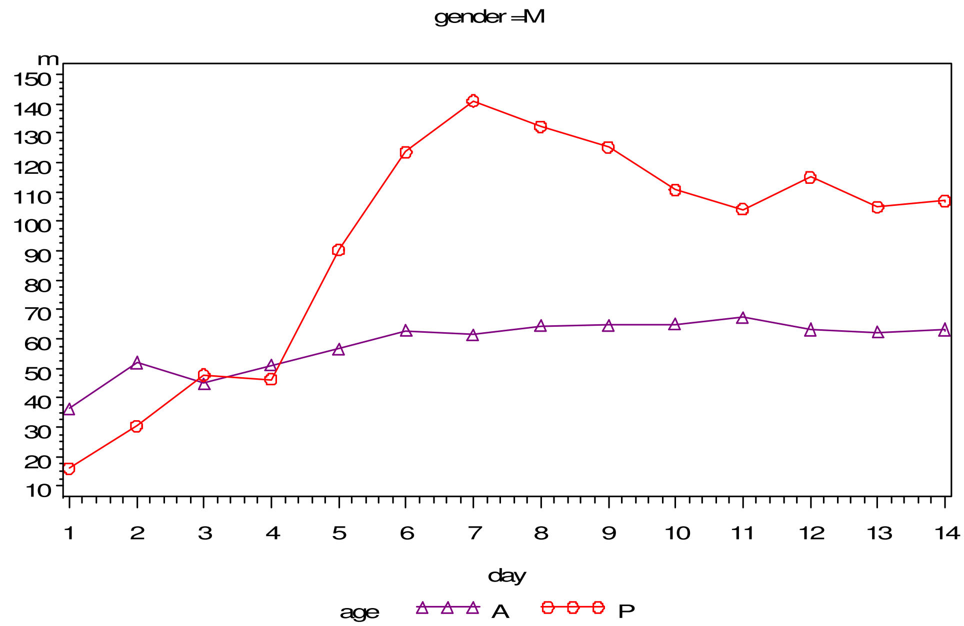

Figure 1.7 Mean behaviors for males at each occasion

Figure 1.4 and Figure 1.5 are plots of the mean response for each gender by age group. For the adults near the beginning of the study the males' behavior increased at a faster rate than the females until day 8. The females mean response increased by 35% from day 7 to day 8 and continued to maintain a higher mean response rate over the males throughout the duration of the study.

For the periadolescent rats the males increased by 95% from day 4 to day 5 and maintained a higher rate over females throughout the duration of the study. The graphs also show within individual changes as well. The mean response by day for each age group by gender is also plotted in Figure 1.6 and Figure 1.7. The graphs also show some within and between subject effects. Both graphs show that regardless of gender the periadolescents mean behavior starts to increase at a faster rate around day four and maintains a higher rate over the adults throughout the study.

1.3 Residual Examination

Residual analysis also plays an important part in the analysis of longitudinal data. Residuals can be used to assess the adequacy of the fitted model and can also indicate the presence of outliers (Fitzmaurice, Laird, Ware, 2004). A scatter plot of the residuals against the predicted mean response can show if there are any systematic trends. A residual plot without trends is good and the normal assumptions i) the random errors have constant variance and ii) the random errors have zero mean are satisfied.

Figure 1.8 has a graphical display of the studentized residuals, the quantile plot, normal histogram, and the residual statistics for the behavior. From the residual plot, it can be seen that most of the residuals are scattered around zero but there also appears to be a slight trend upward and downward at the predicted mean of about 50. The random variation of the residuals is increasing as the fitted value increases, which is an indication that the variance σ^2 is not

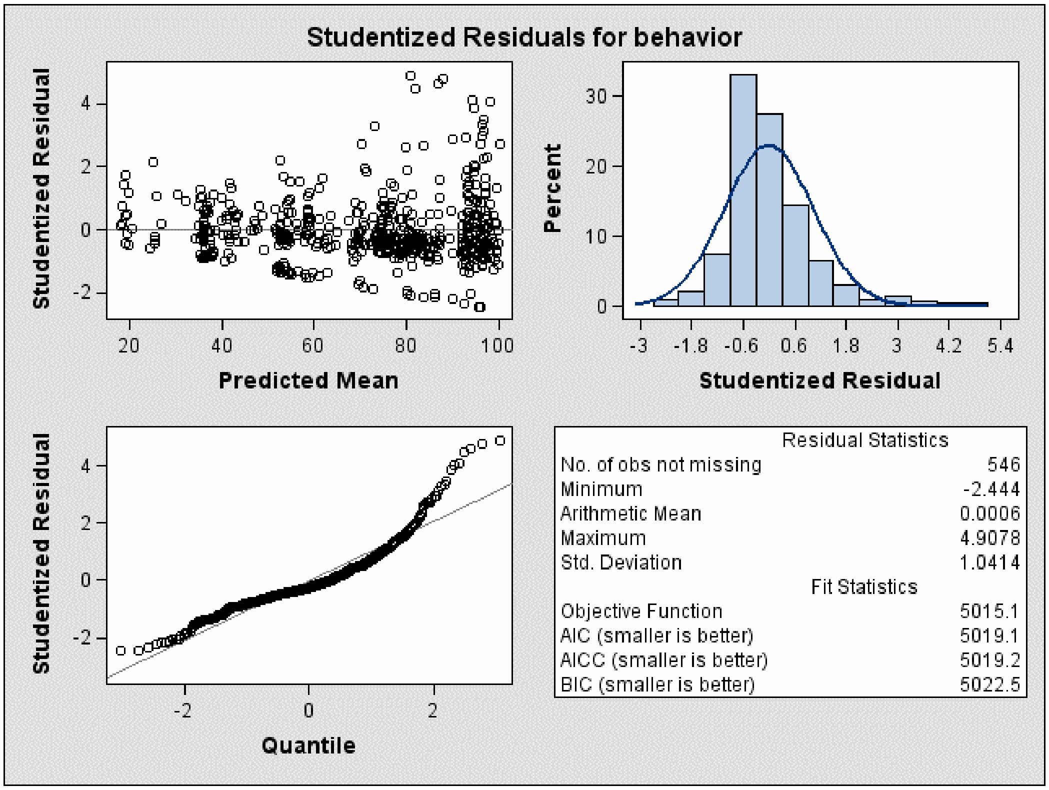

Figure 1.8 Studentized residuals for behavior

constant. The normal histogram is slightly skewed to the right and the Q-Q plot shows a plot of the residuals in sorted order against the value the residuals should have if the distribution of the residuals were normal. The slight curvature in the plot may indicate that the errors are not from a normal distribution or the data has some outliers. This could also be due largely to the fact that the observations from the same subject are not independent and the variance is not constant (correlation exists). It can be concluded that a distribution other than a normal distribution may be a good model for this data set.

The organization of this thesis is as follows; Chapter 2 will provide the goals of longitudinal studies and its notation. The different types of covariance structures will also be discussed. Chapter 3 focuses on ways to analyze longitudinal studies by performing an exploratory analysis and using the univariate repeated measures ANOVA. Chapter 4 reviews the linear mixed effect model and the advantages and disadvantages of using the model. Chapter 5 contains the conclusion of this thesis and will provide recommendations for future work. The code used to analyze the data set is listed in the Appendix.

Chapter Two: LONGITUDINAL DATA

2.1 Objective

Longitudinal data is used to study the pattern of change and the factors that influence those changes both within and between subjects. Subjects could be individuals, animals, and or plants that act as their own controls. Longitudinal data requires special statistical methods because the set of observations on one subject tend to be inter-correlated. This inter-correlation must be accounted to make a valid inference. Another goal is to investigate the effects of important covariates on the patterns of change.

There are two types of patterns: Non-time varying covariates, which could be, gender or age and are considered between (fixed) effects. Time varying covariates such as weight, time or income are considered within (random) effects (Pahwa and Blair, 2002). Measuring the mean response $\mu_{it} = E(Y_{it})$ and seeing how it changes over time will be the primary goal and the secondary goal will be to draw conclusions about the parameters that summarize the characteristics of the covariance or correlation among the repeated measures.

From the above equation, the mean response is allowed to vary over time (which can be seen by its dependence on the subscript t) and changes in the mean response can be related to the individual levels of covariates because of its dependence on the subscript i.

2.2 Notation

Let Y_{it} be the response for the ith subject ($i = 1,...,N$) at the tth occasion where ($t = 1,...,n_i$). The total number of subjects is equal to $\sum_{i}^{N} n_i$, $y_i = n_i \times 1$ is the vector of responses, and $\mathbf{x}_{it} = p \times 1$ is the covariate vector for subject i at time t. The matrix of covariates is $X_i = n_i \times p$ for subject i and will usually include an intercept. For the data used in this paper $i = subject$, $t = 1,...,14$, $y_i = 14 \times 1$, $x_{it} = 4 \times 1$, and $X_i = 14 \times 4$, the fixed effects are age and gender because they do not change throughout the duration of the study and the within individual effects are time and body weight. The data set is also balanced with time meaning all subjects were measured at a common set of occasions and there are no missing data.

2.3 Covariance Structures

Although modeling the correlation structure is not of primary importance it is still however necessary to take into consideration any correlation that may exist when making statistical inference about longitudinal data. Correlation among subjects will probably come from three sources of variability: a) between subject effects, b) within subject effects and c) measurement errors (Fitzmaurice, Laird, and Ware, 2004). An analysis is not valid unless the covariances among the repeated measures are modeled properly.

There are several structures that can be used in the analysis of correlated data with the unstructured (UN) being one of the most commonly used structures. The unstructured structure allows the elements of the covariance matrix to be unconstrained (there are no assumptions being made about the variance and the covariance). This structure is not constrained to be nonnegative definite in order to avoid nonlinear constraints and therefore it must be symmetric and positive definite. The covariance matrix $Cov(Y_i) = \begin{pmatrix} \alpha_1^2 & \sigma_{12} & \sigma_{13} & \dots & \sigma_{1n} \\ \sigma_{21} & \sigma_2^2 & \sigma_{23} & \dots & \sigma_{2n} \\ \sigma_{31} & \sigma_{32} & \sigma_3^2 & \dots & \sigma_{3n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \sigma_{n1} & \sigma_{n2} & \sigma_{n3} & \dots & \sigma_n^2 \end{pmatrix}$ states that the variances across individuals and the correlations are different. This structure is less powerful when there is missing data and/or when the size of the sample is not large enough to estimate an unstructured covariance (the data must be large enough to estimate the $\frac{n(n+1)}{2}$ covariance parameters). Another popular structure is the compound symmetry (CS) $Cov(Y_i) = \begin{pmatrix} 1 & \rho & \rho & \dots & \rho \\ \rho & 1 & \rho & \dots & \rho \\ \rho & \rho & 1 & \dots & \rho \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \rho & \rho & \rho & \dots & 1 \end{pmatrix}$ where $\rho \geq 0$ is the only constraint. This structure states that the correlations between all pairs of measures are the same and the variance is constant across occasions. The compound symmetry is very useful when the mean response is dependent on some combination of population parameters and a single random effect. The biggest disadvantage is its assumption that the correlations between any pair of measurements are the same regardless of time and the variance is constant. Typically, consecutive measurements that are made closer together are more correlated than those that are farther apart. The assumption that the variance is constant is also not valid within longitudinal studies.

The auto regressive (1) [AR(1)] structure

$$Cov(Y_i) = \begin{pmatrix} 1 & \rho & \rho^2 & \dots & \rho^{n-1} \\ \rho & 1 & \rho & \dots & \rho^{n-2} \\ \rho^2 & \rho & 1 & \dots & \rho^{n-3} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \rho^{n-1} & \rho^{n-2} & \rho^{n-3} & \dots & 1 \end{pmatrix}$$ resolves some of the objections the compound symmetry has with successive data and when the measures are equally spaced over time. The AR(1) structures states that the variance is constant and the correlations between two responses that are t measurements apart are ρ^t where $\rho \geq 0$. With this structure, the correlations decrease over time, which is assumed to happen in longitudinal data but most longitudinal studies will not decrease as fast. This structure is only appropriate when the measurements are made at equal time intervals.

The Toeplitz TOEP covariance structure $$Cov(Y_i) = \begin{pmatrix} \sigma^2 & \sigma_1 & \sigma_2 & \dots & \sigma_n \\ \sigma 1 & \sigma^2 & \sigma_1 & \dots & \sigma_{n-1} \\ \sigma_2 & \sigma_1 & \sigma^2 & \dots & \sigma_{n-2} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \sigma_n & \sigma_{n-1} & \sigma_{n-2} & \dots & \sigma^2 \end{pmatrix}$$ assumes pair of responses that are equally spaced in time have the same correlation and the variance does not have to be constant. This structure is also only valid when the measurements are taken at the same time intervals.

The first order factor analytic without the diagonal matrix D [FA0(q)] can be used when the structure is nonnegative definite. When the number of random factors is less than the dimension of the matrix ($q < t$), the structure is nonnegative. This structure can be used to approximate the unstructured matrix in the random statement, where q is equal to the number of random effects.

The variance component VC structure is the default structure for the random and repeated statements used in the mixed models. When used in the random statement a separate variance component is assigned to each effect and when used in the repeated statement, it will specify a heterogeneous variance model. All of the above models can be used with the constraint that the variance is heterogeneous which is true in most longitudinal studies. Ignoring the correlation can cause the inferences about the regression parameters to be incorrect, the estimates of β will be inefficient, and there will be no protection against biases, which is caused by missing data.

2.4 Advantages and Disadvantages

There are several advantages and disadvantages to using longitudinal studies. Some of the advantages are: subjects serving as their own controls which mean the direct study of change can be measured, fewer subjects are required because the measurements are being repeated, between-subject variation is excluded from the error, and longitudinal data can separate aging effects from cohort effects. Some of the disadvantages are: the dependence of the measurements which must be accounted for in the analysis, models are not as well developed; the risk of attrition, carry-over effects, and the improvement or the decline could be caused by treatment or fatigue.

Chapter Three: UNIVARIATE ANALYSIS OF REPEATED MEASURES

There are three main approaches to analyzing longitudinal data:

- Marginal Analysis: where the mean of the response is of importance
- Random Effects Models: used to determine how the regression coefficients change over the individuals
- Transitional Models: where its main focus is to determine how the response variable for a specific subject at time t depends on past values of the response and other variables.

Marginal Models focus on the average of the response variable and how that average changes over time. For the data set used in this thesis using marginal models would answer the question: Does the average lever pressing behavior change over time and does age, gender, bodyweight, and time influence those changes?

A simple analysis of longitudinal data is done by the univariate repeated measures ANOVA. The ANOVA is used to compare and estimate groups in terms of their means and their trends over time. There are several assumptions that must be met in order to use the repeated measures ANOVA.

- The data and errors are normally distributed
- The group comparisons are not used to explain individual growth
- There is no missing data
- The data must also be balanced

If these assumptions are not met the results may be inaccurate.

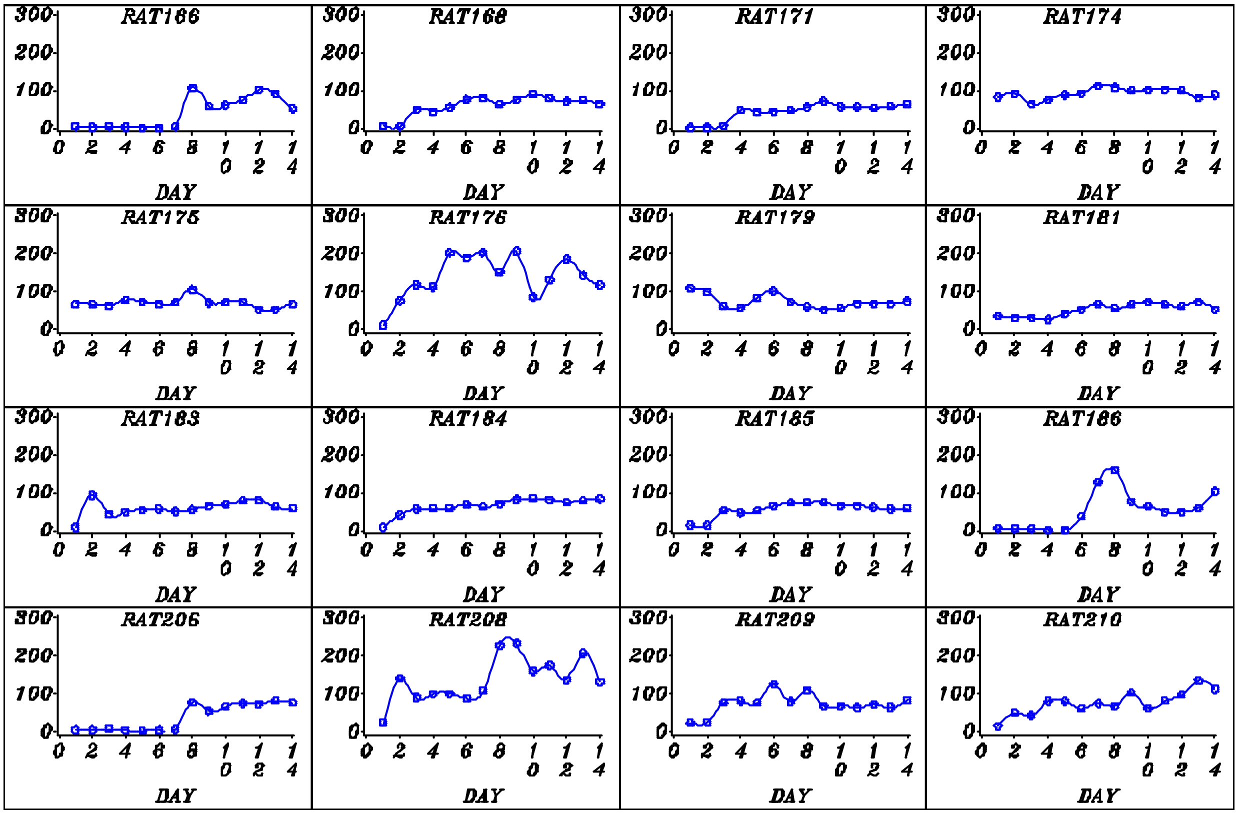

Figure 3.1 Individual behaviors plotted against time

In the univariate repeated measures ANOVA the correlation is assumed to come from the individual specific random effects; this is due to the fact that each subject is assumed to have an underlying level of response that persists over time and influences all measurements on that subject. The times of measurement are treated as a within-subject factor and the effect of time is assumed to be the same for all subjects. The response for the ith subject is assumed to be related to discrete covariates and is assumed to be different from the population mean μ.

Repeated measures ANOVA can be expressed as $y_{ij} = \mu + \tau_i + \nu_j + e_{ij}$, where $E(Y_{ij}) = X'_{ij} = \mu + \nu_j$. The parameter ν_j is the effect of time. The parameter $\tau_i \sim N(0, \sigma_\tau^2)$ is the random subject effect that gives the between-subject variance, and $e_{ij} \sim N(0,\sigma_e^2)$ is a within-subject measurement error and it gives the within-subjects variance. The covariance matrix of the ANOVA has a compound symmetry structure, where the variance and covariance are homogeneous across time and equal to $\sigma_\tau^2 + \sigma_e^2$ and σ_τ^2, respectively. The correlation between two repeated measures is therefore equal to $\dfrac{\sigma_\tau^2}{\sigma_\tau^2 + \sigma_e^2}$.

The first step in analyzing longitudinal data is to create graphs of the group means against time (shown in Chapter 1) and the individual responses against time, which are shown in Figure 3.1. All of the individuals are increasing but not linearly and exhibit significant within subject effects. Some of the rats exhibit a constant mean response profile; which means there was no time effect for those subjects.

Secondly, an analysis of the covariance and correlation matrix should be done to determine what structure is best for the data set. Performing a correlation test on the data for each gender and age group revealed the covariance matrix for the both gender groups exhibited heterogeneous variance and covariance. The correlation matrices appear to have an unstructured structure (where the correlations are all different); or a heterogeneous Toeplitz structure (where the correlations are the same for a pair of responses that are equally separated in time). The correlations for the females appear to be higher than the male correlations. For the age groups, the covariance matrices also exhibit heterogeneous variance and covariance. The covariance is neither increasing nor decreasing in a continuous manner. The correlation matrices for both age groups also resemble a heterogeneous Toeplitz or unstructured structure.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	Pr > F
Model	40	670608.72	16765.22	17.45	<.0001
Error	505	485211.82	960.82		
Corrected Total	545	1155820.546			
Tests of Hypotheses for Mixed Model Analysis of Variance					
SOURCE	**DF**	**Type III SS**	**MEAN SQUARE**	**F VALUE**	**Pr > F**
gender	1	1266.85	1266.85	.34	0.5637
Error	57.33	215358	3756.30		
*Error: 0.2241*MS(rat(gender)) + 0.7759*MS(Error)*					
rat(gender)	37	497018.35	13432.93	13.98	<.0001
* day	1	157324	157324	163.74	<.0001
gender*day	1	153.59	153.59	.16	.6895
Error: MS(Error)	505	485212	960.82		
** This test assumes that one or more other fixed effects are zero*					
	R Square	Coeff Var	Root MSE	Mean behavior	
	0.5802	43.55	30.99	71.17	

Table 3.1 Results for the univariate repeated measures ANOVA

It can be concluded that a model that uses an unstructured or Toeplitz model may fit the data best. Using those structures with heterogeneous variance is also recommended given the design of the covariance matrix.

Table 3.1 shown below gives the results for the dependent variable using the univariate repeated measures ANOVA by gender. The Type III test shows the gender by day interaction as being significant at the .05 level. Therefore, the hypothesis that the groups are the same over time would be rejected (this could also be verified by the graphs of the mean response in Chapter 1) and it can be concluded that the average mean response for the gender groups are not the same over time. The fixed effect gender is not significant at the .05 level but the random effect rat (gender) is significant. The variable gender is treated as our treatment factor. The r-square value of .6404 validates the assumption that correlation exists.

One might question these results because the ANOVA assumes that the covariance matrix has a compound symmetry structure and the variances of the residuals for each of the time points are the same (Kristensen and Hansen, 2004). Plotting the residuals at each occasion as box plots is a good way to see if the residuals are constant. For our study this is not true and the assumption of variance homogeneity has been violated. From previous results, the covariance matrix for the data used in this thesis appears to have a Toeplitz or unstructured structure, which means the results of the ANOVA test, may be invalid because the F ratios may not have an F distribution. Having an F distribution is dependent on the data having a covariance matrix that is similar to a compound symmetry structure. To test whether the assumptions of the univariate repeated measures ANOVA have been violated one can use the sphericity test.

The results from the univariate repeated measures ANOVA that includes the test of sphericity are shown in Table 3.2. From the results of the sphericity test, the hypothesis that the structure of the covariance matrix is a compound symmetry would be rejected. The between- subject variable gender and the within subject effect variable gender*day are also not significant. The results for the between and within variables are based on the assumption that the compound symmetry structure of the covariance matrix is true.

Sphericity Tests							
Variables		**DF**	**Mauchly's Criterion**	**Chi-Square**	**Pr > Chi-Square**		
Transformed Variates		90	1.15E-07	518.78	<.0001		
Orthogonal Components		90	2.47E-06	419.22	<.0001		
Tests of Hypotheses for Between Subject Effects							
SOURCE	**DF**	**Type III SS**	**MEAN SQUARE**	**F VALUE**	**Pr > F**		
gender	1	9650.75	9650.75	0.72	0.4021		
Error	37	497018.35	13432.93				
Univariate Test of Hypothesis for Within Subject Effects						***G-G***	***F-G***
day	13	210431.46	16187.04	18.73	<.0001	<.0001	< .0001
day*gender	13	27065.8	2081.98	2.41	0.0037	.0589	.0498
Error (day)	481	415603	864.04				

Table 3.2 Univariate ANOVA with the test of Sphericity

A univariate repeated measures ANOVA test could be done on the age groups but it is pointless given the fact the covariance matrix for the data set does not exhibit a compound symmetry structure. The univariate test degrees of freedom is adjusted for data sets that do not have a compound symmetry structure, this is printed by two different correction factors. The Greenhouse-Geisser Epsilon (G-G) and the Huynh-Feldt Epsilon (H-F), which agree with the univariate test by showing day as being significant. Although, this adjustment exist it is still however not a very good test for our data set because it does not take into fact that correlation exist, the variance is not constant and requires the data set to have a normal distribution.

Having a compound symmetry structure, examining only the single aspects of the subjects and requiring the covariates to be discrete are some of the disadvantages of using the univariate repeated ANOVA. If the compound symmetry fact was true, some of the advantages of using the univariate ANOVA would be the fact that the test is easy to do, easy to interpret, and it creates a summary of each subjects' time profile.

Chapter Four: MIXED EFFECT MODELS

Mixed linear models are generalizations of the standard linear model used in the GLM procedures. It allows data to exhibit correlation and non-constant variance. It also allows the means to be measured has any other linear model as well as their variances and covariances. The main assumption of linear mixed effect models is that some subset of the regression parameters will vary randomly from one subject to another and therefore accounting for sources of natural heterogeneity in the population (Little, Milliken, Stroup, and Wolfinger, 1996). SAS PROC MIXED transformed the way repeated measures analysis is performed. It can handle data that has the univariate or multivariate layout. PROC MIXED can handle both balanced and unbalanced data. It can also handle missing data and it applies multiple comparison methods to both the between and within subject factors (Dallal, 2002). There are three assumptions of the PROC MIXED analysis:

- The data is normally distributed
- The expected values of the data are linear in trend with respect to certain parameters
- The variances and covariances are in terms of a different set of parameters and exhibit a structure matching one of those that are available in PROC MIXED

There are two sets of parameters in a mixed linear model that specify the complete probability distribution of the data. The parameters of the mean model are referred to as fixed parameters and the variance and covariance parameters are referred to as the covariance parameters. A distinctive feature of linear mixed models is that the mean response is modeled as a combination of population characteristics (which are shared by all subjects) and subject specific effects that are unique to a specific subject.

The population characteristics are called fixed effects and the subject specific effects are called random effects. Covariance parameters are needed in repeated measurements because the data exhibits correlation and changing variability.

The statistical model $E(Y_i) = X\beta$ is the marginal mean response, which is averaged over the distribution of random effects. Inclusion of random effects produces covariances among the responses, states that the $Cov(Y_i) = \Sigma_i$ has a unique random effects structure, and it allows the covariances of the repeated measures to be expressed as functions of time. A linear mixed effect model explicitly distinguishes between within (random) and between (fixed) subject variability.

Producing a standard two-way analysis of variance using PROC Mixed produced the following result:

Covariance Parameter Estimates						
Cov Parm	**Estimate**	**Std Error**	**Z value**	**Pr Z**		
Residual	1706.33	103.75	16.45	< .0001		
Type 3 Test for Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
bw	1	541	1.19	1.19	0.2762	0.2767
age	1	541	2.48	2.48	0.1151	0.1157
gender	1	541	4.79	4.79	0.0287	0.0291
day	1	541	90.53	90.53	< .0002	<.0001
Fit Statistics						
	- 2 Res Log likelihood		5599.9			
	AIC		5601.9			
	AICC		5602			
	BIC		5606.2			

Table 4.1 Results for the standard two way mixed model

The "Covariance Parameter Estimates" table gives the estimate σ^2 for the model and the "Fitted" table lists information about the restricted/residual likelihood along with other values that help determine if the model is a good fit or not. The Type III test results show that gender and day are significant factors in the model at the 5% level.

From the above results, the model does not seem to be a very good fit for this data. This could be due to the fact this model assumes that the data has a normal distribution and the observations are independent with constant variance. The normality assumption is valid because the response values are all real numbers but because the data is being repeated there is a very high probability that the observations on the same subject are correlated and therefore not independent. The correlation between the subjects can be modeled using one of the covariance structures described previously.

One of the simplest ways of modeling correlation is through the use of random effects. Random effect models for longitudinal studies are regression models in which the regression coefficients are allowed to vary across the subjects. Random effects set up a common correlation among all observations having the same level. Random effects not only allow for the trend over time to be described while taking into account that correlation exists between consecutive measurements, it also describes the variation in the baseline measurement and in the rate of change of time. Random effects can be used to build hierarchical models that correlate measurements made on the same level of a random factor (Moser, 2004). The standard mixed model equation is listed below:

$$y = X\beta + Z\gamma + \varepsilon,$$

where **X** is the matrix of fixed effects, β is the unknown fixed parameters, **Z** is the random design matrix and γ is the unknown random parameters (Little, Milliken, Stroup, and Wolfinger, 1996).

The random statement in the PROC MIXED model defines the random effects for the γ vector in the model, which can be used to specify the traditional variance components. The main purpose of the random statement is to define the **Z** matrix for the random effects and to define the structure of **G** matrix, which is the variance – covariance matrix. The **Z** matrix is built just like the **X** matrix for the fixed effects. The model

$$Y_{ij} = \beta_1 + \beta_2 day + b_i + \varepsilon_{it}$$

allows the subject to vary randomly.

This model (randomly varying subject effect) assumes that each subject has an underlying level of response that continues over time. The variable b_i is the random subject effect that describes how the trend over time for the i^{th} subject deviates from the population mean (represents the influence of subject i on its repeated measurements). The above model describes how the response for the i^{th} subject at the t^{th} time differs from the population mean $X_{it}^{'}\beta$ by the subject effect b_i and the within subject measurement error ε_{it}. The subject effect and the measurement error are independent of each other and are believed to vary randomly with a mean of zero and a variance of $Var(b_i) = \sigma_b^2$ for the subject effect and $Var(\varepsilon_{ij}) = \sigma^2$ for measurement error. The model for the randomly varying subject effects produced the following results:

Covariance Parameter Estimates						
Cov Parm	**Subject**	**Estimate**	**Std Error**	**Z value**	**Pr Z**	
Intercept	rat	883.87	218.53	4.04	< .0001	
Residual		959.22	60.31	15.91	< .0001	
Type 3 Test for Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
day	1	506	170.75	170.75	<.0001	<.0001
Solution for Fixed Effects						
Effect	**Estimate**	**Std Error**	**DF**	**t value**	**Pr > |t|**	
Intercept	38.95	5.52	38	7.05	< .0001	
day	4.3	0.329	506	13.07	< .0001	
Fit Statistics						
	- 2 Res Log likelihood		5394.4			
	AIC		5398.4			
	AICC		5398.4			
	BIC		5401.7			

Table 4.2 Results from the randomly varying intercept model

This model used the variance component structure and the restricted maximum likelihood. The G and GCORR matrix produced the variance/covariance matrix and the correlation matrix respectively for the first subject. Allowing day to be random produces an AIC value of 5398.4 and a BIC value of 5401.7, which is much lower than the model without any random slopes. The type 3 test shows day to be significant at the 5% level. The F value is used to test $H_o : \mu_1 = \mu_2 = ... = \mu_{14}$ against H_a since the p-value is less than .05, the null hypothesis would be rejected.

The model

$$Y_{ij} = \beta_1 + \beta_2 day + \beta_3 age + \beta_4 gender + \beta_5 bw + b_i + \varepsilon_{it}$$

includes the other fixed effects while still allowing the subjects to vary randomly. The results give an AIC value of 5377.9 and a BIC value of 5381.3, which is an indication this is a better fit than the model with day being the only fixed effect. The fixed effects day, age, and bodyweight are significant at the 5% level. The results also show that the females' behavior starts off higher than the males and the starting point for the adults is also larger than those for the periadolescents.

Next, we will look at a model that allows for both the intercept and slope to vary randomly.

$$Y_{ij} = \beta_1 + \beta_2 t_{ij} + b_{1i} + b_{2i} + \varepsilon_{ij}$$

is a linear mixed effects model with randomly varying intercepts and slopes among the subjects. Each subject varies at the baseline level of response ($t_{i1} = 0$ in this case t_{i1} is equal to day one) and in changes of their responses over time. The measurement errors allow the response at any occasion to vary randomly above and below the subject specific trajectories (Fitzmaurice, Laird, and Ware, 2004). Let's examine the above mixed effect model with time being the randomly varying slope and the variance component as the structure of the G matrix. Allowing for the intercept and the slope to be random proves to be a better fit than the random subject effect, which is shown by the AIC value of 5341.2 and the BIC value of 5346.2. This model shows that the fixed effect is significant given the variable time (day) as random.

Now, we will look at the same model with the unstructured covariance, the autoregression (1), Toeplitz, and compound symmetry structures. Table 4.4 shows the results of the fit statistics. The model that used the unstructured structure and allowed the intercept and day to vary randomly proved to be a better fit for the model over the other structures (this is determined by the AIC and BIC values). With the unstructured structure only two iterations were needed to find the maximum likelihood where the restricted maximum likelihood was used to estimate the regression coefficients. The model with the compound symmetry needed ten iterations to find the residual/restricted maximum likelihood.

Covariance Parameter Estimates						
Cov Parm	**Subject**	**Estimate**	**Std Error**	**Z value**	**Pr Z**	
Intercept	rat	667.82	199.58	3.35	0.0004	
day	rat	9.15	2.74	3.34	0.0004	
Residual		779.74	51.35	15.19	< .0001	
Type 3 Test for Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
day	1	38	57.25	57.25	<.0001	<.0001
Solution for Fixed Effects						
Effect	**Estimate**	**Std Error**	**DF**	**t value**	**Pr > |t|**	
Intercept	38.95	4.84	38	8.04	< .0001	
day	4.3	0.568	38	7.57	< .0001	
Fit Statistics						
	- 2 Res Log likelihood		5335.2			
	AIC		5341.2			
	AICC		5341.3			
	BIC		5346.2			
Estimated G Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	667.82		
	2	day	Rat166		9.15	
Estimated G Correlation Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	1		
	2	day	Rat166		1	

Table 4.3 Results from the random intercept and slope model

	UN	AR(1)	TOEP	CS
- 2 Res Log likelihood	5331.7	5385.8	5385.6	5385.8
AIC	5339.7	5389.8	5391.6	5389.8
AICC	5339.8	5389.8	5391.6	5389.8
BIC	5346.4	5389.8	5396.5	5393.1

Table 4.4 Fit Statistic Results

With a *p*-value of <.0001, the variable day is significant at the $\alpha = .05$ level of significance for the model.

The above models did not include the other covariates age, gender, and bodyweight. The next model

$$Y_{ij} = \beta_1 + \beta_2 t_{ij} + \beta_3 age + \beta_4 gender + \beta_5 bw + b_{1i} + b_{2i} t_{ij} + \varepsilon_{ij}$$

will include the additional covariates and leaving time as the randomly varying slope. The results for the model that uses the variance component and REML method are given in Table 4.5. This model has a variance of 780.4 and an AIC and BIC value that are lower than the previous models. The fixed effect day is the only one that is significant at the $\alpha = .05$ level.

Analysis of the previous model was done with the unstructured covariance, the autoregression (1), Toeplitz, and compound symmetry structures. The model that uses the UN structure fits the data best. The UN, AR(1), CS, and TOEP produced the following AIC values 5327.6, 5381.3, 5381.3 and 5383.0, respectively. The BIC values were 5334.3, 5384.6, 5384.6 and 5388.0, respectively. The heterogeneous models of the covariance structures and the FA0(2) structure were also tested and produced the same AIC value as the unstructured structure. The unstructure structure produced a variance of 780 and only needed three iterations to maximize the likelihood. The other structures

Covariance Parameter Estimates						
Cov Parm	**Subject**	**Estimate**	**Std Error**	**Z value**	**Pr Z**	
Intercept	rat	730.39	222.63	3.28	0.0005	
day	rat	8.74	2.72	3.21	0.0007	
Residual		780.44	51.47	15.16	< .0001	
Type 3 Test for Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
day	1	38	39.88	39.88	<.0001	<.0001
age	1	467	0.75	0.75	0.386	0.3864
gender	1	467	0	0	0.9936	0.9936
bw	38.95	467	0.76	0.76	0.3845	0.3849
Fit Statistics						
	- 2 Res Log likelihood		5324.1			
	AIC		5330.1			
	AICC		5330.1			
	BIC		5335.1			
Estimated G Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	730.39		
	2	day	Rat166		8.74	
Estimated G Correlation Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	1		
	2	day	Rat166		1	

Table 4.5 Results from the variance component structure model

produced a variance of 945 and needed nine iterations to maximize the likelihood. In all of the models, day was the only fixed effect that was significant.

The model

$$Y_{ij} = \beta_1 + \beta_2 t_{ij} + \beta_3 age + \beta_4 gender + \beta_5 bw + \beta_6 (gender \cdot day) + \beta_7 (age \cdot day) + b_{1i} + b_{2i} t_{ij} + \varepsilon_{ij}$$

will include the gender*day and age*day interaction terms. The interaction terms will determine if the null hypothesis: “changes among time (day) is the same for the groups” is true or not. Table 4.6 shows the results for the above model using the unstructured structure. The model was also tested using the Autoregressive (1) and the Toeplitz structures, which gave AIC values of 5368.7 and 5369.5 and BIC values of 5372.0 and 5374.5 respectively. From the results it is obvious that a model that uses an unstructured structure fits the data set best. This agrees with our results from Chapter 3.

The results shown in Table 4.6 give the covariance and correlation matrices and

Covariance Parameter Estimates						
Cov Parm	**Subject**	**Estimate**	**Std Error**	**Z value**	**Pr Z**	
UN(1,1)	rat	863.95	263.03	3.28	0.0005	
UN(2,1)	rat	-36.17	22.12	-1.64	0.102	
UN(2,2)	rat	8.87	2.9	3.06	0.0011	
Residual		771.06	50.42	15.29	< .0001	
Test of Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
day	1	36	43.16	43.16	<.0001	<.0001
age	1	467	0.27	0.27	0.61	0.61
gender	1	467	0.48	0.48	0.49	0.49
bw	1	467	0.12	0.12	0.73	0.73
day*gender	1	467	0.03	0.03	0.87	0.87
day*age	1	467	8.56	8.56	0.0034	0.0036
Fit Statistics						
	- 2 Res Log likelihood		5307.1			
	AIC		5315.1			
	AICC		5315.2			
	BIC		5321.8			
Estimated G Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	863.95	-36.17	
	2	day	Rat166	-36.17	8.87	
Estimated G Correlation Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	1	-0.413	
	2	day	Rat166	-0.413	1	

Table 4.6 Results from the unstructured structure model

show day and age*day as being significant at the $\alpha = .05$ level. The age*day term will be discarded because age alone is not significant. The results also show that the null hypothesis would not be rejected and it can be concluded that the trends in the mean response over time are the same in the gender groups (because of the short study period and this is a linear model we can not conclude that there is no difference within the gender groups). Including the interaction terms in the model reduced the AIC value and proved to be a better fit than the models used previously at the beginning of the chapter.

The assumption of a linear transformation between y and the regressors is not always valid. Looking back at the figures in Chapter 1 it can be seen that the response variable may have a nonlinear over time. A nonlinear function can be linearized by using a suitable transformation (Montgomery, Peck, and Vining, 2001). A commonly applied transformation for positive value data is to take the log of the value. The transformed values will then have a full range $(-\infty, \infty)$, which allows a method based on normal distributions to become more reasonable, Crowder and Hand (1990). Taking the log of the response variable will linearize the above model.

The new intrinsically linear model will be

$$\ln(Y_{ij}) = \beta_1 + \beta_2 t_{ij} + \beta_3 bw + \beta_4 age + \beta_5 gender + \beta_6 (age \cdot day) + \beta_7 (gender \cdot day) + \beta_8 (bw \cdot day) + b_{1i} + b_{2i} t_{ij} + \varepsilon_{ij}$$

which implies that the multiplicative error term in the original model is log normally distributed. Taking the log of the response variable is a special case of the Box – Cox method where $\lambda = 0$. The parameters of the model and λ can be estimated simultaneously by the method of maximum likelihood which is explained in Box and Cox [1964] (Montgomery, Peck, and Vining, 2001). Kristensen and Hansen, 2004 says log-transforming the data reduces the overall variability and may help reduce the problem of variance heterogeneity.

Figure 4.2 shows a graph of the residuals for the transformed response variable. The fit statistics show that taking the log transformation improved the fit of the model. Table 4.7 shows that taken the log of the response and analyzing the model with the new response variable is a much better fit for this data set. The AIC and BIC values dropped significantly. The AIC values for the Autoregressive (1), Toeplitz, produced AIC values of 1393.3 and 1322.6 and BIC values of 1396.7 and 1327.5, respectively. These results confirm the analysis that was done in Chapter 3. The results in Table 4.7 still show day and the interaction variable (age*day) as the only fixed factors being significant at the .05 level. The p-value for the age*day did however increase from the previous results and is no longer significant at the $\alpha = .01$ level. Removing the insignificant term age produced an AIC value of 1144.7 and a BIC value of 1151.3, which is a slight increase from the full model.

Other useful power transformations for y^{λ}, are $\lambda = -1, -\frac{1}{2}\ and\ \frac{1}{2}$. The fit statistic results are shown in Table 4.8. The results also show that the log transformation will give the best fit. Figure 4.1 shows a plot of the predicted values as a function of day. Based on our results the best model for our data set is

$$\ln(Y_{ij}) = \beta_1 + \beta_2 t_{ij} + \beta_3 bw + \beta_4 gender + \beta_5 (gender \cdot day) + \beta_6 (bw \cdot day) + b_{1i} + b_{2i} t_{ij} + \varepsilon_{ij}$$

and the results are listed in Table 4.10. Plots of the final model versus the observed response values for some of the subjects are shown in Figure 4.5. Figure 4.5 contains a plot of both the female and male rats from both age groups. The predicted responses also show the males acquiring faster than the females and maintain a higher rate throughout the test. Within the males the periadolescent rats have a steeper slope, which is an indication that they (periadolescent males) acquired faster than the adults and maintained a higher rate throughout the duration of the study. Within the females there does not seem to be much of a difference between the periadolescent and adult rats. The predicted values are almost mirror images of each other. This is an indication the means for both age groups are probably the same or the difference between the two is very small.

	-1	$-\frac{1}{2}$	0	$\frac{1}{2}$
- 2 Res Log likelihood	-627	-732.2	1132.5	2259.5
AIC	-619	-724.2	1140.5	2267.5
AICC	-618	-724.1	1140.5	2267.6
BIC	-612.3	-717.5	1147.1	2274.1

Table 4.7 Fit Statistics for the Box-Cox Method

Covariance Parameter Estimates						
Cov Parm	**Subject**	**Estimate**	**Std Error**	**Z value**	**Pr Z**	
UN(1,1)	rat	1.54	0.393	3.92	< .0001	
UN(2,1)	rat	-0.107	0.0296	-3.62	0.0003	
UN(2,2)	rat	0.0085	0.0024	3.54	0.0002	
Residual		0.324	0.021	15.09	< .0001	
Test of Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
bw	1	460	13.16	13.16	0.0003	0.0003
age	1	460	3.02	3.02	0.0824	0.0831
gender	1	460	5.94	5.94	0.0148	0.0151
day	1	36	47.81	47.81	< .0001	< .0001
day*age	1	460	7.37	7.37	0.0066	0.0069
day*gender	1	460	16.6	0	< .0001	< .0001
day*bw	1	460	30.2	30.2	< .0001	< .0001
Fit Statistics						
	2 Res Log likelihood		1132.5			
	AIC		1140.5			
	AICC		1140.5			
	BIC		1147.1			
Estimated G Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	1.54	-0.107	
	2	day	Rat166	-0.107	0.0085	
Estimated G Correlation Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	1	-0.9347	
	2	day	Rat166	-0.9347	1	

Table 4.8 Results for the logarithmic transformation of the response variable

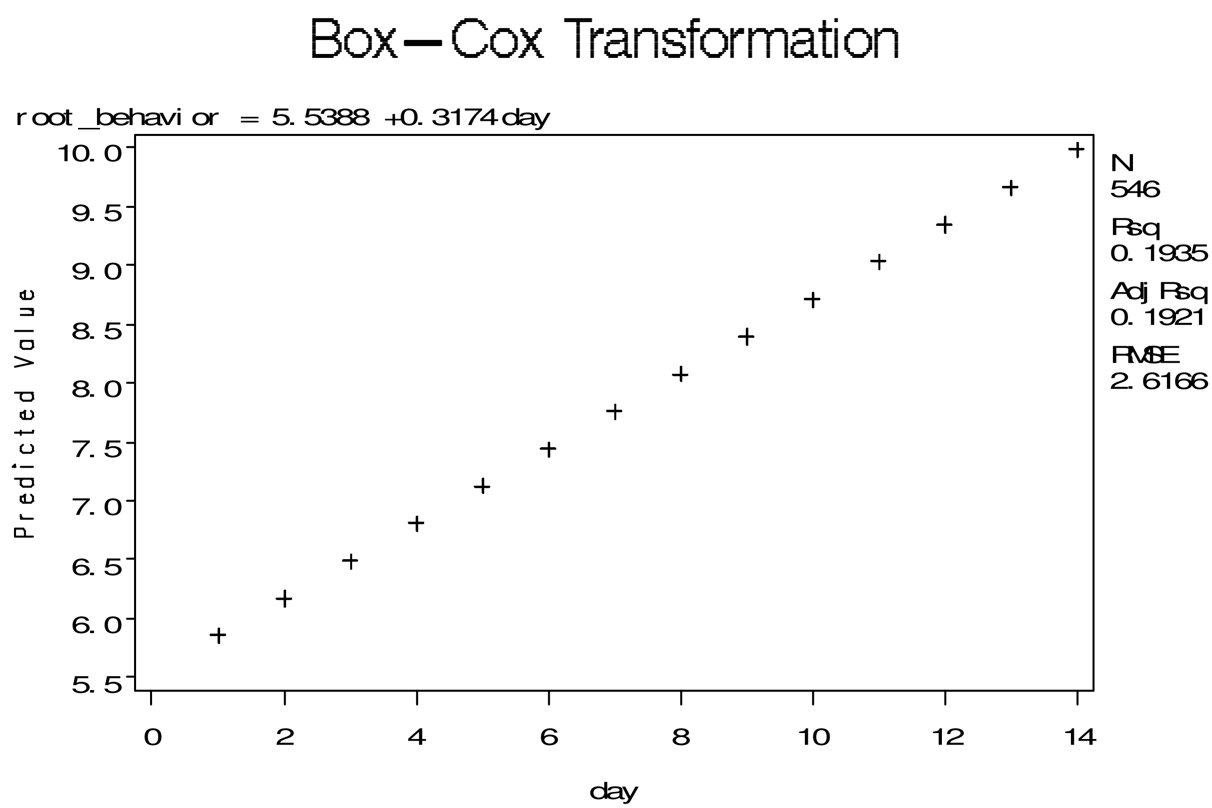

Figure 4.1 Predicted values of the response as a function of day

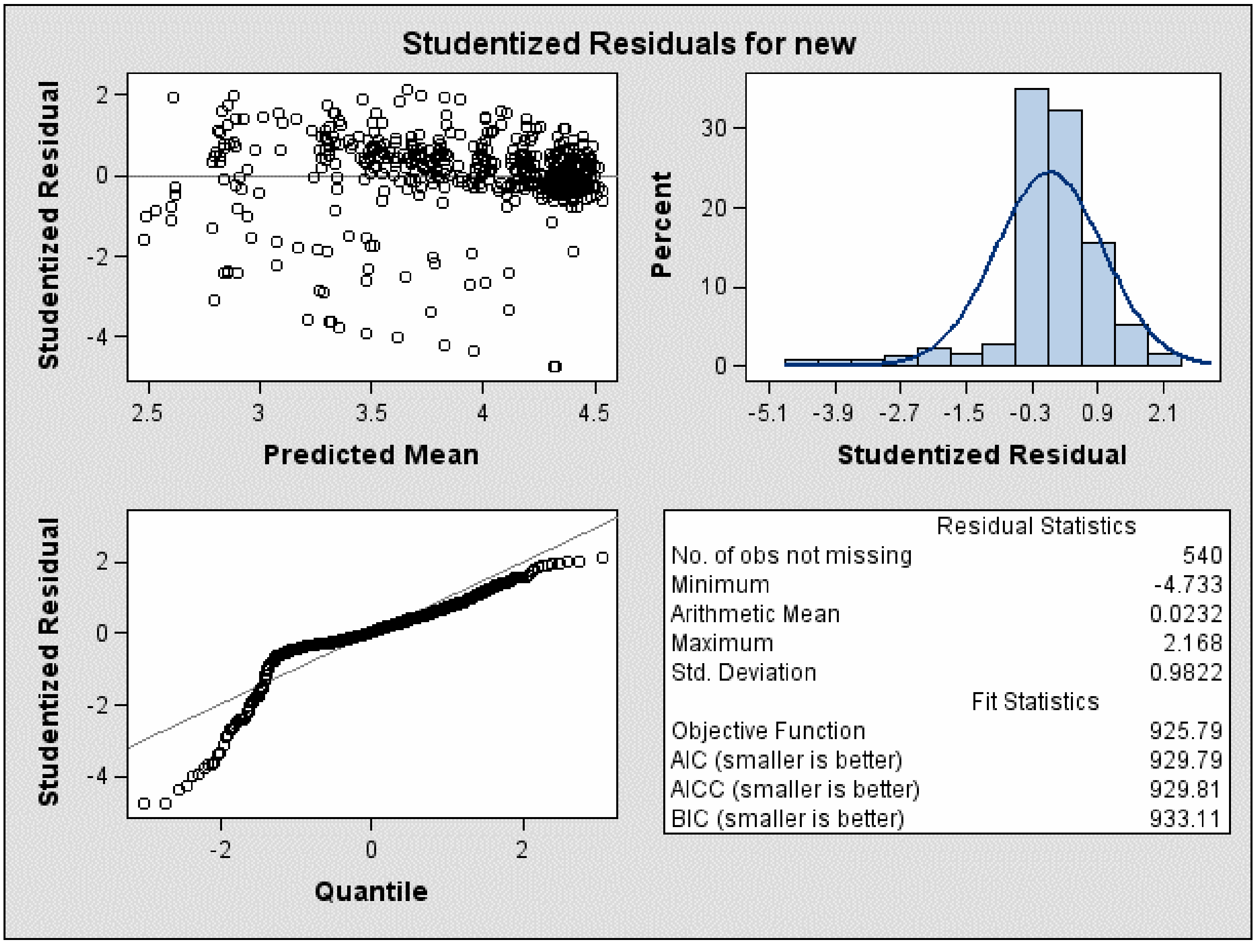

Figure 4.2 Residuals for the transformed response variable

Now, we will do one more transformation to see if it fits the data better and to also verify how conclusion about taking the log of the response variable. Based on Figure 4.3 and Figure 4.4 a polynomial transformation may be a good fit the data but we will need to look at the model in its entirety to determine if it is better than the log transformation.

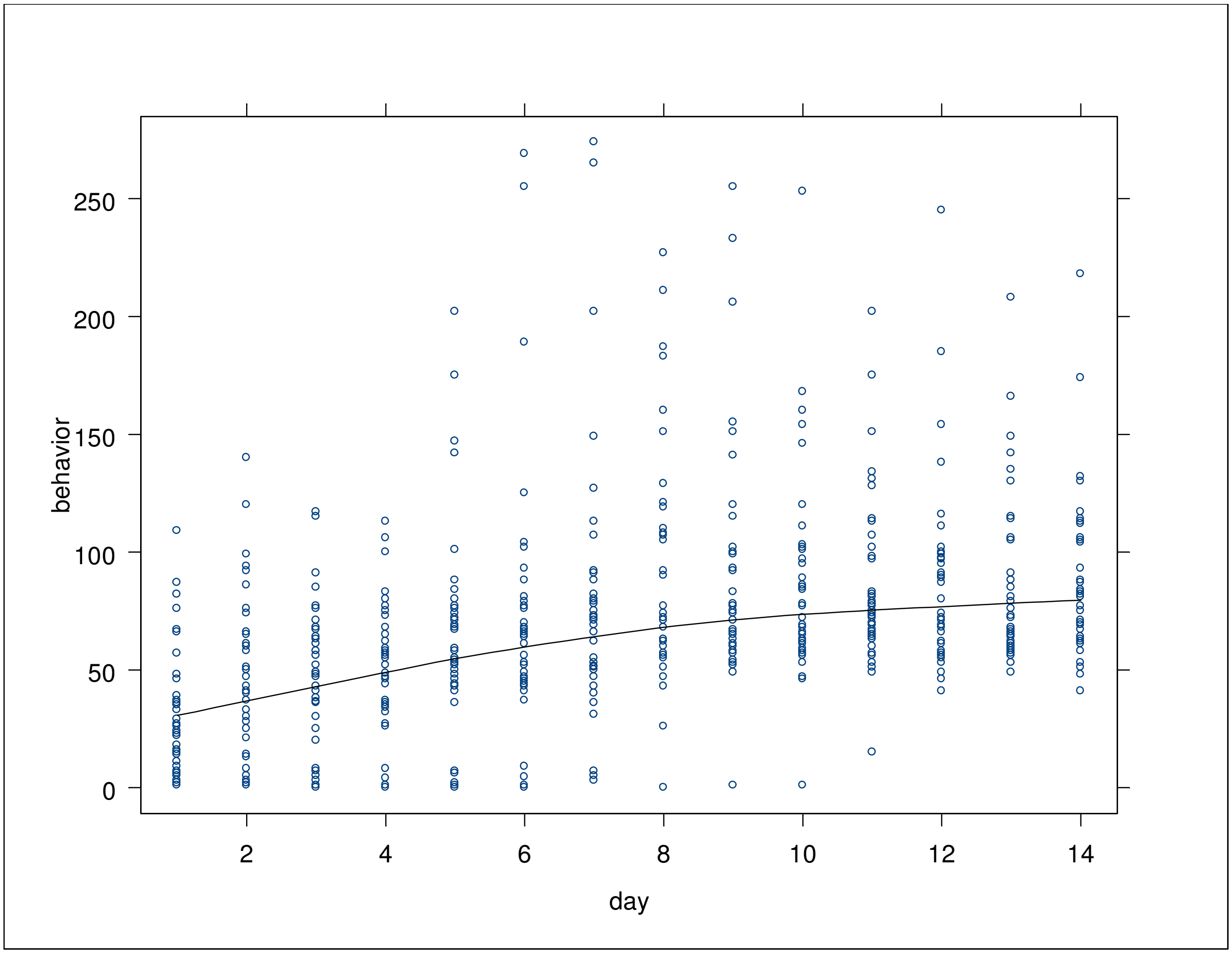

Figure 4.3 Scatter plot of behavior versus day (polynomial transformation)

The model for the polynomial transformation is $Y_{ij} = \beta_o + \beta_1 day + \beta_2 day^2 + \varepsilon_{ij}$. In this model we wish to estimate the intercept, the slope coefficient for the linear day term, and the slope coefficient for the quadratic day (squared) term. The results listed in Table 4.9 show day and daysq are significant at the $\alpha = .05$ level but the AIC and BIC values are much higher than the AIC and BIC values for the Box – Cox method previously done. Therefore we can conclude our final model (log transformation of the response variable) shown in Figure 4.10 is the best.

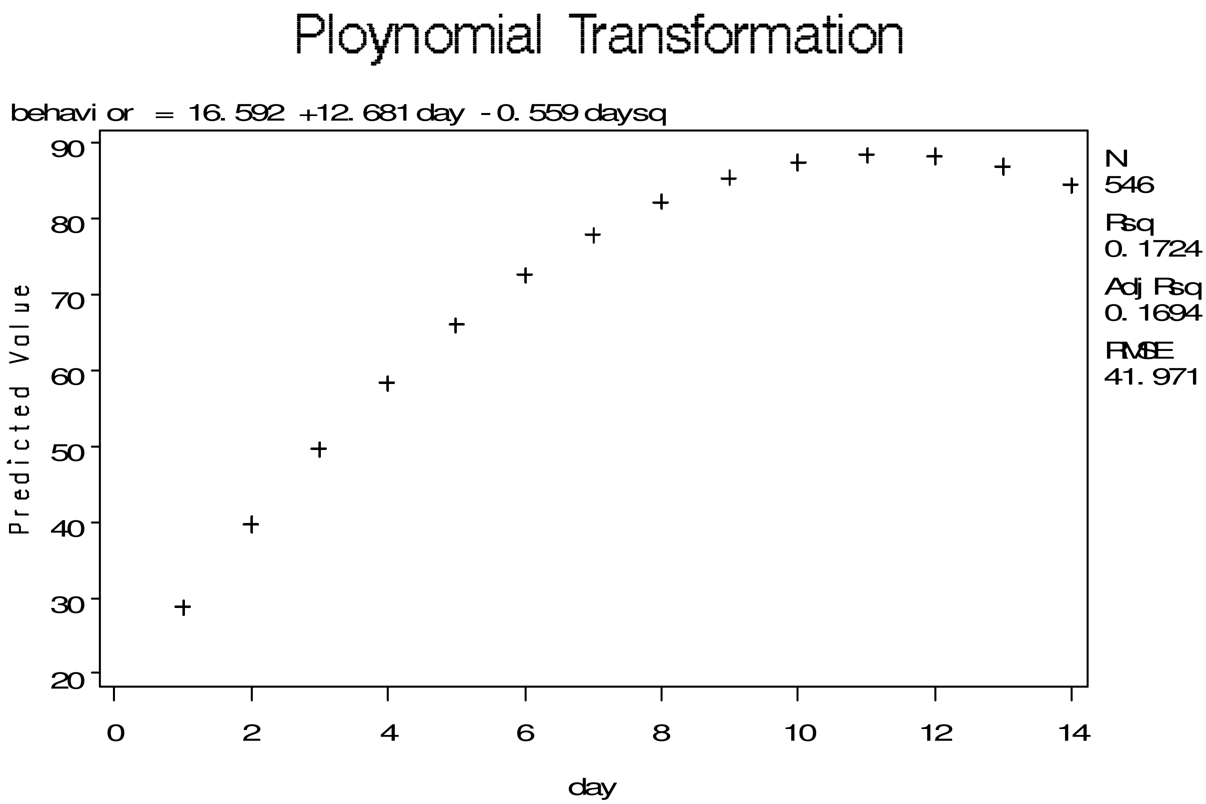

Figure 4.4 Predicted values of the response as a function of day

Some of the disadvantages of using the Mixed Model are: Date will need to be continuous and normally distributed and mixed models only model the data as polynomials. Overall, mixed models are probably the best (linear model) because it allows the data to have both fixed and random effects, it can handle the assumption of dependence among the subjects and provides a large variety of useful covariance structures.

Covariance Parameter Estimates						
Cov Parm	**Subject**	**Estimate**	**Std Error**	**Z value**	**Pr Z**	
UN(1,1)	rat	830.59	241.87	3.43	0.0003	
UN(2,1)	rat	-37.88	22.35	-1.69	0.0901	
UN(2,2)	rat	11.38	3.32	3.43	0.0003	
Residual		695.98	45.55	15.28	< .0001	
Type 3 Test of Fixed Effects						
Effect	**Num DF**	**Den DF**	**Chi - Square**	**F VALUE**	**Pr > Chi - Square**	**Pr > F**
day	1	38	91.93	91.93	< .0001	<.0001
daysq	1	467	50.98	50.98	< .0001	< .0001
Solution for Fixed Effects						
Effect	**Estimate**	**Std Error**	**DF**	**t value**	**Pr > |t|**	
Intercept	16.59	6.06	38	2.74	0.0094	
day	12.68	1.32	38	9.59	< .0001	
daysq	-0.559	0.078	467	-7.14	< .0001	
Fit Statistics						
	- 2 Res Log likelihood		5286.2			
	AIC		5294.5			
	AICC		5294.6			
	BIC		5301.1			
Estimated G Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	830.59	-37.88	
	2	day	Rat166	-37.88	11.38	
Estimated G Correlation Matrix						
	Row	**Effect**	**rat**	**Col1**	**Col2**	
	1	Intercept	Rat166	1	-0.39	
	2	day	Rat166	-0.39	1	

Table 4.9 Results for the polynomial transformation model

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Std Error	Z value	Pr Z
UN(1,1)	rat	1.57	0.4066	3.87	< .0001
UN(2,1)	rat	-0.111	0.0309	-3.58	0.0003
UN(2,2)	rat	0.0087	0.0025	3.47	0.0003
Residual		0.3310	0.022	15.11	< .0001

Test of Fixed Effects

Effect	Num DF	Den DF	Chi - Square	F VALUE	Pr > Chi - Square	Pr > F
bw	1	460	12.28	12.28	0.0005	0.0005
gender	1	460	3.20	3.20	0.0738	0.0745
day	1	37	56.45	56.45	< .0001	< .0001
day*gender	1	460	8.45	8.45	.0037	.0038
day*bw	1	460	27.09	27.09	< .0001	< .0001

Fit Statistics

2 Res Log likelihood	1136.7
AIC	1144.7
AICC	1144.7
BIC	1151.3

Estimated G Matrix

Row	Effect	rat	Col1	Col2
1	Intercept	Rat166	1.57	-0.111
2	day	Rat166	-0.111	0.0088

Estimated G Correlation Matrix

Row	Effect	rat	Col1	Col2
1	Intercept	Rat166	1	-0.945
2	day	Rat166	-0.945	1

Table 4.10 Final Model

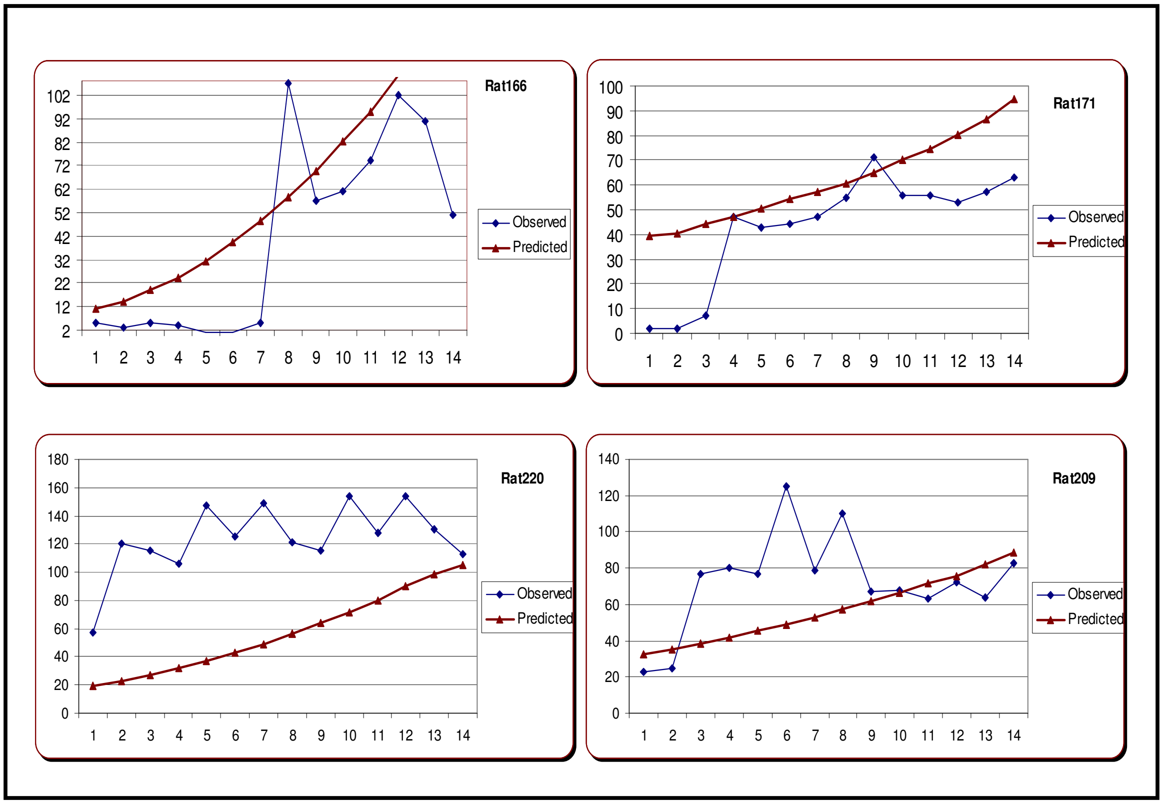

Figure 4.5 Final model versus observed responses

CHAPTER FIVE: CONCLUSION

The primary purpose of this thesis was to focus on data that arises from repeated measurement on a cross section of subjects and describe models that are used to analyze the unique data. Subjects can be humans, animals, houses, and or plants. The desire to use longitudinal data has grown increasingly within the biological sciences, engineering, government, social sciences, education, and other physical sciences.

Longitudinal data (repeated measures) consist of observations taken on the same subject repeatedly over time. Its goal is to determine if the mean response changes over time and what factors influence those changes. An observer may also want to know if there is a difference in the mean response between two or more groups and if the change is positive or negative.

The first step in analyzing repeated observations is to do an exploratory analysis by creating graphs to see if there is a change and doing a simple linear regression analysis. Graphs of the average response over time can be very helpful. One can tell if the trends between two or more groups are the same, if there are between subject and within subject effects, if the change in the response is linear or not, and if the variance increases as the study progress. From the graphs one can see that there is significant within and between subject effects, the variance is increasing, and there is a nonlinear positive change within the average response. The graphs also showed that the mean response is not the same within the age groups, but the differences in the mean response within the gender groups are minimum to none (the hypothesis $\mu_M = \mu_F$ would not be rejected).

Performing a simple linear regression analysis is also very helpful and enlightening. Performing a simple correlation test allows the observer to determine what covariance structure would work best for their given data set. Performing a correlation test on the data set used in this thesis showed that the correlation matrix resembled an unstructured or Toeplitz structure. Doing a simple correlation test in the beginning of the study saves time and decreases the probability of having inaccurate results.

The univariate repeated measures ANOVA test is used to test repeated measures but only if the data set has a normal distribution, linear, balanced (all observations are measured at the same time), and the correlation matrix of the data set has a compound symmetry structure. This way of testing has been in place for years but because of the strong requirements this way of testing may not be beneficial. If the correlation matrix does not have a compound symmetry structure, the results of the ANOVA test may be inaccurate and false conclusions may be drawn. The assumption of sphericity is also very strong and maybe unrealistic for repeated measurements because the variance is usually not constant over time (Hedeker and Gibbons, 2006).

The linear mixed effect model is also used to analyze repeated measures. The mixed effect model allows for both fixed and random effects. For the rat data set, the random covariate was day because it did not remain the same throughout the study. Allowing for day to be random proved to be a better fit versus the model that had no random effects. One big disadvantage of using the mixed model is it also requires the response to enter linearly. From the graphs, it can be seen the response is not linear over time. The response variable was transformed by taking its log and the mixed model was used still allowing for day to vary randomly reduced the AIC value significantly.

Transformation of the response variable allows the response to become intrinsically linear. Transforming the response variable and including the interaction terms produced the best results. The models used in this thesis required the response to enter linearly and from the graphs that were produced, it is obvious the response is nonlinear over time. Future work for the rat data set should include using a nonlinear model because it allows the response to enter nonlinearly. The nlmix model used in SAS or SPLUS is designed to assist with the modeling of nonlinear repeated measurements.

Nonlinear models are fully parametric and models the within subject covariance structure in great detail. Nonlinear models are extension of mixed models but allow the data to have a normal, binomial, or Poisson distribution. The nonlinear model is used to estimate the fixed parameters of the nonlinear mixed effects model and the density of the random effects jointly by maximum likelihood. "The density of the random effects is assumed to be smooth but is otherwise unrestricted. The method uses a series expansion that follows from the smoothness assumption to represent the density and quadrature to compute the likelihood. Simulation from this representation is easy and may be used as an alternative to quadrature. Standard algorithms are used for optimization. Empirical Bayes estimates of random coefficients are obtained by computing posterior modes." (Davidian and Giltinan, 1995). Disadvantages of using this model are: i) very long computation times, ii) initial values may be difficult to find, iii) best suited for models with a single random effect and iv) can not handle nested or crossed random effects.

After much testing our results showed the variable age was significant and the null hypothesis stating the means are the same for the fourteen days, averaged over the groups would be rejected. The plot of the mean behaviors for the age group shows there is a difference between the adults and the periadolescents. The periadolescents behavior increases at a much faster rate and maintains a higher average response rate over time.

The age*day interaction term was also significant; this deals with the null hypothesis that the changes in the mean response over time is the same for the age groups. This null hypothesis would also be rejected. These results were obtained from the linear mixed effects model.

From the time plots, the male periadolescent rats had a flat mean response trajectory over time; this means there was no main effect of time for those rats. The female periadolescents and adult rats mean responses were somewhat parallel this is an indication the mean response for the females regardless of age are similar and because the graphs are not flat there was some time effect (these results were also obtained from the linear mixed effect models).

The gender and gender*day variables were not significant and we can conclude the means were the same for the gender groups and the changes among time was the same for the groups. Body weight was also not significant and did not contribute to the increase in the rat's behavior.

Using the log transformation on the response variable showed all covariates (gender, day, bodyweight, gender*day and bodyweight*day) except for age were significant. This means that there were some differences within their means over time. The null hypothesis: "the mean responses are the same in the adults and periadolescents" would not be rejected for the age variable. From the graphs that includes the predicted responses there appears to be some change within the ages for the males but not for the females. The graphs also confirm the results that there is some difference within the gender group. So, we can conclude that there was some difference in the age groups for the male rats but within the females there was probably little to none. Since the age variable was found to be insignificant it was removed from the model; given the fact the observation period was short and this was a small sample size we will not conclude that age does not influence the rats' behavior. Increasing the observation period, sample size and using a nonlinear model will probably be the best route for future studies.

References

Crowder, M.J. & Hand, D.J. (1990). *Analysis of Repeated Measures*. New York: Chapman and Hall.

Dallal, Gerard E. (2002). *Repeated Measures Analysis of Variance Part II: After SAS's Mixed Procedure*. < www.tufts.edu/~gdalla1/repeat2.htm > (June 21, 2006).

Davidian, Marie & Giltinan, David M. (1995). Nonlinear Models for Repeated Measurement Data, New York: Chapman and Hall.

Everitt, B.S (1995). The analysis of repeated measures: a practical review with examples. *The Statistician*, Vol 44, No. 1, 113-135.

Fitzmaurice, G.M., Laird, N.M., & Ware, J.H. (2004). Applied Longitudinal Analysis, New Jersey: Wiley.

Frees, F.W. (2004) Longitudinal and Panel Data: Analysis and Applications in the Social Sciences, New York: Cambridge.

Hedeker, Donald & Gibbons, Robert. (2006). Longitudinal Data Analysis, New Jersey: Wiley.

Kristensen, Michael & Hansen, Thomas (2004). *Statistical analysis of repeated measures in physiological research: a tutorial*. Advan Physiol Edu, 28:2-14, 2004, <www.ajpadvan.physiology.org > (February 28, 2006).

Lindsey, J.K., (1999). Models for Repeated Measurements, Oxford: Oxford University Press

Littel, R.C., Milliken, G.A., Stroup, W.W., & Wolfinger, R.D. (2006). SAS for Mixed Models, Cary, NC: SAS Institute, Inc.

Montgomery, Douglas C., Peck, Elizabeth A. & Vining, G. (2001). *Introduction to Linear Regression Analysis*. New York: John Wiley and Sons, Inc.

Moser, E. Barry (2004). *Repeated Measures Modeling With PROC MIXED*. SUGI 29, paper 188-29, May 2004, <www2.sas.com/proceedings/sugi29/188-29.pdf > (December 8, 2006).

Pahwa, Punam & Blair, Saskatchewan T. (2002). *Statistical Analysis of Longitudinal Data*. CACRC, newsletter, April 2002, < www.cacr.ca/news/2002/0204pahwa.htm > (July 21, 2006).

Shahbazi, Mahin (2005). *Age and Sex Differences in the Acquisition and Maintenance of Intravenous Amphetamine Self-Administration in rats*. Georgia State University, Electronic Thesis and Dissertation Database, August 2005, (September 13, 2006).

Ware, James H. (1985). Linear Models for the Analysis of Longitudinal Studies. *The American Statistician*, 39, 95-101.

Appendix

****Obtain the means for each rat and the correlations****;

proc sort data=libname.rats out=rats4;
by gender;
run;

ods html;
title "Sample Covariance and Correlation Matrices by Gender";
proc corr data=rats4 cov;
by gender;
var day1-day14; ****data is in the multivariate layout****
run;
ods html close;

Univariate Repeated Measures ANOVA test

****univariate layout****
****test is for gender group****

proc sort data=libnames.rats2 out=rats5;
by gender;
run;

ods html;
proc glm data=rats;
class rat gender;
*model behavior=gender rat(gender) day day*gender/;*
random rat(gender) / test;
run;
ods html close;
quit;

****nouni -suppresses individual ANOVA, printe ask for the test of sphericity, and the nom command means no multivariate just univariate under the assumption that the covariance structure is correct ****;

ods html;
proc glm data=rats4;
class gender rat;
*model day1-day14 = gender/nouni; ***data is in the multivariate layout****
repeated day/printe nom;
run;
ods html close;
quit;

**** **PROC MIXED** - covtest provides the estimates of the std errors of the estimated variance components, type = option lets you specify what structure to use for the covariance matrix, options G and GCORR requests the estimates of the variances and covariances be displayed ****

```
ods html;
proc mixed data=libname.rats2 covtest;
class rat age gender;
model behavior = day bw age gender/solution chisq;
random intercept day/type=un subject=rat g gcorr;
run;
ods html close;
```