• Consider a simplified version of Ricardo’s analysis that includes the following factors of interest (essentially, fixing ACh-dosage and age):

1) genotype (WT/KO)
2) Tempol drug (yes/no)

```
<table>
<thead>
<tr>
<th>drug</th>
<th>yes</th>
<th>no</th>
</tr>
</thead>
<tbody>
<tr>
<td>genotype</td>
<td>WT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KO</td>
<td></td>
</tr>
</tbody>
</table>
```

We get to observe each mouse under both levels of drug, but a mouse belongs in only one genotype category (i.e. a mouse is nested in a genotype.)
Assume we have 5 mice of each genotype giving us N=20 observations (5 observations in each of the 4 ‘cells’ below).

<table>
<thead>
<tr>
<th>drug</th>
<th>yes</th>
<th>no</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>genotype</strong></td>
<td>WT</td>
<td>KO</td>
</tr>
</tbody>
</table>

- What type of analysis is appropriate?
  - The crucial element is to recognize that we do not have independent observations.
  - If initially perceiving this in the two-way ANOVA framework... note that the error term used to test *genotype* is different than the *error* term used to test *drug*.
  - Split-plot? Repeated measures? Paired *t*-test?

- To me, the general structure looks a lot like a split-plot design. One way to design a split-plot...
– Levels of whole-plot factor (i.e. *genotype* here) are randomly assigned to whole plots (i.e. mice).

– Each whole plot is then divided into split plots, and the levels of the split-plot factor (i.e. *drug*) are randomly assigned to the split plots within each whole plot.

– This is not quite a true ‘split plot’ because we can’t randomly assign genotype to each mouse, and it looks like Ricardo ALWAYS performed the *drug=NO* first and then did *drug=YES*, but the idea is similar... we have correlation between observations due to ‘repeated measures’ on each mouse, and each mouse is nested in a whole-plot factor level.
− If you can recognize this connection to the split plot, it can be easy to write out the ANOVA table and determine appropriate tests.

− I would write the ANOVA table as:

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>genotype</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>mouse(genotype)</td>
<td>8</td>
<td>← random term</td>
</tr>
<tr>
<td>drug</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>drug*genotype</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>s.p. error</td>
<td>8</td>
<td>← random term</td>
</tr>
<tr>
<td>c.total</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

* At the whole plot level, we essentially have a two-sample $t$-test between WT and KO. With 5 mice in each genotype group, there are 8 df for error.

* The split plot error is also the $mouse(genotype)*drug$ interaction term which has $8 \times 1 = 8$ df.
Statistical Consulting Topics

Split Plot vs. Repeated Measures

• The two analyses listed above are closely related.

• They are similar in that individuals are usually nested in one factor, and observed on multiple levels of another factor.
  – Like mice are nested in a genotype and then observed at all drug levels.
  – Or patients nested in a treatment and then observed at multiple time points.

• These studies often use terminology like “within subjects factor” (drug) and “between subjects factor” (genotype).
• They can be **different** because we model their correlation structure differently.

– In a true **split-plot analysis**, the levels of the split-plot factor are assigned at random to a split-plot unit, and we usually model the correlation structure as a *block diagonal* with the within-subject correlation as **compound symmetry**.

\[
\text{var}(Y) = \begin{pmatrix}
W & 0 & \ldots & 0 \\
0 & W & \ldots & \\
\ldots & \ldots & \ldots & 0 \\
0 & \ldots & 0 & W
\end{pmatrix}
\]

where

\[
W = \begin{pmatrix}
\sigma^2 + \sigma_s^2 & \sigma_s^2 & \sigma_s^2 \\
\sigma_s^2 & \sigma^2 + \sigma_s^2 & \sigma_s^2 \\
\sigma_s^2 & \sigma_s^2 & \sigma^2 + \sigma_s^2
\end{pmatrix}
\]

if there were 3 measurements per subject.
In a repeated measures analysis, we often model the correlation of observations over time. The correlation structure is still block diagonal (independence between subjects), but the within-subject correlation reflects the differing levels of correlations between observations. We might use an AR(1) structure, for example.

\[
\text{var}(Y) = 
\begin{pmatrix}
W & 0 & \ldots & 0 \\
0 & W & \ldots & \ldots \\
\ldots & \ldots & \ldots & 0 \\
0 & \ldots & 0 & W
\end{pmatrix}
\]

where

\[
W = \sigma^2 \begin{pmatrix} 1 & \rho & \rho^2 \\
\rho & 1 & \rho \\
\rho^2 & \rho & 1 \end{pmatrix}
\]

if there were 3 equally-spaced measurements per subject.
• I see the main difference between these two analyses as the ability (or lack of ability) to randomly assign levels to the split-plot units.

  – The level of *time* usually can’t be randomly assigned and is just an innate characteristic of an observation → repeated measures.

  – If there are only two levels of the “within subject factor”, then the end result of the differing correlation structures is the same.

  \[ W = \sigma^2 \begin{pmatrix} 1 & \rho \\ \rho & 1 \end{pmatrix} \quad \text{or} \quad W = \begin{pmatrix} (\sigma^2 + \sigma_s^2) & \sigma_s^2 \\ \sigma_s^2 & (\sigma^2 + \sigma_s^2) \end{pmatrix} \]

• The crucial element for both of these analyses is similar, that we need to include correlation between observations on the same subject or experimental unit in our model, and use appropriate ‘nesting’ statements in SAS.
Building from here...

• What if we have this same amount of data for a ‘young’ set of mice and an ‘old’ set of mice?
  – Is a mouse nested in \textit{age}?
  – Is a mouse nested in specific \textit{age} and \textit{genotype} combination?
  – Still have only two observations per mouse.

• What about the \textit{dosage}?
  – One mouse (with a specific \textit{age} and \textit{genotype}) is observed over all dosages for each drug level.
  – Based on Ricardo’s description, it looks like they were done in sequential order, at 5 minutes apart.
  – Is it linear in dose?
  – We now have 10 observations per mouse.