Modeling Spatial Learning in Rats Based on Morris Water Maze Experiments

Christel Faes, Marc Aerts
Center for Statistics, Hasselt University, Agoralaan, Diepenbeek, Belgium
Email: christel.faes@uhasselt.be

Helena Geys, Luc De Schaepdrijver
Johnson and Johnson, PRD Biometrics and Clinical Informatics, Beerse, Belgium

February 19, 2008

Abstract

The Morris water maze, developed by Morris (1984), is a behavioral experiment designed to test the spatial memory. When repeating the experiment several times, the changes in time (latency) and distance (path) taken to reach the platform are indicators for the learning and memory abilities of the rat. In juvenile toxicity studies, it is of interest to test whether dosing juvenile rats with some compound of interest has an effect on its learning ability. The traditional analysis uses non-parametric tests to check for a possible dose-effect. However, due to the many tests performed, this approach lacks power. Here, an alternative method is proposed, accounting for the longitudinal design of the study, the right-censoring of observations when animals did not find the platform and the correlation between the time and distance taken to reach the platform.

Keywords: Juvenile Toxicity Study, Morris Water Maze, Censoring, Longitudinal Data

1 Introduction

When a pharmaceutical company brings a new medicine on the market it must be assured that the product is safe for intended use and in the event of accidental misuse. Laboratory animals continue to play a vital and necessary role in the safety and risk assessment of new medicines for important areas of human health care. Since the 1960s, the U.S. Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) stimulated reproductive and
developmental toxicity research to better protect people against exposures to agents that cause developmental toxicity. Since then, many different experimental methods for investigating toxic effects of chemicals on reproduction and development are in use. A combination of three studies is typically used, assessing specific types of effects: fertility studies, embryo-fetal developmental toxicity studies and pre- and post-developmental toxicity studies. In this paper, we concentrate on the hazard identification and dose-response evaluation of the Morris water maze in juvenile toxicity studies. Juvenile toxicity studies are designed to study potential adverse effects following exposure of xenobiotics during critical periods of organ development. In these studies, mother animals are bred, giving birth to young animals. Typically, a number of these animals are selected and exposed to the chemical of interest. Parameters that are examined include growth and development, serum chemistry and haematology, gross pathology and histopathology, physical and sexual developmental landmarks and behavioral assessment such as locomotor activity, auditory startle response and learning and memory.

Several types of behavioral experiments, testing the learning and memory of the developing animals, can be conducted. One procedure, which is often used, is the Morris water maze, developed by Morris (1984). It is a behavioral experiment designed to test spatial memory. In this experiment, a rat is placed into a circular pool divided in quadrants which contains a platform, hidden a few millimeters below the water surface. The rat is placed in the tank and must learn the location of the submerged platform through a series of trials. The time (latency) and distance (path) taken to reach the platform are indicators for the learning and memory abilities of the rat.

Analyzing data from the Morris water maze raises a number of statistical challenges. First, since the experiment is repeated at several time points, we are dealing with a longitudinal design. Second, when a rat does not reach the platform after 60 seconds, the rat was guided to the platform. This means that the outcomes of interest are right-censored. Further, multiple outcomes, of different nature, are of interest in this study (time and distance taken to reach
the platform). In addition, since laboratory studies involve considerable amounts of time and money, as well as large numbers of animals, it is essential that the most appropriate and efficient statistical models are used (Williams and Ryan 1996).

In this paper, focus is on the risk assessment of a chemical, based on the Morris water maze experiment. A description of the data is given in Section 2. In Section 3 a discussion about the statistical analysis which is typically performed is given. In Section 4 an improved multivariate analysis is proposed for the risk assessment of the chemical, based on the time and distance taken to reach the platform. In Section 5, the results are presented.

2 The Morris Water Maze

In a juvenile study with a central nervous system active compounds, pups were exposed from day 12 of age until day 50 of age. A first set of animals (Set I) was tested for learning and memory using the Morris water maze during the treatment period, at age of 46 (± 2) days. A second set of animals (Set II) was tested 14 days after the treatment period, at age of 69 (± 2) days. The dose levels used were a control group and three treated groups (low, mid and high dose groups corresponding to 0.04, 0.16 and 0.63 mg/kg/day, respectively). Each treatment group consists of 12 male and 12 female rats.

The Morris water maze is a circular pool filled with water made cloudy by the addition of powdered milk. The pool has a submerged platform a few millimeters below the water surface. Visual cues, such as colored shapes, are placed around the pool in plain sight of the animal to assist the rats in their spatial orientation. An illustration of the Morris water maze is given in Figure 1. The pool is subdivided in 4 quadrants, as illustrated in the figure, with the platform in quadrant 4. Each rat was first placed onto the platform for 15 seconds to triangulate its position in relation to the spatial cues. After this, the rat was placed in the water. The rat will swim around the pool in search of the platform. Various parameters were recorded, including the number of quadrants the rat had entered (path) and the time taken for the rat to reach
the platform \textit{(latency)}. If 60 seconds elapsed and the rat had not found the platform, the rat was guided to the platform. This experiment was repeated three times per day, with 30 minutes between the end of the trial and the beginning of the next trial. This procedure was repeated at 4 days, each day starting at a different point. The letters A to D in Figure 1 indicate the starting position of the rats at days 1 to 4, respectively.

The earliest and classic measure of learning is latency, which is the time it takes to find the platform. The rat’s escape from the water reinforces its desire to quickly find the platform, and on subsequent trials (with the platform in the same position) the rat should be able to locate the platform more rapidly. However, rats might also guess an area and swim a search pattern, getting to the platform quite quickly. Therefore it is important to in addition take the path in quadrants into account as well.

Figure 2 shows the average time (latency) and average number of quadrants (path) for the rats to reach the platform, per dose group at the different days. The top two rows correspond with rats which were tested during the treatment period (Set I). The bottom two rows correspond with rats which were tested after the treatment period (Set II). From the graphs it is clear that
both the average latency and path decrease in time. Also, both parameters show very similar
trends, indicating possibly large correlation between the two endpoints. There are, however,
no clear differences between the different dose groups, neither for Set I or Set II.

3 The Standard Analysis

Typically, the following procedure is used to study the results of the Morris water maze.
(i) First, summary statistics of the time to reach the platform (latency) are calculated. Also
percentages of animals completing the maze were looked at. (ii) Second, the time-to-events
(latency) were analyzed using the Wilcoxon Test with exact probabilities. Animals failing to
complete the maze were given the value of (maximum allowable time) +1, here the value 61.
(iii) The Jonckheere Trend test was used to examine if a dose related trend was present in
latency using the group ordering 1, 2, 3 and 4. (iv) Further, the frequency of successfully
completing the maze was analyzed using the Fisher exact test. (v) Finally, the Cochran
Armitage trend test was used to look for a dose related trend in the frequency of successful
completion using the group ordering 1, 2, 3 and 4. Details about these nonparametric tests
can be found in, e.g., Lehmann and d’ Abrera (1975).

All tests were performed separately at each session and run, and also separately for each sex.
Tests were 2 sided and not adjusted for multiple testing. Results are summarized in Table 1.
The table displays the type of test used, the hypothesis tested, the gender, the number of tests
performed (due to the different sessions and runs) and number (percentage) of significant test
results for, respectively, Set 1 and Set 2 animals. Due to the large number of tests being
performed inference was based on consistent effects being seen over the different time periods
and the sexes. It seems there are more significant effects in females in Set 1, in comparison
with the effects in males. But, since only few effects are significant, it is concluded that there
is no important effect of the test article on the development of the rat.

While use of these non-parametric methods is easy, this procedure ignores many aspects in the
Figure 2: The average profiles of latency and path per dose group. The top two rows present the average latency and path per day in Set 1. The bottom two rows present the average latency and path per day in Set 2.
Table 1: The standard analysis. Summary of the results of Wilcoxon test and Jonckheere test for the latency, and Fisher’s exact test and CMH test for the response completing.

<table>
<thead>
<tr>
<th>Response: latency</th>
<th>Test</th>
<th>Hypothesis</th>
<th>Sex</th>
<th># Tests</th>
<th># Sign Tests (%)</th>
<th># Sign Tests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilcoxon test</td>
<td>group 2 vs group 1</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 3 vs group 1</td>
<td>M</td>
<td>12</td>
<td>1 (8.3%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 4 vs group 1</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 2 vs group 1</td>
<td>F</td>
<td>12</td>
<td>1 (8.3%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 3 vs group 1</td>
<td>F</td>
<td>12</td>
<td>2 (16.7%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 4 vs group 1</td>
<td>F</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>Jonckheere test</td>
<td>trend</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trend</td>
<td>F</td>
<td>12</td>
<td>3 (25.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response: completing</th>
<th>Test</th>
<th>Hypothesis</th>
<th>Sex</th>
<th># Tests</th>
<th># Sign Tests (%)</th>
<th># Sign Tests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fisher’s exact test</td>
<td>group 2 vs group 1</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 3 vs group 1</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 4 vs group 1</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 2 vs group 1</td>
<td>F</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 3 vs group 1</td>
<td>F</td>
<td>12</td>
<td>1 (8.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>group 4 vs group 1</td>
<td>F</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>CMH test</td>
<td>trend</td>
<td>M</td>
<td>12</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trend</td>
<td>F</td>
<td>12</td>
<td>1 (8.3%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

data and does not use the data in an efficient way. First, the response is analyzed separately at each time point. However, correlation among the measurements at different time points is very likely to be present because of the learning process of the animal. In the standard analysis, the association of the measurements at different endpoints is ignored and lost. This also results in a problem of multiple testing. As an alternative, one can account for the longitudinal nature of the data, accounting for the possible correlation between endpoints during successive experiments. Second, when animals fail to find the platform within 60 seconds, the latency is set to a fixed value (61 seconds). This (unrealistically) assumes that the rats which were guided
to the platform, would have reached it after 61 seconds. This causes the standard analysis to be subject to bias. More advanced methods account for the information that measurements are censored at a certain point in time. Here, use of a parametric method is proposed, accounting for the longitudinal structure of the data and the censoring of observations. In addition, the number of quadrants (path), also being an important indicator for the memory and learning of the animal, will be investigated for possible dose-effects in the same analysis.

4 A New Dose-Response Approach

In this section, an alternative dose-response approach for the Morris water maze experiment is proposed. It is a likelihood-based procedure, accounting for the complex design of the study. First, a dose-response model for the latency variable is given. Second, a dose-response model for the path variable is explained. Finally, these two models are then combined in a joint model.

4.1 Dose-Response Analysis of Latency

First, a dose-response model is proposed to describe the relationship between the latency and dose. The latency is the time for the rat to reach the platform, also known as a time-to-event variable. Several methods to analyze event times exist in literature. Event times are very often the endpoint of interest in clinical trials. Examples include the time to recurrence of cancer or survival time. Event times are always prone to censoring. An appropriate statistical analysis for event time data should account for the censoring in the data. Survival methods include for example the non-parametric life table analysis or Kaplan-Meier survival curves, and the parametric exponential, Weibull or log-normal regression models (Hougaard, 2000). Here, a parametric dose-response model, describing the relationship among latency and dose, is proposed, to handle the non-standard situation of successive time-to-event outcomes.
Let $t_{ij}$ be the latency of rat $i$ ($i = 1, \ldots, N$) at experiment $j$ ($j = 1, \ldots, J$), and $\delta_{ij}$ be a censoring indicator which equals 0 if the latency was censored, and 1 otherwise. In the water maze, censoring was conducted at a fixed time point, namely at 60 seconds. A censored observation therefore always has $t_{ij} = 60$ and $\delta_{ij} = 0$. First, assume that the responses at the different runs $j$ are independent. Under the assumption of right censored data, there are two possible contributions to the likelihood for the runtime $t_{ij}$: (i) if the event occurred at time $t_{ij}$ ($0 \leq t_{ij} \leq 60$), the contribution is

$$L_{ij} = f(T = t_{ij}),$$

and (ii) if it is censored at time $t_{ij} = 60$, the contribution is

$$L_{ij} = S(t_{ij}) = P(T \geq t_{ij}) = P(T \geq 60).$$

Indeed, the method accounts for the knowledge that the event did not yet occur up to the censoring time. Assuming a Weibull distribution for the time-to-event,

$$T \sim Weibull(\lambda, \kappa)$$

with $\lambda$ a scale parameter and $\kappa$ a shape parameter, the likelihood over all observations is

$$\ell = \prod_i \prod_j \ell_{ij} = \prod_i \prod_j (\kappa \lambda t_{ij}^{\kappa-1} e^{-\lambda t_{ij}^\kappa})^{\delta_{ij}} (e^{-\lambda t_{ij}^\kappa})^{1-\delta_{ij}} = \prod_i \prod_j (\kappa \lambda t_{ij}^{\kappa-1})^{\delta_{ij}} (e^{-\lambda t_{ij}^\kappa}).$$

When the shape parameter $\kappa$ equals 1, than the Weibull model reduces to the exponential model. Based on the proposed model, the proportion of animals reaching the platform before time $t$ is given by

$$P(T < t) = 1 - \exp(-\lambda t^\kappa),$$

and the mean time to reach the platform is equal to

$$E[T] = \lambda^{-1/\kappa} \Gamma \left(\frac{1}{\kappa} + 1\right),$$
with $\Gamma(.)$ the gamma-function. Note that the scale parameter $\lambda$ is proportional to the mean time to reach the platform. In order to specify the dose-response relationship between the latency and dose (and other possible covariates), $\lambda$ is estimated as in an exponential regression as

$$
\lambda = \exp(X_i\beta),
$$

with $X_i$ a ($J \times p$)-dimensional matrices of known covariate values for subject $i$ (e.g. dose) and $\beta$ a $p$-dimensional vector of unknown fixed effects regression coefficients.

However, the responses of the same animal at different runs and days are possibly correlated. To account for possible correlation of successive event-times, rat-specific effects $b_i$, also called random effect, on the scale parameter $\lambda$ are included:

$$
\lambda = \exp(X_i\beta + Z_i b_i),
$$

with $Z_i$ a ($J \times q$)-dimensional matrices of known covariate values for subject $i$ and $b_i$ a $q$-dimensional vector of unknown random effects. It is assumed that $b_i$ follows a multivariate normal distribution with mean 0 and variance-covariance matrix $D$. This is commonly called a generalized linear mixed model (Molenberghs and Verbeke, 2005), or in the context of censored data a frailty model.

Based on this model accounting for the longitudinal nature of the data, the mean latency, or time to reach the platform, is given as

$$
E[T|\beta, \kappa, D] = \int_{\mathbb{R}^q} \lambda(\beta, b) \Gamma[(1/\kappa) + 1] f(b) db,
$$

with $\Gamma(.)$ the gamma-function and $f(b)$ a multivariate normal distribution with mean 0 and variance-covariance matrix $D$. This can be easily calculated numerically (Molenberghs and Verbeke, 2005). Similarly, the probability of being censored at 60 seconds is given as

$$
P(T > 60|\beta, \kappa, D) = \int_{\mathbb{R}^q} \exp(-\lambda(\beta, b)60^\kappa) f(b) db.
$$
4.2 Dose-Response Analysis of the Path

Second, a dose-response model describing the relationship between the dose and the path (number of quadrants) is formulated. Let $q_{ij}$ be the number of quadrants for rat $i$ at run $j$. Ignoring the correlation due to the repeated measurements, a Poisson distribution for the number of quadrants can be assumed:

$$Q_{ij} \sim \text{Poisson}(\mu_{t_{ij}})$$

with $t_{ij}$ the time in search for the platform and $\mu$ the average number of quadrants per time unit. Thus, if the rat reached the platform at time $t_{ij}$ ($0 \leq t_{ij} \leq 60$), the contribution to the likelihood is

$$L_{ij} = f(Q = q_{ij}|T = t_{ij}) = (\mu_{t_{ij}})^{q_{ij}} \exp(\mu_{t_{ij}})/q_{ij}!;$$

if the rat did not reach the platform at 60 seconds, the contribution is

$$L_{ij} = f(Q = q_{ij}|T = 60) = (\mu_{60})^{q_{ij}} \exp(\mu_{60})/q_{ij}!.$$

The dose-response relationship in terms of covariates $X_i$ is specified via the rate $\mu$:

$$\mu = \exp (X_i\beta).$$

To account for possible correlation at different runs and days, a random effect on the mean parameter $\mu$ is included,

$$\mu_{ij} = \exp (X_i\beta + Z_i b_i),$$

with $Z_i$ a ($J \times q$)-dimensional matrices of known covariate values for subject $i$ and $b_i$ a $q$-dimensional vector of unknown random effects. Based on this model, the mean number of quadrants per second is given as

$$E[\mu|\beta, D] = \int_{\mathbb{R}^q} \exp (X_i\beta + Z_i b_i) f(b)db,$$

11
4.3 Joint Dose-Response Analysis

The latency and path are measured on the same rats, during each experiment. It is possible that they influence each other, for example shorter swimming times can be related to faster swimming (number of quadrants per second). To account for such effect, we will estimate the dose-effect of latency and path jointly in one model.

Combining the dose-response models for the latency and path, we can specify the following model:

\[
T_{ij} = \text{Weibull}(\lambda_{ij}, \kappa), \\
\lambda_{ij} = \exp(X_{1ij}\beta_1 + Z_{1i}b_{1i}), \\
Q_{ij}|T_{ij} = \text{Poisson}(\mu T_{ij}), \\
\mu_{ij} = \exp(X_{2ij}\beta_2 + Z_{2i}b_{2i}),
\]

\[
(b_1, b_2)' \sim N(0, D),
\]

linking the two dose-response models by assuming that the random effect \((b_1, b_2)\) follow a joint normal distribution. In this way, we account for the possible association between the path and latency, and a more efficient estimation method follows. To account for the censoring of observations, similar likelihood expressions as given in previous sections are used.

5 Results

The water maze experiment is repeated 3 times per day and at 4 days. Animals were randomized in two sets, the first set which was tested during the treatment period and the second set which was tested after the treatment period.

Model selection starts by assuming independence among the two outcomes of interest. To account for possible correlations due to the longitudinal structure of the data, a random intercept on both outcomes was considered. In a later step, correlations between latency and
path are accounted for. For both the latency and path, a dose-response model with dose, day, time and gender as covariates, including all pairwise interactions, and all interactions with set is considered. Day and time are included as continuous covariates, whereas dose was included as a categorical covariate. All interactions with the set are included in the model, since this allows to model all data jointly (from both experimental groups) in which way we can also study differences in these two groups. This results in a dose-response model with 39 and 40 parameters for, respectively, the path and latency. These models were simplified using a stepwise procedure based on AIC, stepwise deleting the most non-significant effect. This resulted in models with 13 and 20 parameters for, respectively, the path and latency. These two models are then combined into a joint model by linking the two random intercepts using a bivariate normal distribution with general variance-covariance matrix. This resulted in a model with 34 parameters with an AIC value of \(-35905\), which is better than a model assuming independence among the latency and path (AIC equal to \(-35864\)).

Table 2 shows the parameter estimates of the final model, corresponding to the path and latency. For the path, there is a significant decrease of the number of quadrants per second at the high dose level. This is only present when the experiment is conducted during the period of dosing (Set 1), and not when the experiment is done two weeks after dosing. The learning effect is visible in both studies, reflected by the increase of quadrants per second with time and day. The dose has no effect on this learning effect (no significant interaction of dose with time or day). For the latency, the dosing has an effect on the learning effect, during the period of dosing. In the low dose group, the learning effect from day to day is largest, as compared to animals in the high dose group. This effect is not seen in the Set 2 animals. For the Set 1 animals, a dose-gender interaction is also noted, indicating that females react more on the dose in comparison with the male rats. This was also seen in the traditional analysis, although there was no formal way of testing for such an effect. Note that the correlation between the number of quadrants per minute and the time taken to reach the platform are highly correlated. The higher the rate at which rats change quadrant (number of quadrants
per minute), the longer it takes for the rats to reach the platform.

In Figure 3 the resulting profiles are illustrated for male animals in Set 1. The top row shows the estimated number of quadrants per minute, for the different dose levels. The number of quadrants clearly increases when repeating the experiment. This shows the learning effect of the animal, swimming faster when it explores the location of the platform. There is also a clear effect of the dose given to the animal, with higher doses showing higher swimming speed as compared to animals in low dose groups. Since lines are parallel, the learning effect in the different dose groups is not different. The second row in Figure 3 shows the mean estimated time to reach the platform, in case the animals would not be censored after 60 seconds. There is a clear decrease in time to each the platform when the experiment is repeated in time. If the animals would not be stopped swimming after 1 minute, they would need (on average) 2 minutes to reach the platform during the first experiment. There seems to be small differences in time to reach the platform, among dose groups. The bottom row of Figure 3 shows the estimated probability of censoring, or the estimated probability that animals need more then 60 seconds to reach the platform. There is a clear decreasing trend with time, and only a minor effect of dose.

6 Discussion

In this paper, a parametric approach to model data from the Morris’ Water Maze is given. The traditional methods, using several non-parametric test are used on the same dataset. The proposed method has higher power as compared with the traditionally used method in detecting possible significant effects. While the procedure is more time-consuming as compared to the non-parametric method, the proposed method accounts for all aspects in the data: censoring, correlations, bivariate outcomes.
Table 2: Parameters estimates for the joint model for latency and path.

| Parameter | Estimate | St.Error | p-value | | Estimate | St.Error | p-value |
|-----------|----------|----------|---------| |----------|----------|---------|
| **Path**  |          |          |         | |          |          |         |
| Intercept | -1.211   | 0.035    | <.001   | | -1.301   | 0.024    | <.001   |
| Dose=1    | -0.070   | 0.037    | 0.062   | |          |          |         |
| Dose=2    | -0.062   | 0.037    | 0.095   | |          |          |         |
| Dose=3    | -0.124   | 0.037    | 0.001   | |          |          |         |
| Time      | 0.065    | 0.019    | 0.001   | | 0.113    | 0.019    | <.001   |
| Day       | 0.094    | 0.014    | <.001   | | 0.130    | 0.014    | <.001   |
| Gender    | -0.052   | 0.026    | 0.052   | |          |          |         |
| Time*Day  | -0.020   | 0.012    | 0.088   | | -0.034   | 0.012    | <.001   |
| **Latency** |          |          |         | |          |          |         |
| Intercept | -5.193   | 0.232    | <.001   | | -5.238   | 0.150    | <.001   |
| Dose=1    | -0.046   | 0.272    | 0.867   | |          |          |         |
| Dose=2    | 0.039    | 0.272    | 0.885   | |          |          |         |
| Dose=3    | -0.091   | 0.276    | 0.743   | |          |          |         |
| Time      | 0.583    | 0.077    | <.001   | | 0.549    | 0.077    | <.001   |
| Day       | 0.575    | 0.071    | <.001   | | 0.733    | 0.052    | <.001   |
| Gender    | 0.374    | 0.222    | 0.094   | |          |          |         |
| Dose=1*day| 0.292    | 0.087    | 0.001   | |          |          |         |
| Dose=2*day| 0.171    | 0.088    | 0.054   | |          |          |         |
| Dose=3*day| 0.121    | 0.087    | 0.165   | |          |          |         |
| Dose=1*gender| -0.698   | 0.305    | 0.023   | |          |          |         |
| Dose=2*gender| -0.611   | 0.304    | 0.046   | |          |          |         |
| Dose=3*gender| -0.337   | 0.306    | 0.273   | |          |          |         |
| Time*day  | -0.132   | 0.038    | 0.001   | | -0.092   | 0.037    | 0.014   |
| κ         | 1.113    | 0.021    | <.0001  | | 1.113    | 0.021    | <.001   |

Random Effect Variance and Correlation

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>St.Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.E. RI Path</td>
<td>0.078</td>
<td>0.011</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>S.. RI Latency</td>
<td>0.492</td>
<td>0.039</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.908</td>
<td>0.112</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
Figure 3: The estimated profiles of latency and path per dose group for the male Set 1 rats. The top row presents the estimated number of quadrants per minute per day. The second row shows the estimated mean time to reach the platform (in seconds) and the third row presents the estimated probability of censoring.
References


