Modeling Spatial Learning in Rats Based on Morris Water Maze Experiments

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Risk Assessment

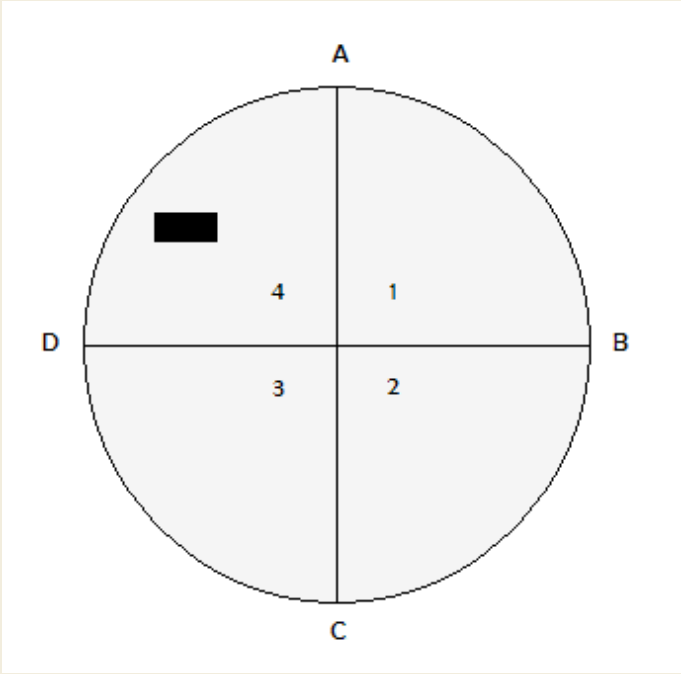
- A new medicine must assured to be safe
- Laboratory animals are used for the risk assessment
- A combination of three studies is typically used:
  - Fertility studies
  - Embryo-fetal developmental toxicity studies
  - Pre- and post-developmental toxicity studies

Juvenile Toxicity Studies

- Study the potential adverse effects following exposure during critical periods of organ development
- Young animals are exposed to the chemical of interest
- Important parameter that are examined are learning and memory
- Morris water maze (Morris, 1984) is a behavioral experiment, testing the spatial learning and memory of the developing animals

Morris Water Maze

- A rat is placed into a circular pool
- The pool contains a platform, hidden a few millimeters below the water surface
- The rat 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 of the rat



The Experiment

- A central nervous system active compound was tested
- Pups were exposed from day 12 of age until day 50 of age
- Set 1: tested for learning and memory during the treatment period
- Set 2: tested 14 days after the treatment period
- Control group and three treated groups (low, mid and high dose groups)
- 12 male and 12 female rats per treatment group

Procedure

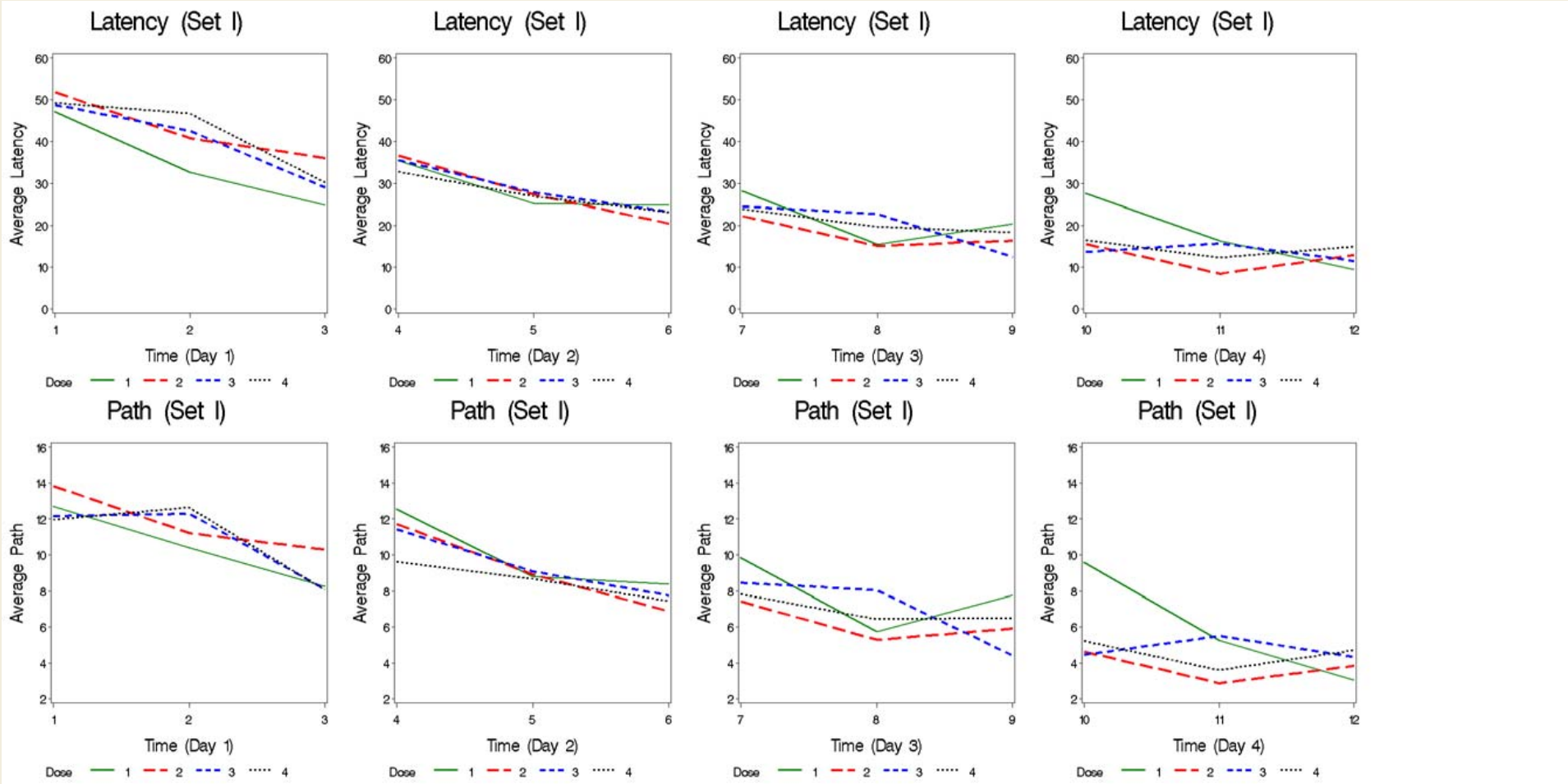
1. Rat is placed onto the platform for 15 seconds
2. The rat is placed in the water
3. The rat will swim around the pool in search of the platform
4. If 60 s elapsed and rat had not found the platform, the rat was guided to the platform
5. (2)-(4) is repeated three times (with a 30 minutes break)
6. (1)-(4) is repeated at 4 days, each day starting at a different point (A-D)

Outcomes of Interest

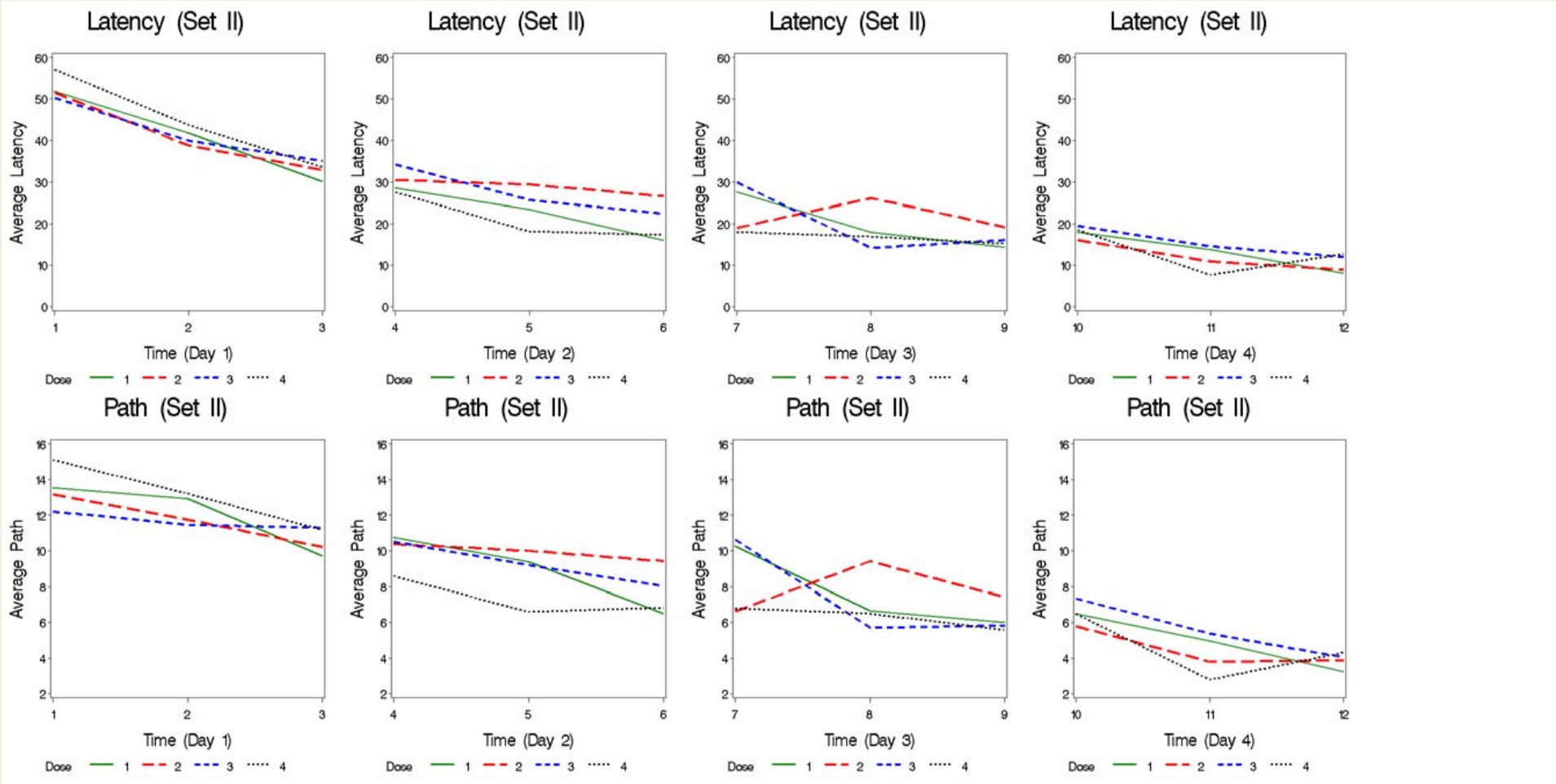
The rat's escape from the water reinforces its desire to quickly find the platform, and on subsequent trials the rat should be able to locate the platform more rapidly

- Latency:
  - Measured as the time (in seconds) to reach the platform
- Path
  - Measured as the number of quadrants
  - Rats might guess an area and swim a search pattern, getting to the platform quite quickly. Therefore, path has to be taken into account as well.

Set I


Latency (Set I)
Average Latency
Time (Day 1)
Time (Day 2)
Time (Day 3)
Time (Day 4)
Dose 1 2 3 4
Path (Set I)
Average Path

Set II


Latency (Set II)
Average Latency
Time (Day 1)
Time (Day 2)
Time (Day 3)
Time (Day 4)
Dose 1 2 3 4
Path (Set II)
Average Path

Standard Analysis

1. *Summary statistics* of the time to reach the platform (latency)
2. *Percentages* of animals completing the maze are calculated
3. Time-to-events (latency) were analyzed using *Wilcoxon Test* with exact probabilities
   (animals failing to complete the maze are given value 61)
4. The *Jonckheere Trend test* was used to examine if a dose related trend was present in the latency
5. Frequency of successfully completing the maze was analyzed using *Fisher exact test*
6. *Cochran Armitage trend test* used to look for a dose related trend in the frequency of successful completion

All test were performed separately at each session and run, and also separately for each sex.

Results Standard Analysis

Response: latency				Set 1	Set 2
Test	Hypothesis	Sex	Tests	Sign Tests	Sign Tests
Wilcoxon test	group 2 vs group 1	M	12	0 (0.0%)	1 (8.3%)
	group 3 vs group 1	M	12	1 (8.3%)	1 (8.3%)
	group 4 vs group 1	M	12	0 (0.0%)	1 (8.3%)
	group 2 vs group 1	F	12	1 (8.3%)	1 (8.3%)
	group 3 vs group 1	F	12	2 (16.7%)	1 (8.3%)
	group 4 vs group 1	F	12	0 (0.0%)	0 (0.0%)
Jonckheere test	trend	M	12	0 (0.0%)	1 (8.3%)
	trend	F	12	3 (25.0%)	0 (0.0%)
Response: completing				Set 1	Set 2
Test	Hypothesis	Sex	Tests	Sign Tests	Sign Tests
Fisher's exact test	group 2 vs group 1	M	12	0 (0.0%)	0 (0.0%)
	group 3 vs group 1	M	12	0 (0.0%)	0 (0.0%)
	group 4 vs group 1	M	12	0 (0.0%)	0 (0.0%)
	group 2 vs group 1	F	12	0 (0.0%)	0 (0.0%)
	group 3 vs group 1	F	12	1 (8.3%)	0 (0.0%)
	group 4 vs group 1	F	12	0 (0.0%)	0 (0.0%)
CMH test	trend	M	12	0 (0.0%)	0 (0.0%)
	trend	F	12	1 (8.3%)	0 (0.0%)

Results Standard Analysis

- Due to the large number of tests being performed inference is 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.
- Only few effects are significant, thus no important effect of the test article on the development of the rat

But...

- The standard procedure ignores many aspects in the data
- It does not use the data in an efficient way

Challenges

- Longitudinal design (experiment is repeated at several time points)
- Right-censoring (when rat does not reach the platform after 60 s, it is guided to the platform)
- Multiple outcomes, of different nature (time and distance taken to reach the platform)
- An efficient and appropriate statistical method

Dose-Response Analysis of Latency

- t_{ij} is the latency of rat i at experiment j
- δ_{ij} is the censoring indicator (0 if censored, 1 otherwise)
- There are two possible contributions to the likelihood:
  1. If the event occurred at time t_{ij}, the contribution is
  $$L_{ij} = f(T = t_{ij})$$
  2. If it is censored at time $t_{ij} = 60$, the contribution is
  $$L_{ij} = S(t_{ij}) = P(T \geq t_{ij}) = P(T \geq 60)$$
- Assuming a Weibull model, the likelihood is

$$\begin{aligned} \ell &= \Pi_i \Pi_j \left(\kappa\lambda t_{ij}^{\kappa-1} e^{-\lambda t_{ij}^{\kappa}}\right)^{\delta_{ij}} \left(e^{-\lambda t_{ij}^{\kappa}}\right)^{1-\delta_{ij}} \\ &= \Pi_i \Pi_j \left(\kappa\lambda t_{ij}^{\kappa-1}\right)^{\delta_{ij}} \left(e^{-\lambda t_{ij}^{\kappa}}\right). \end{aligned}$$

Dose-Response Analysis of Latency

- To specify the dose-response relationship, the scale parameter λ is estimated as in an exponential regression

$$\lambda = \exp(X_i \boldsymbol{\beta})$$

- To account for possible correlation of successive event-times, rat-specific effects are included:

$$\lambda = \exp(X_i \boldsymbol{\beta} + Z_i \boldsymbol{b}_i)$$

with $\boldsymbol{b}_i \sim N(0, D)$

Dose-Response Analysis of Latency

- The mean latency, or time to reach the platform, is given as

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

  with $\Gamma(.)$ the gamma-function and $f(\boldsymbol{b})$ a multivariate normal distribution with mean 0 and variance-covariance matrix D.
- The probability of being censored at 60 seconds is given as

$$P(T > 60|\boldsymbol{\beta}, \kappa, D) = \int_{\mathbb{R}^q} \exp(-\lambda(\boldsymbol{\beta}, \boldsymbol{b})60^{\kappa}) f(\boldsymbol{b}) d\boldsymbol{b}.$$

- This can be easily calculated numerically

Dose-Response Analysis of Path

- q_{ij} is the number of quadrants of rat i at experiment j
- t_{ij} is the time for rat i at run j
- A Poisson distribution for the number of quadrants is assumed:

$$Q \sim Poisson(\mu t_{ij})$$

- The likelihood contributions are:
  1. If the rat reached the platform at time t_{ij}, the contribution is

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

  2. If it is censored at time $t_{ij} = 60$, the contribution is

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

Dose-Response Analysis of Path

- The dose-response relationship is specified via the rate μ:

$$\mu_{ij} = \exp(X_i \boldsymbol{\beta})$$

- To account for possible correlation of successive event-times, rat-specific effects on the mean parameter are included:

$$\mu_{ij} = \exp(X_i \boldsymbol{\beta} + Z_i \boldsymbol{b}_i)$$

with $\boldsymbol{b}_i \sim N(0, D)$

Dose-Response Analysis of Path

- The mean number of quadrants per second is given as

$$E[\mu|\boldsymbol{\beta}, D] = \int_{\mathbb{R}^q} \exp\left(X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i\right) f(\boldsymbol{b}) d\boldsymbol{b},$$

  with $f(\boldsymbol{b})$ a multivariate normal distribution with mean 0 and variance-covariance matrix D.
- This can be easily calculated numerically

Joint Dose-Response Analysis

- Latency and path are measured on the same rates, 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 effects, we estimate the dose-effect of latency and path jointly in on model:

$$\begin{aligned} T_{ij} &= Weibull(\lambda_{ij}, \kappa) \\ \lambda_{ij} &= \exp(X_{1ij}\boldsymbol{\beta}_1 + Z_{1i}\boldsymbol{b}_{1i}) \\ Q_{ij}|T_{ij} &= Poisson(\mu T_{ij}) \\ \mu_{ij} &= \exp(X_{2ij}\boldsymbol{\beta}_2 + Z_{2i}\boldsymbol{b}_{2i}) \\ (\boldsymbol{b}_1, \boldsymbol{b}_2)' &\sim N(0, D) \end{aligned}$$

Model Selection

- For both the latency and path: dose, day, time and gender are considered as covariates
- Pairwise interactions are included as well
- Dose included as categorical variable; day and time as continuous variable
- All interactions with 'Set' are included in the model (allowing to model all data jointly)
- This results in a model with 39 and 40 parameters for, resp., the path and latency
- Use of stepwise procedure based on AIC, stepwise deleting the most non-significant effects

Conclusions: Path

Parameter	Estimate	St.Error	p-value	Estimate	St.Error	p-value
Path		Set 1			Set 2	
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

- Significant decrease of the number of quadrants per second at the highest dose level (during period of dosing)
- No dose effect when the experiment is done two weeks after dosing
- Clear learning effect in both groups (decrease of number of quadrants per second)
- Dose has no effect on this learning effect

Conclusions: Latency

Parameter	Estimate	St.Error	p-value	Estimate	St.Error	p-value
Latency		Set 1			Set 2	
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

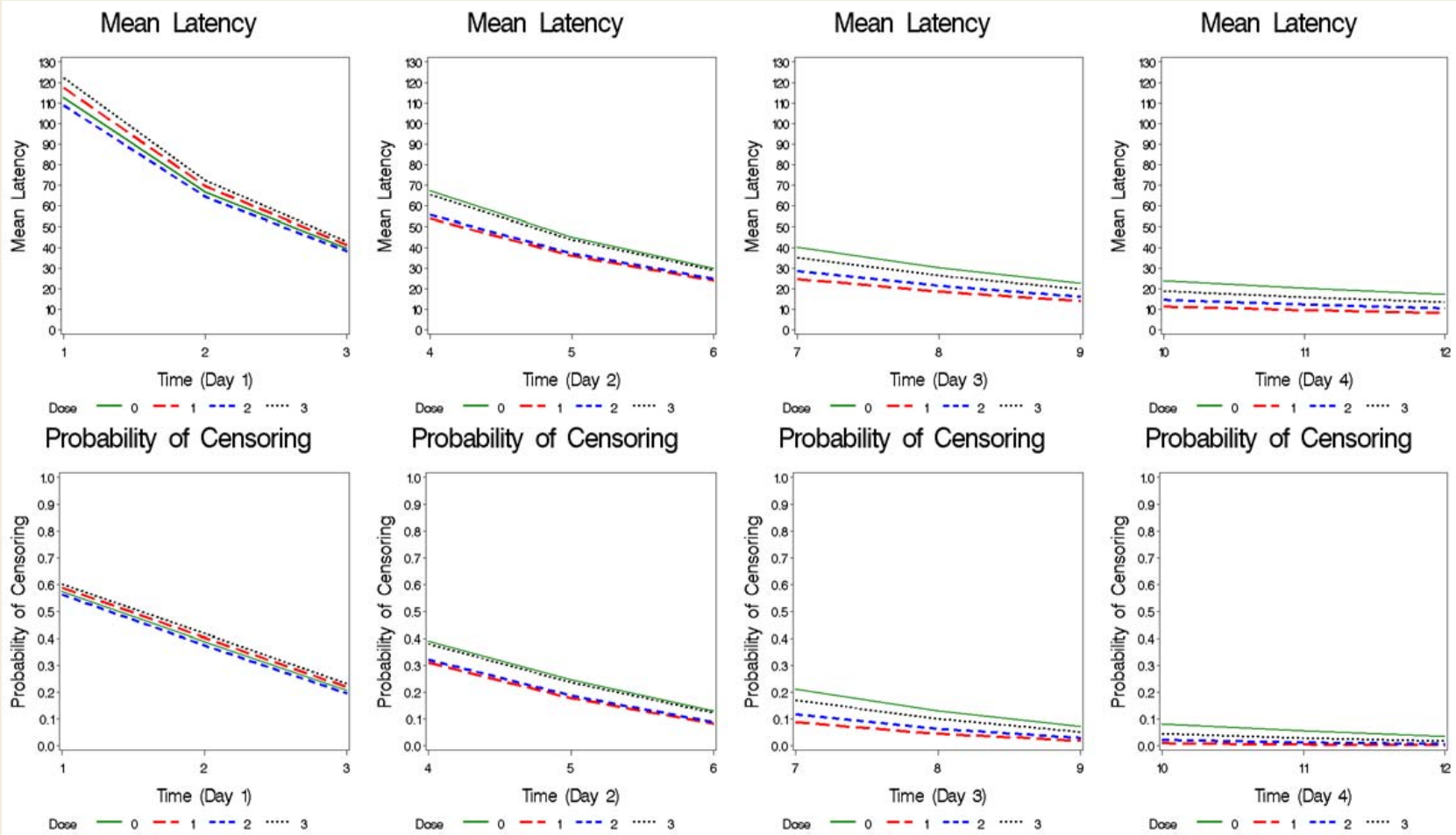
- Dosing has effect on learning, during the period of dosing
- For Set 1 animals, a dose-gender interaction is also noted

Conclusions: Correlations

Parameter	Estimate	St.Error	p-value
Random Effect Variance and Correlation			
S.E. RI Path	0.078	0.011	$<.001$
S.E. RI Latency	0.492	0.039	$<.001$
Correlation	0.908	0.112	$<.001$

- The number of quadrants per second and time taken to reach the platform are highly correlated: the smaller the rate at which rats change quadrant, the longer it takes for the rats to reach the platform.

Estimated Mean Latency and Censoring Probability



Conclusions

- The proposed method accounts for all aspects in the data: censoring, correlations, bivariate outcomes
- It is more time-consuming, but has higher power in detecting possible significant effects