

# A Web-based Simulation of a Wet-lab Experiment Modelling Parkinson's Disease in Rats

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**Abstract:** *The results of several studies pertaining to 6-OHDA lesions of the nigrostriatal dopamine system and the resulting rotational behavior are analyzed using meta-analysis. The studies are then combined to produce reasonable data for a web-based simulation which mimics the resulting rotational behavior found in the wet-lab experiment.*

**Introduction:** In this paper we describe the experiment to be modelled, summarize results found in the existing, published literature, describe the analysis used to combine the results from several experiments, show the computations involved, and explain the data used in the web-based simulation.

**Background:** In 1971 Dr. Urban Ungerstedt published his ground-breaking research detailing the connection between nigrostriatal dopamine (DA) system damage and intensity of rotational behavior in rats injected with d-amphetamine (AMPH) (Ungerstedt, 1971). Since then, this experiment has been replicated using apomorphine (APO), and has become a well-known rat model of Parkinson's disease.

In this experiment, the substantia nigra of the rat brain is injected with the neurotoxin 6-hydroxydopamine (6-OHDA). The toxin causes destruction of dopaminergic neurons within the substantia nigra, severely hindering the nigrostriatal dopamine system, and causing an acute DA loss. Depending on the site of the 6-OHDA injection, the amount of nerve damages varies.

Within the classroom setting, a two-week recovery period usually occurs after the lesion is administered to a rat. Rotational testing then occurs for one week. The lesioned rats are injected with APO or AMPH and the resulting rotational behavior is observed. Rotations contraversive to the side of the lesion are seen when APO is used, due to the stimulation of the postsynaptic DA receptors by the drug (Seeman & Niznik, 1990). AMPH causes rotation in the ipsiversive direction

by causing the release of DA stored in the presynaptic terminal and preventing DA reuptake (Yurek & Hipkens, 1993). The number of directed rotations for each testing period is recorded for each test rat. Also, prior to surgery, the rats are injected with APO or AMPH and the results are recorded for comparison.

**The analysis:** Meta-analysis is a method used to determine if several independent studies can be collectively interpreted and if the results can be combined. The first step is gathering and collecting the data from articles based on various criteria. Next, the analysis is performed and the results interpreted in order to obtain a meaningful simulation.

**Data collection:** Several articles in the existing literature were used and chosen based on consistency of data reporting. Each article needed to contain the average number of rotations for a given time period, the area of the lesion, the amount of denervation in the substantia nigra, whether AMPH or APO was administered, the standard deviation of the data, and the number of rats in the sample size. Often the standard error of the mean (SEM) was reported along with the sample size instead. Since the SEM is the standard deviation divided by the square root of the sample size, the standard deviation could easily be determined.

This basic information was not always apparent and when possible, the data was inferred from graphs presented. The software TechDig was used to help maximize accuracy when interpreting information from the graphs. Using a downloaded picture of the graph (scanned or pdf) and manually entered scales for the axes, the user selects a data point and the program returns the coordinates. The data generated by TechDig was similar to earlier visual estimations.

The number of rotations reported in each article was usually given as an average over all rats in the study over all days of testing along with a standard deviation. After determining which rate was being used for reporting, all data was converted to the number of rotations in a ten minute time period. The reported (or estimated) standard deviation was converted to be consistent with this rate.

**The computations:** Once the data was standardized, meta-analysis was used to analyze the data and test for homogeneity between studies. The first step in meta-analysis is to determine an appropriate effect size. The effect size quantifies the relationship between variables in an experiment. In this analysis the effect

size type used was the arithmetic mean, the average number of rotations of each rat in a 10 minute period with respect to amount of damage to the substantia nigra and type of drug administered. Clearly, this effect size statistic best represents the pertinent information contained in each study. Effect size analysis typically begins using a fixed effects model. If there is reason to believe that this model is not appropriate, other models are available. In this case, a random effects model proved to be better suited to the modelling of the 6-OHDA experiment. The details of each model and the reason for the choice of the random effects model are given below. Both models follow the same procedure as outlined in Chapter 7 of *Practical Meta-Analysis* by Lipsey and Wilson.

**Nerve damage to the substantia nigra greater than 90%** The following statistics are calculated to determine (1) if the mean effect size is statistically significant and (2) if the population formed from each study is homogeneous. The data and calculations for 90% or higher damage to the substantia nigra with APO administered are shown in detail. Explanations of the meta-analytical terms are found below and the following table gives the authors of the selected studies with the relevant quantities used in performing the meta-analysis for the fixed effects model. For further information on an individual study see the references.

- $N$  is the sample size of the study.
- $SD$  is the standard deviation of the data in the study
- $ES$  is the effect size (number of rotations reported in a 10 minute period divided by the sample size). Hence, this is the average number of rotations per rat in the study.
- $SE$  is the standard error of the mean,  $\frac{SD}{\sqrt{N}}$ .
- $v$  is a weighted variance  $\frac{SD^2}{N}$
- $\omega$  is the inverse variance weight,  $1/v$
- $\omega^2$ ,  $\omega * ES$ , and  $\omega * ES^2$  are self evident

Fixed effects model statistics:

Study	<i>N</i>	<i>SD</i>	<i>ES</i>	<i>SE</i>	$\nu$	$\omega$	$\omega^2$	$\omega * ES$	$\omega * ES^2$
Reglodi	12	38.57	62.10	11.13	123.95	0.01	.0001	.5	31.11
Kirik	30	9.07	79.33	1.66	2.74	.36	.1328	28.91	2293.26
Kashihara	6	7.16	66.4	2.92	8.55	0.12	0.0137	7.77	515.67
Metz	14	10.33	62.00	2.76	7.62	0.13	0.0172	8.14	504.53
Kondo	6	14.65	101.00	5.98	35.78	0.03	0.0008	2.82	285.12
Carmen	30	40.48	95.06	7.39	54.61	0.02	0.0003	1.74	165.46
Garrett	8	3.78	126.5	1.34	1.79	0.56	0.3130	70.77	8952.31
Column totals			592.39	33.18	235.04	1.23	0.4778	120.64	12747.45

In order to test for a significant mean effect size as well as homogeneity of the population, we compute the following.

<i>WM</i>	98.38
<i>SE<sub>WM</sub></i>	0.90
<i>WM<sub>l</sub></i>	96.61
<i>WM<sub>u</sub></i>	100.15
<i>z test</i>	108.94
<i>Q</i>	878.90

*WM* is the weighted mean effect size,

$$WM = \frac{\sum (w_i ES_i)}{\sum w_i}$$

This is a weighted average of the various effect sizes, taking into account sample size and standard deviation of individual studies. Note that multiplication of an individual effect size by  $w_i$  (which is the number of rats in the  $i^{th}$  study divided by its variance) gives more weight to those studies with larger sample size and less weight to those with large standard deviations.

*SE<sub>WM</sub>* is the standard error of the weighted mean effect size, computed by finding  $\sqrt{1 / \sum w_i}$ .

*WM<sub>l</sub>* and *WM<sub>u</sub>* give the confidence interval of the weighted mean effect size,

$$WM_l = WM - z_{(1-\alpha)}(SE_{WM})$$

$$WM_u = WM + z_{(1-\alpha)}(SE_{WM})$$

where  $z_{(1-\alpha)}$  is the critical value for the  $z$  distribution with confidence level  $\alpha$ . We used  $z = 1.96$  with  $\alpha = .05$ . Because the confidence interval,  $(WM_l, WM_u)$  does not contain 0, the weighted mean effect size is statistically significant at  $p \leq \alpha$ . We also performed a direct test of the significance of the weighted mean effect size, computing a  $z$  test (here  $z = \frac{|WM|}{SE_{WM}}$ ). Since the  $z$  value here exceeds 1.96, statistical significance is obtained for  $p \leq .05$ .

The  $Q$ -score is computed to test the null hypothesis that the population is homogeneous.  $Q$  is distributed as a  $\chi^2$  value with 6 (number of studies - 1) degrees of freedom. The critical value for a confidence level of  $\alpha = 0.05$  is 12.6. Our  $Q$  value is well above this critical value and we reject the null hypothesis of homogeneity.

Because of this, we determined that the fixed effects model may not be appropriate and this led us to examine the random effects model. A random effects model takes into consideration variability between studies due to differences in individuals that cannot be measured separately from the experimental results. Thus, this model takes into account the variability inherent to individuals in the population. In the current setting, on site 6-OHDA experiments suggest a wide range in the number of rotations - all being perfectly normal. Therefore we concluded that a random effects model would account for this variability and give more accurate results.

In the random effects model, the inverse variance weight takes into account not only variance in the study, but also that of individuals in the population. The variance weight is the sum of  $\nu_\theta$  and  $\nu_i$  where  $\nu_\theta$  is an estimate of the random effects variance - computed by

$$\nu_\theta = \frac{Q - (k - 1)}{\sum w_i - (\sum w_i^2 / \sum w_i)}$$

where  $Q$  is the value of the homogeneity test already derived,  $k$  is the number of effect sizes (number of studies) and the  $w_i$  are the inverse variances computed as in the fixed effects model. The relevant statistics for this model are shown below. Recall that the amount of damage is greater than 90% and the drug administered is APO.

$WM$	84.86
$SE_{WM}$	12.39
$WM_l$	60.56
$WM_u$	109.15
$z$ test	6.85
$Q$	3.29

Clearly, the effect size is significant as the interval  $(WM_l, WM_u)$  does not contain 0 and the value from the  $z$ -test is again above 1.96. However, the value of  $Q$  is below the critical value of 12.96 and we fail to reject the null hypothesis of homogeneity. Meta-analysis using the random effects model in the case of administering AMPH with more than 90% damage to the substantia nigra was also performed and the relevant statistics are:

$WM$	117.1
$SE_{WM}$	15.45
$WM_l$	86.77
$WM_u$	147.34
$z$ test	7.58
$Q$	7.83

Again we see that our effect size is statistically significant and we fail to reject the null hypothesis of homogeneity.

**Nerve damage to the substantia nigra less than 90%:** Similar analyses were conducted for studies where the amount of damage was less than 90% with both APO and AMPH being administered. In these cases, the data was not as reliable and there were wide fluctuations in the number of rotations regardless of drug administered. These studies did not appear in the literature with the same frequency as those where the damage was greater than 90%.

We prepared data for pre-surgery rotations based on experiments done in Dr. Ramirez's neuroscience course. This data is not generally reported in the literature.

Given the meta-analysis with damage greater than 90%, we concluded that the population from which the studies were drawn is homogeneous. We summarize our findings below including the numbers we used for the mean and standard deviations in the computer simulation.

**Summary:** What follows is the summary of all means and standard deviations used in the simulation.

Pre-surgery with no damage.

	Direction:	Contraversive	Ipsiversive
Apomorphine	Mean:	2.1	2.9
	Standard Deviation:	.46	.63
Amphetamine	Mean:	3.3	2.56
	Standard Deviation:	.95	.5

Post surgery, with drugs:

	Amount of Damage:	0-50%	50-75%	75-90%	90-100%
Apomorphine (contraversive rotation)	Mean:	3.5	9.7	13.3	84.9
	Standard Deviation:	2.53	6.94	10.56	12.39
Amphetamine (ipsiversive rotation)	Mean:	23.1	38.6	109.6	117.1
	Standard Deviation:	13.09	20.26	38.00	15.45

Post surgery rotation in the direction opposite of that expected with respect to the drug administered.

	Ipsiversive rotation (Apomorphine)	Contraversive rotation (Amphetamine)
Mean:	1.27	.11
Standard Deviation:	.33	.13

The means shown represent the average number of rotations of each rat over the 7 day experiment. This is the information most commonly found in the literature. The fact that the simulation requires only a mean and standard deviation is discussed in the next section. The means for APO or AMPH administered with damage greater than 90% are the weighted mean effect size from the random effects model meta-analysis. The outcome from the analysis and the availability of plenty of meaningful data gave solid statistics in these two categories. The other categories have not appeared in the literature with the same intensity and availability. Here we calculated the means for use in the simulation by weighting the average of the reported means in the literature (i.e., the mean =  $\frac{\sum(n_i * ES_i)}{\sum n_i}$  where  $n_i$  is the number of rats in a given study). The numbers used for the simulation in these cases may not be as statistically significant as those for the greater than 90% damage, but given the consistency for the results in the greater than

90% category, and the wide spread fluctuations in the lower damage categories, calculating the means in this way seems reasonable.

## The simulation:

**Overview:** In order to generate a web based simulation of the rotational behavior, the main piece of information needed is the number of rotations of a rat in a 10 minute period averaged over a 7 day period. This data is the information most available in the literature. The output depends on the amount of damage and the type of drug administered, both of which the user enters in the simulation. The user can also enter the number of rats to be used in the web-based "experiment".

Once it was determined using meta-analysis that the effect size was from a homogeneous population, the task was to reproduce data - in the form of number of rotations in a 10 minute period which would reflect the results found in real life experiments. In nearly all instances, the raw data - number of rotations of each rat on each day was not available. Therefore, it was impossible to determine if there is an underlying distribution to the number of rotations each day in individual rats. However, the central limit theorem tells us that the means of a population are approximately normally distributed with mean that of the population and standard deviation equal to the standard deviation of the population divided by the square root of the sample size. The latter is called the standard error of the mean. Thus to produce reasonable simulated data, we need to report only on average number of rotations of an individual rat over a 7 day period rather than the number of rotations of an individual rat on a particular day. We use  $n = 7$  as the sample size and report this as an average number of rotations of an individual rat over a 7 day period. The calculations are then repeated for the number of rats in the simulated study. These averages are from an approximately normally distributed random variable with means and standard deviations dependent on drug administered and amount of damage as given in the previous section.

**The calculations:** To produce data from a normal distribution, we first produce numbers from a standard normal distribution (mean 0 and standard deviation 1). We used the polar Box-Muller method, found for example in Brandt or Knuth to generate normally distributed random numbers. The method produces two numbers from a standard normal distribution per iteration. We compute as

many numbers as needed in each simulation. Two numbers are chosen using a random number generator - here we use the C# random generator and call them  $x$  and  $y$ .

Next we compute  $s = x^2 + y^2$ . If this quantity is greater than or equal to 1, those numbers  $(x, y)$  are rejected and two new values are computed. Otherwise, continue on. Thus, the random numbers when taken as an ordered pair  $(x, y)$  lie within the unit circle. We now compute the two numbers,  $v$  and  $w$  which come from a standard normal distribution by

$$\begin{aligned} v &= x \sqrt{\frac{-2 \ln s}{s}} \\ w &= y \sqrt{\frac{-2 \ln s}{s}} \end{aligned}$$

This is the polar Box-Muller transformation. Finally,  $v$  and  $w$  are converted to numbers  $v'$  and  $w'$  from a normal distribution with mean  $\mu$  and standard deviation  $\sigma$  by

$$\begin{aligned} v' &= \frac{v - \mu}{\sigma} \\ w' &= \frac{w - \mu}{\sigma} \end{aligned}$$

This process is repeated until there are enough numbers to produce data for the number of rats in the simulation. The values of  $\mu$  and  $\sigma$  are constant within each category. If a user chooses 55% damage and administers APO,  $\mu = 9.7$  and  $\sigma = 6.94$ . These same numbers are used if the users chooses 70% damage, again administering APO.

**Summary:** Users perform the web-based experiment based on the Ungerstedt model using the data generated. After we performed the meta-analysis of results found in the literature, we determined reasonable parameters from those results. Those parameters were then used to generate the data. The user can then determine if there is an effect on rotational behavior due to the surgery, give an average and standard deviation for their own simulation, and clearly see the differences between effects of apomorphine and amphetamine. Because of the nature of the

algorithm used to generate the data, the web based simulation gives different numbers each time the simulation is run, just as in the real life experiment.

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