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AN ALGORITHM FOR DETECTING PHENOTYPIC MUTANTS FOR THE JAX NEUROSCIENCE MUTAGENESIS FACILITY

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Abstract. The Mutagroup at Jackson Labs is interested in generating new mouse models for studying neurological disease by producing mutations in mice by injecting them with ENU. The group proposes to produce large numbers of potential mutants and screen them for phenotypic anomalies. In this report we propose a statistical algorithm to flag phenotypic deviants. We have applied the algorithm to a pilot data set collected by Dr. Kevin Seburn on mice placed in cages equipped with monitoring devices. Aiming for a 5% false positive rate, the algorithm was able to detect 18 of the 27 mutant mice it was presented.

1. Introduction. The goal of the ENU Mutagenesis group at Jackson Labs is to induce mutations in mice by injecting with them with ENU. Because of the nature of ENU it is believed that it will give rise to a large number of mice that show altered behavior.

Since the project aims to produce large numbers of possibly mutant mice, it is necessary to develop methodology that will be able to screen large numbers of possibly mutant mice. Also, since many mutants may not show obvious visible signs, it is necessary to have a method that is sensitive to non-visual clues.

In this report we will describe a mutant detection algorithm based on multivariate statistical analysis that will be able to screen large number of potential mutants based on data on physiological and/or behavioral data collected on the mice. The proposed algorithm is first *trained* on a set of normal mice of the same strain as that of the background of the mutagenized mice. Then, based on the data collected in the cages, the algorithm computes a distance between the test mice and the normal mice. If this distance is “too large” the mouse in question is flagged as a likely mutant. The cutoff distance for flagging can be adjusted for a desired rate of false positives.

We trained the algorithm on a set of normal mice. Then we applied it to a set of normal mice (not in the training set) and a another set consisting of mostly known mutant mice, some normal mice from a different strain and some normal mice. Using a cutoff corresponding to a 5% false positive rate, the algorithm flagged 1 out of 19 in the control test set as mutant and 18 out of 27 in the mutant test set.

Section 2 describes the algorithm. The results after applying the algorithm on the pilot data set are presented in Section 3. Technical statistical details are contained in Section 4. Section 5 concludes.

2. The mutant detection algorithm. First, the investigator selects what characteristics of the data to focus on and what data summaries to use. The only statistical requirement is that the data after summarization are approximately Gaussian. Suppose we decide to use p summaries for each mouse, then each mouse would be summarized by an array of numbers of length p .

The second step is to compute summaries for the normal mice from the background strain. We will use the sample mean vector and the sample covariance matrix as summaries. In symbols, suppose $\underline{x}_1, \underline{x}_2, \dots, \underline{x}_n$ are the n data vector summaries

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from the control mice. The sample mean is

$$\bar{\underline{x}} = \frac{1}{n} \sum_{i=1}^n \underline{x}_i$$

and the sample covariance is

$$S = \frac{1}{n-1} \sum_{i=1}^n (\underline{x}_i - \bar{\underline{x}})(\underline{x}_i - \bar{\underline{x}})'$$

The sample mean vector and covariance matrix may also be used for strain characterization.

Suppose \underline{y} is the summary from a potentially mutant mouse. Calculate for the test mouse the *Mahalanobis distance*

$$d(\underline{y}) = (\underline{y} - \bar{\underline{x}})' S^{-1} (\underline{y} - \bar{\underline{x}})$$

The greater the distance, stronger the evidence that the data came from a mutant mouse.

To evaluate the strength of the evidence calculate the *outlier score*

$$s(\underline{y}) = P(\chi_p^2 > d(\underline{y})).$$

Suppose we can tolerate a false positive rate of α , then flag those mice with outlier score smaller than α . The false positive rate is adjustable and should be adjusted according to the needs of the investigator.

3. Results. Dr. Kevin Seburn's lab has developed protocols and expertise to monitor possibly mutant mice for behavioral and/or physiological anomalies. In the most current protocol, mice placed in cages equipped with monitoring devices are observed for about three days. Various physiological and behavioral measurements are made (see Appendix for a list).

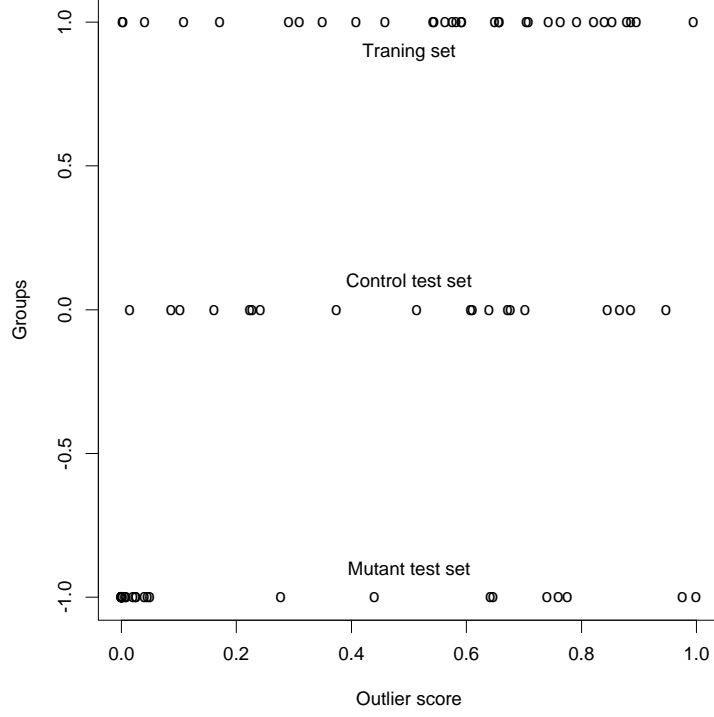
The mice studied were separated into three sets. The first set, the *training set*, consisted of 32 normal mice from the C57BL6/J strain. We suspect that there may be a small batch to batch variation in the data collected. To make it representative, we put at least one mouse from each experimental batch into the training set. The second set was a test set with 19 control mice. The third set was the test set containing 27 mice. Most mice in this set were known mutants, some were mice from a different strain and there are a few suspected normals. The algorithm was trained with the first set and then applied to the other two sets. We wanted to see if the algorithm had the desired false positive rate on the second set and a bigger positive rate on the third.

After substantial exploratory analysis and deliberation, we decided to concentrate on 3 variables, the RER (Respiratory Exchange Rate), number of vertical beam breaks (measuring rearing activity) and number of sequential horizontal beam breaks (measuring ambulatory activity). Mice, being nocturnal, are more active at night, so we made means of each of the variables for the lighted and darkened periods in the lab. To satisfy the Gaussian distribution assumption, we took logarithms of all variables before we made means¹.

Figure 3 plots, for the three test sets, the computer outlier scores. We can see that for the control groups, the first and second sets, the outlier scores are approximately

¹Since activity measurements can be 0 at times, for the ambulatory and rearing activity measurements, we added 1 to the measurements before taking logs to avoid taking logs of zero.

FIG. 3.1. *Plot of the outlier scores for the three groups of mice.*

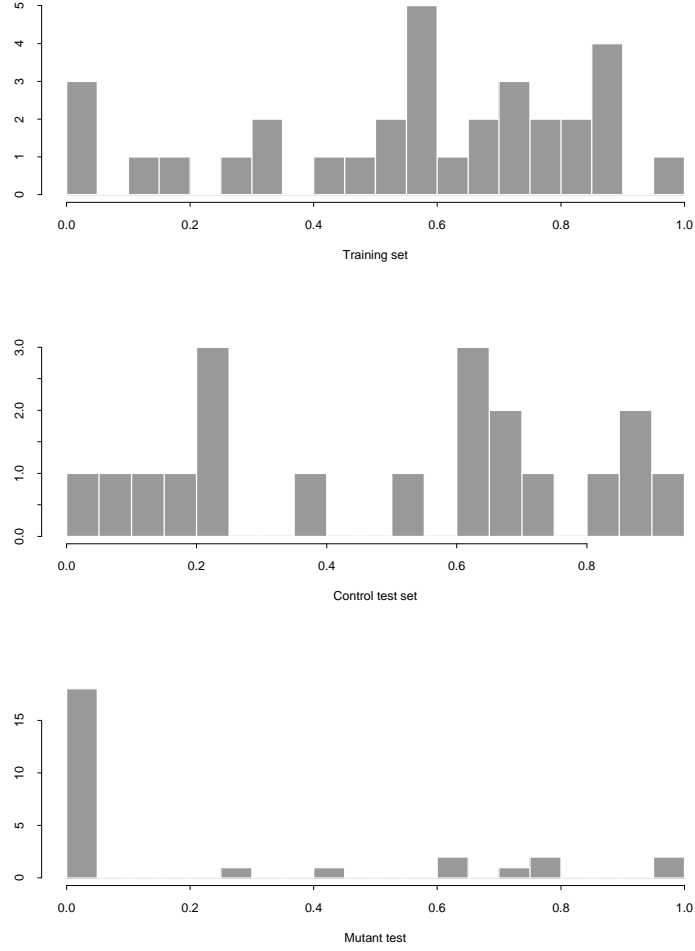


evenly distributed between 0 and 1 (as is expected from statistical theory). For the third set, the test set with mostly mutant mice, the outlier scores are mostly concentrated near 0, indicating that most of them are flagged as outliers. Figure 3, which plots the histograms of the outlier scores makes the distributions a little more clear. Using a cutoff corresponding to a 5% false positive rate, we flagged 1 out of 19 in the test set with normal mice and 18 out of 27 of the test set with mutants. Table 3 shows details about the mutants.

The false positive rate of 1 in 9 in the control test set is consistent with the desired false positive rate of 5%. In addition, the algorithm was able to flag most of the mutants in the mutant test set. There are a few puzzles to be solved. Why were the DRD3 mice so different in their behavior? But the overall picture is that the algorithm seems to do a good job in picking out mutant mice. This gives us hope that it will perform well in the future when it has more data to train on and more mice to screen.

4. Technical details. We assume that the data from the normal mice from the background strain, $\underline{X}_1, \underline{X}_2, \dots, \underline{X}_n$, are n iid sample from a p -variate normal distribution with mean vector μ and variance matrix Σ . This is our training set. If

FIG. 3.2. *Histograms of the outlier scores for the three groups of mice.*



\underline{Y} is another observation from the same distribution, then

$$\underline{Y} - \bar{\underline{X}} \sim N_p(\underline{0}, (1 + \frac{1}{n})\Sigma).$$

Therefore,

$$(4.1) \quad (\underline{Y} - \bar{\underline{X}})'((1 + \frac{1}{n})S)^{-1}(\underline{Y} - \bar{\underline{X}}) \sim T_{n-1,p}^2,$$

where $T_{n,p}^2$ denotes a *Hotelling's* T^2 distribution with n and p degrees of freedom.

When the size of the training set, n is large, the LHS in (4.1) is approximately equal to the Mahalanobis distance of the point \underline{Y} from $\bar{\underline{X}}$ with S as the metric. Also, for large n , Hotelling's T^2 with n and p degrees of freedom is approximately χ^2 with p degrees of freedom. Thus, when the training set is large enough, and the observation \underline{Y} comes from the same distribution as the training set. the Mahalanobis distance of the point from the sample mean of the training set, $\bar{\underline{X}}$, with metric defined in terms of

TABLE 3.1
Table of mice in the mutant test set and their outlier scores.

Date-Cage	Outlier score	Notes
071799-1	0.0000*	Tubby
071799-2	0.0000*	Tubby
072399-1	0.0005*	129SV
072399-2	0.0000*	129SV
073099-2	0.0015*	A1J
080399-1	0.0002*	MDX
080399-2	0.7604	MDX
080699-1	0.0000*	A/J
080699-2	0.0002*	A/J
091599-5	0.9994	DRD3
091599-6	0.0000*	DRD3
091599-8	0.9755	DRD3
101899-1	0.6460	B6ST1
101899-3	0.6417	B6ST1
101899-4	0.7756	B6ST1
101899-5	0.2772	B6ST1
110899-1	0.0054*	Het/Het
110899-2	0.0404*	Het/+
110899-3	0.0077*	Het/Het
110899-4	0.0449*	Het/+
110899-5	0.0252*	Het/Het
110899-6	0.0252*	Het/Het
110899-7	0.0490*	Het/Het
110899-8	0.7402	Het/+
111999-6	0.0002*	FMR1
111999-7	0.4405	FMR1
111999-8	0.0202*	FMR1

the sample covariance matrix, S , is approximately distributed as a χ_p^2 . This justifies the χ^2 distribution used in Section 2.

5. Discussion. The encouraging results from our pilot study indicate that the proposed algorithm is very promising. As more data is collected, we will have a better idea of its performance.

5.1. Strain characterization. An interesting bi-product of the algorithm is that it provides us with a way of characterizing strains too. The strain characteristics are summarized by the sample mean and covariance of the training set data after appropriate transformation².

The algorithms not only flags mutant mice but also normal mice from other strains. Thus, to detect mutant mice from strains other than the C54BL6/J strain used in this report, we will have to first build a database of those strains.

5.2. Batch and other environmental effects. Since the statistical algorithm only analyzes numbers devoid of the context, for it to be successful, the data collection

²The transformations are important, else the mean and covariance will not be valid as strain characterization summaries

procedure has to be closely monitored so that there are no "process drifts". The algorithm could flag a change in environmental conditions instead of mice behavior, if there are changes in the lab conditions.

We also recommend that each batch of experiments contain some normal mice to help us adjust and monitor environmental conditions of the cages. The algorithm may also be further tuned with the help of the control mice. This is an avenue for further study.

5.3. Phenotypic domains. In our trial set we used 6 number summaries for each mice. There is potential for using many more summaries, each focusing on different aspects of mouse behavior. However, there are limitations on the number of summaries we can use in any given run of the algorithm because, for p summaries, we have to estimate $p(p+1)/2$ parameters given only np numbers, assuming a sample size of n .

Dr. Patsy Nishina suggested that we divide the number summaries according phenotypic domains. Next, we can train and run the algorithm on the variables in each of the domains. This is a good suggestion and will be of great help in interpreting the mutants detected.

If we divide the data into k phenotypic domains and calculate the outlier score for a particular mouse for each of the domains, we will get k scores s_1, s_2, \dots, s_k . The *overall outlier score* for the mouse will *not* their maximum, but their sum,

$$s = \sum_{j=1}^k s_j.$$

This is a consequence of *Bonferroni's Inequality*. It follows that if a false positive rate used for each of the domains is α , then the overall false positive rate will be $k\alpha$.

5.4. Other considerations. Under the proposed breeding mechanism for the Mutagroup the probability that a mouse in the G3 generation will be recessive for a mutation is 1/8. So far, we have not used this information in our analyses. It is possible that this may lead to refinements of the algorithm or ways to control errors.

Appendix A. Variables recorded in the cages.

- Sample: Sample number
- Time Code: Time in proportion of the day
- Time: Time in hh:mm:ss format
- VO2: Volume of oxygen consumed per unit body weight per unit time
- O2 in: Oxygen content of air going in
- O2 out: Oxygen content of air going out
- DO2: Difference of the above two
- Acc O2: Cumulative of the above column
- VCO2: Volume of carbon dioxide consumed per unit body weight per unit time
- CO2 in: Carbon dioxide content of air going in
- CO2 out: Carbon dioxide content of air going out
- DCO2: Difference of the above two
- Acc CO2: Cumulative of the above column
- RER: Respiratory Exchange Ratio VCO2: VO2
- Heat: Energy consumed (formula not known to me)
- Flow: How many litres of air flowing into cage; should be constant
- Hor: Counts of movement without displacement
- Vert: Counts of vertical movement indicative of rearing
- Amb: Counts of breaking beams in sequence; indicative of ambulation

- DrinkA: Water drinking from source A
- DrinkB: Water drinking from source B

Appendix B. Computer programs. Splus was used for the statistical computing. Bash shell under the Cygnus system was used for scripting and text file processing. This document was edited under Emacs and then processed under the MiKTeX distribution of L^AT_EX.

Appendix C. Raw results. What follows are the raw results from the computer programs used to perform the statistical analyses. These are provided for reference.

The following shows the outlier scores, the date and cage of the mice for each of the three data sets, the training set, the control test set and the mutant test set.

```
> source("/Mutant/trainandvalidate.s")
[1] "Training set outlier scores: "
073099-1 092199-5 092199-6 092199-7 092199-8 091599-1 091599-2
  0.0405  0.7044  0.0031  0.0018  0.7079  0.5627  0.5434
091599-3 091599-4 092799-5 092799-6 092799-7 092799-8 100599-1
  0.3495  0.7425  0.8398  0.5754  0.5419  0.8532  0.4079
100599-2 100599-3 100599-4 101299-1 101299-2 101299-3 101299-4
  0.9944  0.5918  0.7636  0.8212  0.6569  0.4584  0.8857
100199-1 100199-2 100199-3 100199-4 100199-5 100199-6 100199-7
  0.582  0.8788  0.656  0.1708  0.7916  0.1086  0.2913
100199-8 101899-6 101899-7 101899-8
  0.6496  0.8953  0.5909  0.3096
[1] "Test set outlier scores: "
092199-1 092199-2 092199-3 092199-4 092799-1 092799-2 092799-3
  0.6394  0.5135  0.6075  0.2273  0.3739  0.8451  0.161
092799-4 100599-5 100599-6 100599-7 100599-8 101299-5 101299-6
  0.702  0.6761  0.0143  0.6106  0.947  0.1019  0.8661
101299-7 101299-8 111999-1 111999-3 111999-4
  0.0862  0.2229  0.6717  0.8861  0.2416
[1] "Test set outlier scores: "
071799-1 071799-2 072399-1 072399-2 073099-2 080399-1 080399-2
  0 0 0.0005 0 0.0015 0.0002 0.7604
080699-1 080699-2 091599-5 091599-6 091599-8 101899-1 101899-3
  0 0.0002 0.9994 0 0.9755 0.646 0.6417
101899-4 101899-5 110899-1 110899-2 110899-3 110899-4 110899-5
  0.7756 0.2772 0.0054 0.0404 0.0077 0.0449 0.0252
110899-6 110899-7 110899-8 111999-6 111999-7 111999-8
  0.0252 0.049 0.7402 0.0002 0.4405 0.0202
```

Next we provide the data summaries used for the analyses. These are the means on the log scale in the following order: RER (night and day), Rearing activity (night and day), and Ambulatory activity (night and day). The final column is the outlier score.

```
> result
$train:
      x.1      x.2      x.3      x.4      x.5      x.6      X2
073099-1 -0.3102 -0.1669 2.8754 4.7008 3.5156 5.1237 0.0405
092199-5 -0.2999 -0.2394 2.2824 4.5301 4.1947 6.4103 0.7044
092199-6 -0.3594 -0.3976 4.8392 5.4759 4.0156 6.1429 0.0031
092199-7 -0.3289 -0.2362 0.8375 2.6053 1.4688 3.3651 0.0018
```


092199-8	-0.3333	-0.3386	2.5516	4.5349	3.8625	6.1256	0.7079
091599-1	-0.3620	-0.2983	2.5054	4.7360	4.0182	6.0941	0.5627
091599-2	-0.3027	-0.2959	2.0826	4.0471	4.5706	5.8266	0.5434
091599-3	-0.3684	-0.3123	2.3548	4.0271	4.5400	6.2574	0.3495
091599-4	-0.3289	-0.2170	2.4773	3.8770	3.7334	5.3972	0.7425
092799-5	-0.3421	-0.2929	2.1603	4.6441	4.4214	6.6482	0.8398
092799-6	-0.3848	-0.2132	2.1381	4.4945	3.8973	6.2642	0.5754
092799-7	-0.3704	-0.2188	2.1598	4.7399	3.8941	6.2875	0.5419
092799-8	-0.3035	-0.2190	2.6521	4.9808	4.6746	6.6754	0.8532
100599-1	-0.3612	-0.2534	1.6622	4.2704	3.8806	6.5589	0.4079
100599-2	-0.3291	-0.2511	2.5166	4.5816	4.5200	6.2975	0.9944
100599-3	-0.3116	-0.1512	2.1200	4.6865	4.5254	6.6227	0.5918
100599-4	-0.3759	-0.2759	2.8184	4.4387	4.3144	6.3205	0.7636
101299-1	-0.3463	-0.3462	2.9883	4.7860	4.6797	6.5461	0.8212
101299-2	-0.3441	-0.2354	3.0233	4.3327	4.5674	6.1941	0.6569
101299-3	-0.3671	-0.2608	2.8671	4.3235	4.6005	6.1020	0.4584
101299-4	-0.3420	-0.2257	2.9294	4.9707	4.0937	6.3498	0.8857
100199-1	-0.2883	-0.2575	3.3750	5.0747	5.2771	6.7106	0.5820
100199-2	-0.2964	-0.2863	2.5146	4.7873	4.7078	6.6166	0.8788
100199-3	-0.2798	-0.3053	2.1357	4.3835	4.5398	6.1814	0.6560
100199-4	-0.2734	-0.3630	2.1897	4.5188	4.8496	6.3191	0.1708
100199-5	-0.3423	-0.1921	2.6496	4.4548	4.3261	6.0031	0.7916
100199-6	-0.3040	-0.3674	3.1753	3.7631	4.0248	5.0803	0.1086
100199-7	-0.3320	-0.1649	2.0630	4.1403	3.8659	6.2459	0.2913
100199-8	-0.3179	-0.2346	3.4538	5.1373	5.1007	6.6352	0.6496
101899-6	-0.2890	-0.2617	2.4746	4.3076	4.5248	5.9683	0.8953
101899-7	-0.3389	-0.2684	2.4494	4.7997	3.5916	6.1662	0.5909
101899-8	-0.3140	-0.2142	2.2434	4.1248	4.2142	6.3576	0.3096

\$control:

	x.1	x.2	x.3	x.4	x.5	x.6	X2
092199-1	-0.3497	-0.2851	2.1793	4.8299	4.2490	6.5855	0.6394
092199-2	-0.3133	-0.2442	2.0605	3.8035	3.8748	5.8362	0.5135
092199-3	-0.3479	-0.2187	2.7221	5.1525	4.7732	6.8132	0.6075
092199-4	-0.3322	-0.2373	2.9256	4.6871	4.2131	5.5891	0.2273
092799-1	-0.2888	-0.2267	2.1284	3.7873	4.3867	5.8688	0.3739
092799-2	-0.3398	-0.2376	1.7244	4.3663	4.0636	6.2584	0.8451
092799-3	-0.3542	-0.1538	2.5770	4.2621	4.7032	6.2282	0.1610
092799-4	-0.3261	-0.2783	2.4248	4.1198	4.5722	5.8684	0.7020
100599-5	-0.3007	-0.1968	2.4174	4.6186	4.2734	6.4228	0.6761
100599-6	-0.3747	-0.3517	1.9154	4.5397	3.8653	5.9663	0.0143
100599-7	-0.3435	-0.3226	2.2681	4.5809	4.4700	6.3462	0.6106
100599-8	-0.3244	-0.2895	2.8909	5.0398	4.7488	6.7064	0.9470
101299-5	-0.2892	-0.1901	2.6881	4.0314	4.1538	5.0844	0.1019
101299-6	-0.3474	-0.2209	1.7833	4.2102	4.1127	6.1594	0.8661
101299-7	-0.3774	-0.3104	3.1379	4.4089	4.7568	6.0203	0.0862
101299-8	-0.3561	-0.3037	2.5754	4.7633	4.1563	5.9469	0.2229
111999-1	-0.3549	-0.3057	2.4444	4.6286	4.3160	6.2131	0.6717
111999-3	-0.3142	-0.2562	2.0484	3.8748	4.2206	5.8059	0.8861

```
111999-4 -0.3016 -0.2378 1.6260 3.2223 3.6213 5.1326 0.2416
```

```
$test:
```

	x.1	x.2	x.3	x.4	x.5	x.6	X2
071799-1	-0.4114	-0.3729	2.2306	4.2528	2.6676	4.7422	0.0000
071799-2	-0.4024	-0.4275	1.6014	3.8635	2.4881	4.7956	0.0000
072399-1	-0.3799	-0.3635	1.5343	2.4782	2.3587	3.8762	0.0005
072399-2	-0.2641	-0.1696	0.5349	1.7902	2.3170	4.1487	0.0000
073099-2	-0.3474	-0.1861	2.6268	4.2674	2.7721	4.3064	0.0015
080399-1	-0.2827	-0.2656	3.2885	5.0967	3.8705	5.0052	0.0002
080399-2	-0.3000	-0.2035	2.3508	4.1013	4.2118	5.6414	0.7604
080699-1	-0.3771	-0.2979	3.2948	4.1273	3.9021	4.4079	0.0000
080699-2	-0.3815	-0.1894	2.7462	4.0971	2.3474	4.0179	0.0002
091599-5	-0.3198	-0.2730	2.5858	4.6359	4.3714	6.2651	0.9994
091599-6	-0.3452	-0.2700	5.8033	6.3303	4.2143	6.7021	0.0000
091599-8	-0.3264	-0.2991	3.1116	4.9118	4.5852	6.3832	0.9755
101899-1	-0.3561	-0.2553	3.0824	4.7212	4.6427	6.2082	0.6460
101899-3	-0.2736	-0.2686	2.6451	4.5855	4.3426	6.0821	0.6417
101899-4	-0.3438	-0.2619	2.5499	4.9870	4.2694	6.8013	0.7756
101899-5	-0.3468	-0.2316	2.7874	4.1611	4.9210	6.3599	0.2772
110899-1	-0.2777	-0.2168	3.7349	4.4963	5.2558	5.7228	0.0054
110899-2	-0.2458	-0.2273	3.4601	5.2838	5.6587	6.7912	0.0404
110899-3	-0.2528	-0.2805	4.2294	6.0130	6.2029	7.5240	0.0077
110899-4	-0.2656	-0.2431	3.3860	4.4390	5.4230	6.1940	0.0449
110899-5	-0.2337	-0.3316	3.2181	5.1804	4.8943	6.3895	0.0252
110899-6	-0.2747	-0.3191	3.5189	5.9473	5.3677	7.2030	0.0252
110899-7	-0.3147	-0.3208	2.0083	4.8011	3.3851	5.9497	0.0490
110899-8	-0.3472	-0.3022	2.1504	4.3431	4.3749	6.1810	0.7402
111999-6	-0.4045	-0.2630	2.2985	4.6954	4.8889	6.4499	0.0002
111999-7	-0.2863	-0.1990	2.9119	4.5768	4.9739	6.2853	0.4405
111999-8	-0.3746	-0.2837	1.6537	3.1923	3.7731	5.0917	0.0202

Next we provide the data summaries used for the analyses transformed back to their original scales. They are in the following order: RER (night and day), Rearing activity (night and day), and Ambulatory activity (night and day). The final column is not meaningful and should be ignored.

```
> round(exp(result$train),3)
```

	x.1	x.2	x.3	x.4	x.5	x.6	X2
073099-1	0.733	0.846	17.733	110.035	33.636	167.956	1.041
092199-5	0.741	0.787	9.800	92.768	66.334	608.076	2.023
092199-6	0.698	0.672	126.368	238.865	55.457	465.401	1.003
092199-7	0.720	0.790	2.311	13.535	4.344	28.936	1.002
092199-8	0.717	0.713	12.828	93.214	47.584	457.419	2.030
091599-1	0.696	0.742	12.248	113.977	55.601	443.235	1.755
091599-2	0.739	0.744	8.025	57.231	96.602	339.203	1.722
091599-3	0.692	0.732	10.536	56.098	93.691	521.860	1.418
091599-4	0.720	0.805	11.909	48.279	41.821	220.787	2.101
092799-5	0.710	0.746	8.674	103.970	83.213	771.395	2.316
092799-6	0.681	0.808	8.483	89.523	49.269	525.421	1.778
092799-7	0.690	0.803	8.669	114.423	49.112	537.807	1.719

```

092799-8 0.738 0.803 14.184 145.591 107.190 792.664 2.347
100599-1 0.697 0.776 5.271 71.550 48.453 705.495 1.504
100599-2 0.720 0.778 12.386 97.671 91.836 543.212 2.703
100599-3 0.732 0.860 8.331 108.473 92.333 751.973 1.807
100599-4 0.687 0.759 16.750 84.665 74.769 555.851 2.146
101299-1 0.707 0.707 19.852 119.821 107.738 696.522 2.273
101299-2 0.709 0.790 20.559 76.150 96.293 489.850 1.929
101299-3 0.693 0.770 17.586 75.452 99.534 446.750 1.582
101299-4 0.710 0.798 18.716 144.128 59.961 572.378 2.425
100199-1 0.750 0.773 29.224 159.924 195.801 821.063 1.790
100199-2 0.743 0.751 12.362 119.977 110.808 747.400 2.408
100199-3 0.756 0.737 8.463 80.118 93.672 483.669 1.927
100199-4 0.761 0.696 8.933 91.725 127.689 555.073 1.186
100199-5 0.710 0.825 14.148 86.039 75.649 404.681 2.207
100199-6 0.738 0.693 23.934 43.082 55.969 160.822 1.115
100199-7 0.717 0.848 7.870 62.822 47.746 515.893 1.338
100199-8 0.728 0.791 31.620 170.255 164.137 761.431 1.915
101899-6 0.749 0.770 11.877 74.262 92.277 390.841 2.448
101899-7 0.713 0.765 11.581 121.474 36.292 476.372 1.806
101899-8 0.731 0.807 9.425 61.855 67.640 576.860 1.363

```

```
> round(exp(result$control),3)
```

```

      x.1  x.2  x.3  x.4  x.5  x.6  X2
092199-1 0.705 0.752 8.840 125.198 70.035 724.513 1.895
092199-2 0.731 0.783 7.850 44.858 48.173 342.475 1.671
092199-3 0.706 0.804 15.212 172.863 118.297 909.777 1.836
092199-4 0.717 0.789 18.645 108.538 67.566 267.495 1.255
092799-1 0.749 0.797 8.401 44.137 80.375 353.824 1.453
092799-2 0.712 0.789 5.609 78.752 58.183 522.382 2.328
092799-3 0.702 0.857 13.158 70.959 110.300 506.842 1.175
092799-4 0.722 0.757 11.300 61.547 96.757 353.683 2.018
100599-5 0.740 0.821 11.217 101.352 71.765 615.725 1.966
100599-6 0.687 0.703 6.790 93.663 47.718 390.060 1.014
100599-7 0.709 0.724 9.661 97.602 87.357 570.321 1.842
100599-8 0.723 0.749 18.010 154.439 115.446 817.622 2.578
101299-5 0.749 0.827 14.704 56.340 63.676 161.483 1.107
101299-6 0.707 0.802 5.949 67.370 61.111 473.144 2.378
101299-7 0.686 0.733 23.055 82.179 116.373 411.702 1.090
101299-8 0.700 0.738 13.137 117.132 63.835 382.566 1.250
111999-1 0.701 0.737 11.524 102.371 74.888 499.247 1.958
111999-3 0.730 0.774 7.755 48.173 68.074 332.254 2.426
111999-4 0.740 0.788 5.083 25.086 37.386 169.457 1.273

```

```
> round(exp(result$test),3)
```

```

      x.1  x.2  x.3  x.4  x.5  x.6  X2
071799-1 0.663 0.689 9.305 70.302 14.405 114.686 1.000
071799-2 0.669 0.652 4.960 47.632 12.038 120.977 1.000
072399-1 0.684 0.695 4.638 11.920 10.577 48.241 1.001
072399-2 0.768 0.844 1.707 5.991 10.145 63.352 1.000
073099-2 0.707 0.830 13.829 71.336 15.992 74.173 1.002
080399-1 0.754 0.767 26.803 163.482 47.966 149.187 1.000

```

080399-2	0.741	0.816	10.494	60.419	67.478	281.857	2.139
080699-1	0.686	0.742	26.972	62.010	49.506	82.097	1.000
080699-2	0.683	0.827	15.583	60.166	10.458	55.584	1.000
091599-5	0.726	0.761	13.274	103.121	79.154	525.894	2.717
091599-6	0.708	0.763	331.391	561.325	67.647	814.114	1.000
091599-8	0.722	0.741	22.457	135.884	98.023	591.818	2.652
101899-1	0.700	0.775	21.811	112.303	103.824	496.806	1.908
101899-3	0.761	0.764	14.085	98.052	76.907	437.948	1.900
101899-4	0.709	0.770	12.806	146.496	71.479	899.015	2.172
101899-5	0.707	0.793	16.239	64.142	137.140	578.189	1.319
110899-1	0.758	0.805	41.884	89.685	191.675	305.760	1.005
110899-2	0.782	0.797	31.820	197.118	286.776	889.981	1.041
110899-3	0.777	0.755	68.676	408.708	494.180	1851.960	1.008
110899-4	0.767	0.784	29.548	84.690	226.558	489.801	1.046
110899-5	0.792	0.718	24.981	177.754	133.527	595.559	1.026
110899-6	0.760	0.727	33.747	382.719	214.369	1343.455	1.026
110899-7	0.730	0.726	7.451	121.644	29.521	383.638	1.050
110899-8	0.707	0.739	8.588	76.946	79.432	483.475	2.096
111999-6	0.667	0.769	9.959	109.443	132.807	632.639	1.000
111999-7	0.751	0.820	18.392	97.203	144.590	536.625	1.553
111999-8	0.688	0.753	5.226	24.344	43.515	162.666	1.020

Appendix D. Addendum: 24 February 2000. We decided to take the logs of the measurements *after* taking the means for each mouse. The resulting measurements are thus logs of average activity values. In the original version, we had taken logs before averaging, which is not physiologically meaningful. The algorithm did not change, only the measurement fed to it did.

Before we started our investigation, the Mutagroup had proposed an algorithm for detecting mutants. By this algorithm, a mouse was flagged as mutant if it was more than 3 sd's away from the mean for at least *one* measurement or more than 2 sd's from the mean for at least *two* measurements. Based on results shown here, we made the following recommendation to the Mutagroup.

“We recommend that the Distance Algorithm (DA) be used for flagging mutants and the Standard Deviation Algorithm (SDA) be used for interpretation.

Based on the data collected on mutants so far, the SDA and the DA perform comparably. Both use 6 summary measurements collected on each animal. In the future, more summaries may be calculated. By the nature of the SDA, the false positive rate of the SDA will increase with the number of measurements used. The DA does not suffer from this defect and according to statistical theory, it is at least as powerful as the SDA if both are aiming for the same false positive rate.”