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Exponential Data-Analysis of Passive-Avoidance Behavior in Rats and Mice

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ALLWEIS, C., D. CHERNICHOVSKY AND D. SHULZ. *Exponential data-analysis of passive-avoidance behavior in rats and mice.* PHARMACOL BIOCHEM BEHAV 31(4) 803–806, 1988.—Data obtained with the passive-avoidance task are usually presented as the median values of the latencies to respond. In an earlier publication we described a better way of presenting such data based on the observation that the complement of the cumulative distribution of step-through latencies can be closely fitted by a simple exponential function. Thus the "step-through rate constant" (STRC) is concise and accurate quantitative description of population behavior in this test. In this paper we present two examples of the application of this procedure. In the first, variation in the interval between training and testing in rats changes the STRCs of the different groups. In the second (based on data published by Flood *et al.*) administration of cycloheximide is seen to partition the experimental population of mice into two subgroups with different STRCs.

Rats Mice Memory Passive-avoidance Cycloheximide Exponential data-analysis Survival analysis

IN a recent publication we proposed a novel representation and analysis of passive-avoidance data in rats (5). It is based on the observation that the complement of the cumulative distribution of step-through latencies (i.e., the fraction of animals remaining in the safe compartment) decays exponentially with time from the onset of the trial. A remarkably close fit of this complementary distribution was seen when the best-fitting straight line was drawn through the data points plotted on semilogarithmic coordinates. The slope of this line k, which we call "the step-through rate constant" (or alternatively, the $t_{1/2}$ which is equal to 0.69/k) provides an accurate measure of the population behavior in many cases.

The purpose of this paper is to illustrate the utility of this approach in two different circumstances: the first (using rats) in which the form of the distribution function remains unchanged whilst the step-through rate constant changes and the second [taken from data obtained with mice by Flood *et al.* (2)] in which the experimental treatment partitions the population into two subgroups which have different step-through rate constants. In favourable cases, when there is a large difference in the k values, the value of the rate constant for each of the subgroups may be derived from the data using simple graphic or computerized techniques.

METHOD

Experimental

In passive-avoidance training, the animal is allowed or even encouraged to walk or jump from one place or compartment to another and is then shocked. Subsequent testing to determine whether the animal remembers the experience is performed in the same apparatus. This widely-used paradigm is sometimes referred to as an inhibitory avoidance test.

Details of experimental apparatus and procedures for passive-avoidance training have been provided in the references already cited (2,5). In both experimental procedures the raw data consisted of step-through latencies (STLs). In the first example with rats, the manipulated variable was the interval which was allowed to elapse between training and testing. In the second example with mice, the experimental group received cycloheximide (CXM) subcutaneously and the control group received saline.

Data Analysis

The new method of data analysis previously reported will be described only in outline here. By summing all the STLs which are equal to or less than any time t, we obtain a Cumulative Probability Function (CPF). The complementary distribution function of this CPF is the fraction of animals which have not yet stepped-through up to any point t in time, i.e., the Fraction Remaining $F_R(t)$. In the graphic representations of our experimental data, the fraction of animals remaining (i.e., not yet stepped-through) or its logarithm was plotted as a function of time at each piont in time when an

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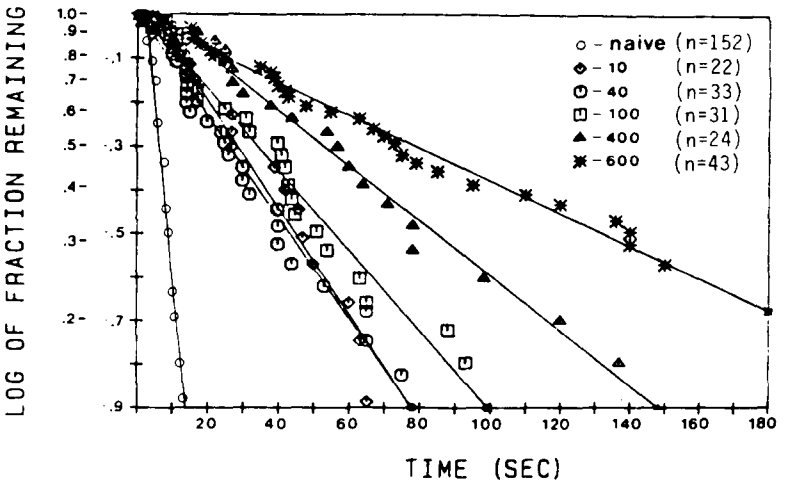

FIG. 1. The fraction of each of the experimental populations remaining (i.e., not yet stepped through) is plotted on the logarithmic ordinate against time from the beginning of the trial. Each point on the graph represents a rat stepping into the dark compartment. The various different symbols represent different train-to-test intervals in minutes as indicated in the key at the top of the graph. The open circles at the extreme left represent the naive group and are plotted in bins for clarity.

animal stepped-through. Thus the time at which each animal steps-through is represented by a point on the graph.

EXAMPLE 1. PASSIVE AVOIDANCE IN RATS: VARYING THE INTERVAL BETWEEN TRAINING AND TESTING

Results

In Fig. 1 we have plotted on semilogarithmic coordinates the fraction of the population remaining in the safe compartments as a function of time, for the naive animals and for the five experimental groups in which different intervals were allowed to elapse between training and testing (1).

Each point represents the value F_R at the time when a rat stepped-through. The best-fitting regression lines were calculated and drawn using SPSS programs. It is clear from the graph that each of the group plots can be fitted closely by a regression line which is a graphic representation of a simple exponential function,

$$F_R(t) = c \cdot e^{-kt} \quad (1)$$

where $F_R(t)$ is the fraction of the total population which has not yet stepped-through at time t.

The significance of the two parameters is as follows. The STRC, k, represents the fraction of the population which has not yet stepped-through at time t which does step-through during the next brief time interval Δt. We arbitrarily use a Δt of 1 sec. The parameter c is a constant whose value is close to 1.0 in these cases. When $c=1$ the exponential trend begins at time zero. When $c>1$ there is a delay before the onset of exponential trend and when $c<1$ some fraction of the population steps-through at t=zero prior to the onset of the exponential trend.

The differences in the slopes of the regression lines correspond to different values of k. Table 1 gives the value of these constants derived from the best-fitting regression lines in Fig. 1. It is clear from these results that rats tested immediately after training have a somewhat larger step-through rate constant and that with the passage of time the value of this constant decreased monotonically.

DISCUSSION

The analysis described here leads to a quantitative measure of the augmentation of the learned response that occurs during the first ten minutes after training. This measure may be expressed as either the change in the population step-through rate constant or as the change in the time at which 50% of the animals in a given group have stepped-through, $t_{1/2}$. Both of these values can be assessed from the best-fitting exponential function. If the group behavior can be adequately described by such a function (as in this case here) there is nothing to choose between these two ways of coding the data since, assuming that $c=1$, these two parameters are related by

$$t_{1/2} = \frac{0.69}{k} \quad (2)$$

In Table 1 we have presented both parameters for the various experimental groups together with their standard errors.

Since the absolute STRCs of naive control populations may vary from one session to antoher, a normalization procedure to correct for this variation is desirable so that results obtained in different sessions may be compared. The required normalization is generally achieved with the following formula.

$$\% \text{ Retention} = \frac{t_{1/2} \text{ test exptl.} - t_{1/2} \text{ train exptl.}}{t_{1/2} \text{ test control} - t_{1/2} \text{ train control}} \cdot 100 \quad (3)$$

The numerator represents the increase in $t_{1/2}$ due to the training procedure in the experimental group, and the de-

TABLE 1

THE EFFECT OF THE DURATION OF THE INTERVAL BETWEEN TRAINING AND TESTING ON $t_{1/2}$ TEST AND ON K_{test}

ΔT train→test	$t_{1/2}$ ± S.E.	K ± S.E.	N
naive	7.0 ± 0.6	0.099 ± 0.0029	152
10 sec	28.7 ± 6.3†	0.024 ± 0.0012	22
40 sec	26.5 ± 4.8*	0.026 ± 0.0007	33
100 sec	34.5 ± 5.5†	0.020 ± 0.0008	31
400 sec	57.0 ± 8.6	0.012 ± 0.0002	24
600 sec	86.2 ± 5.2	0.008 ± 0.0002	43

*$p<0.002$; †$p<0.01$.

The p-values obtained with the Wilcoxon test refer to the significance of difference compared to the 600-sec group.

nominator represents the increase in $t_{1/2}$ due to the training procedure in the control group.

It may readily be seen that this formula positions experimental groups along a dimensionless scale. If the experimental procedure has no effect on retention then the experimental group will be scored as 100% retention. If the experimental procedure prevents retention completely then the experimental group will be scored as zero% retention. Partial effects of the experimental procedure on retention will result in scores distributed proportionately along this scale. Small variations in the absolute values and in the increase in $t_{1/2}$ due to training between different control groups are corrected for by this procedure. It is similar to the well-known "percent savings" score often used in behavioral research.

Figure 2 is a graph of the normalized data plotted against the time which elapsed between training and testing. It shows that the retention level is about 30% initially and remains at that level for about two minutes. Over the next eight minutes, retention rises monotonically to its maximum value.

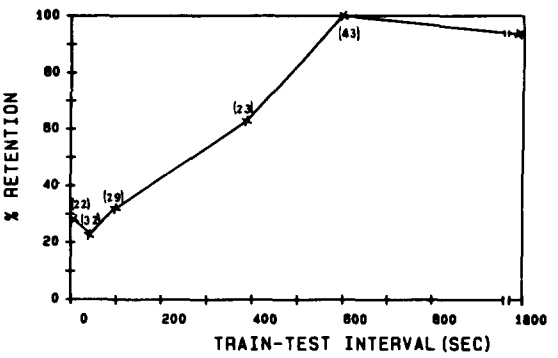

FIG. 2. The effect of train-to-test interval on test performance. Since this experiment did not involve control and experimental groups in the usual sense, the normalization formula was modified as follows:

$$\% \text{ Retention} = \frac{t_{1/2} \text{ test} - t_{1/2} \text{ naive}}{t_{1/2} \text{ maximum test} - t_{1/2} \text{ naive}} \cdot 100$$

This formula positions each group appropriately along a scale which rates a naive group as zero and the group having a maximum $t_{1/2}$ on test (the 600 sec interval train-to-test group) as 100%. Normalized group data (based on step-through rate constants derived from Fig. 1) are plotted against the train-test interval for each of the groups. Retention, which is initially low (about 25%), rises linearly to its maximum value over the period from about 1 min to 10 min after training.

EXAMPLE 2. THE EFFECT OF CXM ON PASSIVE-AVOIDANCE BEHAVIOR

The data used to illustrate the use of this analysis in the case when an exponential function with two terms is required is from Flood *et al.* (2). These workers carried out a very thorough study of the effect of CXM on C57B1 mice using a passive-avoidance paradigm. (We are grateful to the authors for permission to use their data.) We analysed their test data for controls and experimental groups treated with CXM using our new procedure and plotted the outcome of this analysis in Fig. 3.

The control data can be very well-fitted by an exponential function since the experimental points when plotted as described above fall on the logarithmic regression line. However, in the case of the CXM-treated group a biexponential function of the form given in equation (4) is required to fit the data points well.

$$FR_t = c_1 \cdot e^{-k_1 t} + c_2 \cdot e^{-k_2 t} \quad (4)$$

In this relatively simple case when there is no apparent lag in the onset of the exponential declines, c_1 and c_2, the intercepts of the regression lines with the y axis, represent the fractional composition of the population. Convenient methods for estimating the value of the parameters of this equation are described by Riggs (6).

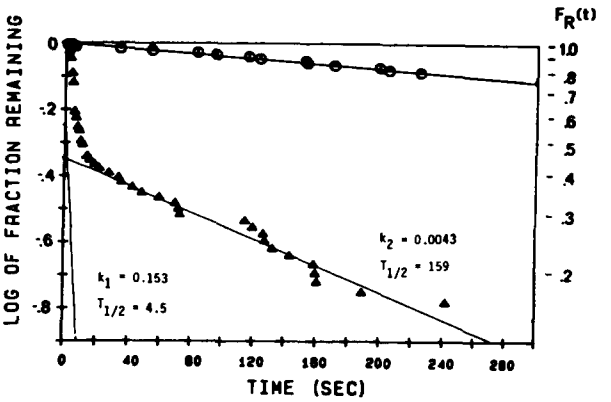

FIG. 3. An exponential analysis of the effect of cycloheximide on passive avoidance learning in mice using data from Flood *et al.* (2). The control test data for untreated mice, ○, can be well fitted by a simple exponential function. However, the test data require a biexponential function. The two best-fitting regression lines were derived graphically as described by Riggs (6).

The result of this analysis, which is presented in Fig. 3, implies that the experimental group is made up of two different populations and that the behavior of each of these populations can be well-described by its step-through rate constant. About one-half of the animals in the experimental group seem to behave on test like the naive group (the first exponential term), whilst the other half have a slope which is

slightly greater than that of the control group on test. It is not clear whether this result is due to some uncontrolled factor in the experimental procedure or due to the presence of the two subpopulations whose susceptibilities to CXM are different.

CONCLUDING REMARKS

The passive-avoidance paradigm is possibly the most widely-used behavioral test in the field of memory research. Its major advantages are its simplicity and the short time needed for both training and testing. Till now, its major disadvantages have been the extraordinary wide spread of the experimental data and the influence of the necessary arbitrary cut-off points in data acquisition on the data analysis.

Since we have shown that both train and test step-through latencies are exponentially distributed within a population, it is no longer necessary to discard most of the data by resorting to a median or mean step-through latency. By means of a simple logarithmic regression analysis of all the available data it is possible in many cases to obtain a single number which characterizes the population behavior more accurately.

The first experiment described here illustrates the application of this analysis to a situation in which the step-through rate constant changes whilst the exponential form of the distribution function remains unchanged. The first step in this analysis is to derive the step-through rate constants for each experimental group from their best-fit regression lines. As might well be expected on theoretical grounds, the closeness of fit of the regression lines to the data points improves as the number of animals in the group is augmented. By replotting the data followed each experiment in a series, it may be possible to repeatedly assess the improvement in fit and terminate the experiment when the regression line has been adequately defined in the context of the other circumstances of the investigation.

Since the focus of interest in most experiments of this kind is the extent to which memory is impaired by a given procedure, the second step in the analysis is to calculate the degree of treatment-induced impairment using the step-through rate constants of naive and trained control rats to delimit a scale appropriate to this purpose. We refer to this scale as % retention. The resulting graph, histogram or table gives a clearer and more valid picture of the results of the experiment than can be obtained from median group values.

Sahgal and Wright (4) have advocated the use of the Information Statistic for the analysis of passive-avoidance data. They state that the distribution of data from this test is bimodal because the scores fall into distinct categories (short and long response latencies) which are separated from each other by a third (medium) category containing significantly fewer scores than would be expected if the data were truly homogenous. Their method has been used by LeBrun *et al.* (3). We prefer the analysis described here for the following reasons.

In applying survival analysis to the passive-avoidance test, one regards the act of stepping-through (or down) as the terminating event for the individual rat and the time which elapsed between the instant the animal was placed in the test apparatus and the time at which it stepped-through (its step-through latency) as its survival time. We have shown that the complement of the cumulative frequency-distribution of STLs within a population can be very closely fitted by exponential functions both for naive and trained animals. This is an empirical finding which invalidates the use of statistical measures of central tendency.

As a matter of convenience data collection is stopped at some arbitrary point in time, the "cut-off point." Since the collection of data ceases with the recording of the last terminating event, those members of the population who have not yet undergone the terminating event do not contribute data. All terminating events, up to and including the last one recorded, contribute to the statistical definition of the form and parameters of the appropriate survival function. Since the remaining animals did not undergo the terminating event, they should not be regarded as the second category of a bimodal distribution because in order for them to constitute a category, the frequency distribution of their STLs would have to be known.

Our paper is also intended to illustrate the extension of this approach to experimental procedures which generate data that cannot be well-fitted by a simple exponential but can be closely fitted by an exponential expression with two terms. In such cases this data analysis enables the investigator to partition the experimental population into two subgroups. He is then in a position to advance hypotheses to account for the partitioning of the population and subject them to experimental testing. Treating such biexponential data as homogeneous may give rise to uncertainties which cannot be satisfactorily resolved by introducing an arbitrary cut-off piont. We illustrate this type of situation using data published by Flood *et al.* (2) on the effect of CXM on memory in mice.

It is clear from the analysis presented here that CXM is effective in producing amnesia in only part of the population subjected to their experimental procedure. The reason for this is not apparent and further experimentation will most likely be needed to elucidate it. Posttest investigation of two well-differentiated subpopulations is feaseable.

The advantage of using our data analysis is evident from this example since a median value in these circumstances is misleading and the definition of amnesia on the basis of an arbitrarily selected step-through latency less convincing than a partitioning of the population into subgroups on the basis of their different behaviors.

There may well be other cases in which drug treatments, brain stimulation, CNS lesions and other procedures partition population behavior in the passive-avoidance test but in the absence of data for each animal it is not possible to examine the literature to determine if this is so.

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