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A Novel Method for Quantifying Passive-Avoidance Behavior Based on the Exponential Distribution of Step-Through Latencies

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SHULZ, D., D. CHERNICHOVSKY AND C. ALLWEIS. *A novel method for quantifying passive-avoidance behavior based on the exponential distribution of step-through latencies.* PHARMACOL BIOCHEM BEHAV **25**(5) 979–983, 1986.—In the extensive literature dealing with the one-trial passive-avoidance task the data are usually represented by the median latency to respond. We propose here a novel representation and analysis of passive-avoidance data which 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 is seen when the best-fitting straight line is drawn through the data points plotted on semilog 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 description of the population behavior as a whole in most cases. In view of the exponential distribution of passive-avoidance data this treatment appears to be more appropriate than the widely-used measures of central tendency, the median and mean. It is applicable to research on the effects of drugs on passive-avoidance memory, and probably appropriate to other behavioral paradigms and species.

Rats Passive-avoidance Memory Exponential data analysis

THE aim of this paper is to propose the use of an exponential statistical description and analysis for data obtained with the one-trial passive-avoidance paradigm.

The data usually recorded with this widely-used paradigm is two step-through latencies (STLs) for each animal; the first for training and the second for test. These STLs vary greatly from animal to animal. In working up the data, the first step usually taken is the reduction of group data to a single value sometimes without any measure of dispersion.

A random sampling of 40 research publications dealing with passive-avoidance behavior from Bammer's review [1] revealed that about 40% of these investigators expressed their results as means and about 60% expressed them as medians. Seven of the papers presented both means and medians for good measure. There was also a small number of authors who declined to use either of these measures and instead preferred to use an arbitrary cut-off point or criterion to demarcate between two states, "remembers" and "forgets." Various non-parametric tests of significance were applied to the control and test data, the most popular being the Mann-Whitney U-test. The STLs of individual animals are rarely given.

The above data-treatments seem to be chosen in view of the non-normal and highly skewed distribution of these STLs. The situation is complicated by arbitrary cut-off of very large STLs.

During the course of an investigation designed to find out if our four-phase model of memory consolidation which was derived with the use of an active-avoidance paradigm [2] was applicable to the passive-avoidance paradigm we used a different statistical data treatment which has a number of advantages. This treatment is based on the observation that the fraction of the animals in a group that had not yet stepped-through as a function of time from the onset of the trial can be fitted very closely indeed by an empirical function of the form:

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

where $F_R(t)$ is the fraction of animals which have not yet stepped-through (i.e., fraction remaining) at any given time t and c is a constant which has a value close to 1. This observation implies an exponential distribution of STLs. Hence the behavior of a group in this task can usually be described

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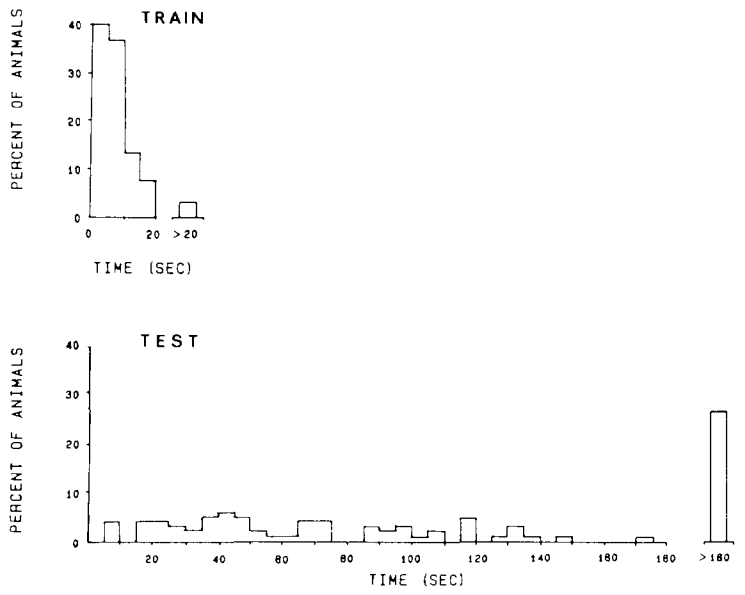

FIG. 1. The distribution of training and test step-through latencies. Five-second bins were used.

by a single parameter, its k value. This was found to be true for both training and test data. The data treatment described here is a special case of survival analysis [3].

METHOD

Experimental Animals

White local male rats (Sabra strain) weighing 120–150 g were used. Animals received from the animal house were kept in groups of 5 to a cage until the learning session (no more than 5 days) and were allowed free access to dry food pellets and water. Rats were first introduced to the experimental room 1 day before training began. All rats were trained in a one-trial step-through passive-avoidance task.

Training Apparatus

The apparatus consisted of a wooden box 61 cm long divided into a small white start box (18×24×41 cm high), and a black shock box (43×24×41 cm high) by a wall with a 9 cm diameter hole at the bottom and a guillotine door. The white start-compartment was illuminated by a 75 Watt lamp situated above it. The black shock compartment was covered with a black ceiling. Both compartments contained separate grid floors consisting of 0.5 cm diameter rods 1 cm apart. The black box grid could be electrified by a high voltage, constant current device. During the training both voltage and current were monitored on an oscilloscope and registered with a Grass Polygraph.

Training Procedure

Rats were placed in the white compartment facing the wall opposite the door with the guillotine door open. Rats were allowed to step through and the latency to cross into the dark compartment with all four paws was recorded (training step-through latency). Immediately after having entered the black box, a constant current footshock (50 cps, 0.7 mA peak-to-peak) was delivered until the rat escaped to the white safe box. The "latency to escape" shock was timed. After the rat had escaped the shock the sliding door was closed and the animal was removed from the apparatus 30 sec later. Rats which did not enter the black box within 20 sec of being placed in the apparatus or did not escape the punishing footshock within 20 sec (3.5%) were discarded from the experiment. Between training and test, animals were housed individually and were not handled or disturbed. All behavioral manipulations were conducted between 9 a.m. and 5 p.m.

Test Procedure

Rats were tested for their retention of the task at several different training test intervals between 30 and 240 min. They were placed in the white start box and the time until the animal crossed into the black box (four paws) was measured. The latency to enter at test will be called: "test step-through latency" (test STL). Animals not entering the black box within 180 sec were removed and assigned a score of >180. An increase in test STL compared to training STL indicates retention of the learned task.

RESULTS

Since detailed analysis failed to show any significant differences between groups tested at different times during the interval from 30 min to 240 min, the training and testing results were pooled separately. The distribution of training and testing latencies obtained is presented in Fig. 1.

It may be noticed that as a result of training there is a great change in the distribution of STLs. The STLs of the trained animals are distributed throughout the whole period of 180 sec of observation, and a significant fraction remain in the safe compartment at the end of this period.

Analysis of Results

In our experiments the infinite number of times from the

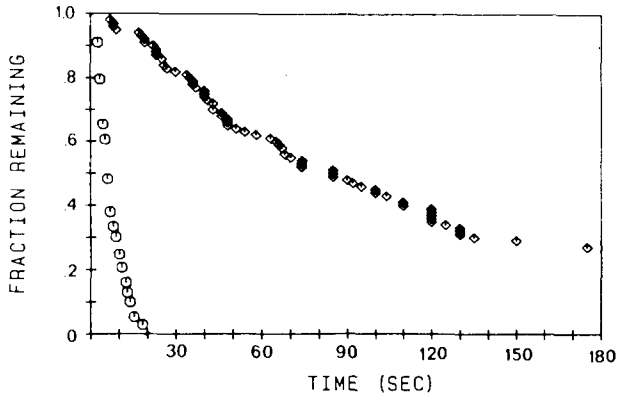

FIG. 2. The fraction of animals remaining plotted as a function of time from the onset of the trial. Circle and rhombus symbols correspond to training and test data respectively.

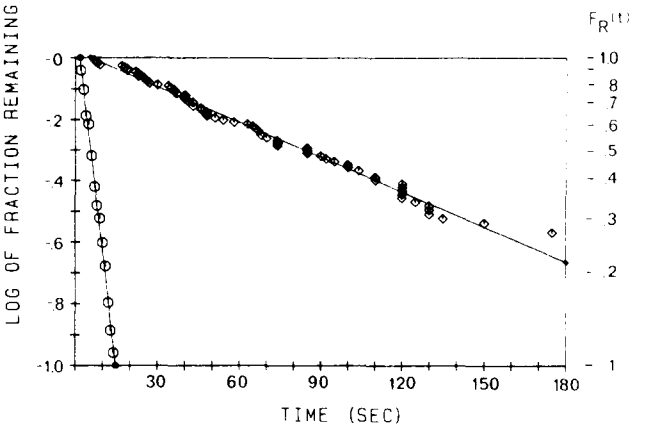

FIG. 3. The log of the fraction of animals remaining plotted as a function of time from the onset of the trial. Values for $F_R(t)$ are also indicated on the same logarithmic scale. Circle and rhombus represent training and test data respectively. $r_{test} = -.994$, standard error of estimate=0.018; $r_{train} = -.988$, standard error of estimate=0.044.

onset of the trial at which an animal may step through may be regarded as a continuous random variable whose distribution function F(t) is a continuous function which represents the probability to step through in less than time t:

$$F(t) = P(STL<t).$$

In practice we sum all STLs which are equal to or less than any time t. The resulting distribution function is often called 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 representation of our experimental data, the fraction of animals remaining was plotted as a function of time at each point in time when an animal stepped-through. Thus the time at which each animal steps-through will be represented as a point on the graph (Fig. 2). Our data is plotted in this way in Fig. 2 except that the training data points represent the fraction of animals stepping-through during each successive second due to the difficulty of plotting a large number of data in such a small area.

The plotted fraction of animals remaining $F_R(t)$ seems to fall exponentially with time, suggesting that the data can be described mathematically by an empirical function of the form:

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

We tested this idea by using a logarithmic transformation, since if the data fits the exponential model then log $F_S(t)$ would decay linearly with time according to the following linear equation:

$$\log F_R(t) = -kt \cdot \log(e) + \log(c) \qquad (2)$$

A regression program was used to draw best-fit lines through the points plotted on a semilogarithmic graph. The plots obtained are presented in Fig. 3. The remarkable close fit of the points to their lines indicated that the exponential model was acceptable both for train and for test data.

The fact that $F_R(t)$ is exponential implies that its Probability Density Function (PDF) (i.e., the probability of animal stepping-through at any point in time) is also exponential. This is so because the PDF is the negative derivative of $F_R(t)$ and hence retains the exponential form although the constants change so that:

$$PDF = c \cdot k \cdot e^{-kt} \qquad (3)$$

The ratio function obtained by dividing the PDF by its $F_R(t)$ represents the fraction of animals stepping-through at a given time divided by the fraction remaining (i.e., not yet stepped-through at that time). It expresses the probability that animals which remain till some t time will step-through at that time. Since in the present case the $F_R(t)$ function and its PDF are exponential this ratio function remains constant over time and is equal to k.

Equation (1) includes a parameter c (which is the intercept of $F_S(t)$ with the Y axis at t=0) the value of which in the relation to the value k determines the delay time of onset of the exponential decay as follows:

$$t_{delay} = \log(c)/k \cdot \log(e) \qquad (4)$$

If c is equal to 1 the exponential process begins without delay. If c is smaller than 1 the delay takes a negative value. The meaning of this is that (1−c) fraction of the population steps through immediately at t=0 prior to the onset of the exponential process.

When c is greater than 1 it signifies a delay before the onset of exponential step-through behavior.

In our case the regression analysis indicated small delays of about 2 sec for training data and about 5 sec at test. To simplify the model, the parameter c can be restricted to 1 since no significant deterioration in the closeness of fit results. However the decision whether to neglect the delays or not depends on the level of accuracy aimed at and whether one is interested in the delays themselves.

If we decide to restrict the value of the parameter c to 1, the behavior of a population in the passive-avoidance paradigm may be adequately and parsimoniously described

by a single parameter, its K-value, which will be termed "the step-through rate constant." Otherwise, two parameters, k and c, are needed to describe the data.

The parameter k may be estimated by two different methods: (1) The maximum likelihood method of estimation:

$$\hat{k} = d / \sum_{i=1}^{n} STL_i \qquad (5)$$

where $\hat{k}$ is the maximum likelihood estimator of k, d is the number of animals which stepped-through within the observation interval within 180 sec or 20 sec for test or training respectively), and $\sum STL_i$ is the sum of observed step-through latencies of all animals in the experiment [3].

(2) The slope of the regression line for the logarithmically transformed data may also be used for k-value estimation. The slope of the best fitting line (b) is equal to k·log(e) in equation (2). Hence k is equal to b/log(e) or 2.3b.

Both methods gave closely comparable k values. The results obtained with the second method only are presented below (Table 1) since it was supplied by the regression program which also gives the value of the intercept if it is different from 0.

The exponential behavior of the population enables us to estimate from the logplot the time at which one-half of the population has already stepped through. We designate this statistically-derived estimate as $T_{1/2}$. If the parameter c is restricted to 1, there is a reciprocal relation between $T_{1/2}$ and k such that:

$$T_{1/2} = 0.69/k \qquad (6)$$

Hence the estimated $T_{1/2}$ is an equally valid measure of the population behavior, and it is also presented in Table 1.

If the parameter c is not restricted to 1 then the relation of T to k is described by the following equation:

$$T_{1/2} = \frac{\log(1/2) - \log(c)}{\log(e) - k} \qquad (7)$$

DISCUSSION

The conventional reduction of passive avoidance data by resort to means or medians seems to be based on the notion that STL is a stimulus-to-response interval and that the individual members of an ideal homogeneous population would all have the same STL in this test. Conversely, variability in STLs within a given population seems to be regarded as unavoidable "noise" of experimental origin superimposed on a signal of definite value. This "noise" which is manifested in the highly-skewed wide dispersion of the experimental data is therefore discarded at the first step of data analysis by resort to means or medians.

We advance the suggestion that since it is possible to describe the behavioral data of the population as a whole very accurately by a rate constant (i.e., the ratio of the number of animals stepping-through at some point in time divided by the number of animals which have not yet stepped-through) it is better to use this "step-through rate constant" or a measure which is based on it rather than use an index of central tendency which is inappropriate to the data distribution.

The "half-time," $T_{1/2}$, described above is an alternative measure of group behavior derived from the step-through rate constant. It is usually a more reliable estimate of the time at which 50% of the population have stepped-through than is a median whose value is subject to fluctuation of the time at which one or two "central" animals of the group happen to step-through. Only providing that (1) the median STL is less than the cut-off STL (180 sec) and that (2) the "central animal" happens to fall close to the regression line, the value of this median will be close to that of the statistically derived $T_{1/2}$.

TABLE 1

VALUES OF THE CONSTANTS DERIVED BY FITTING EXPONENTIAL FUNCTIONS TO TRAINING AND TEST DATA

	Train	Test
No. of rats	97	94
K ± S.E.	0.183 ± 0.0039	0.0086 ± 0.00011
$T_{1/2}$ ± S.E.	6.2 ± 0.9	84.6 ± 7.5
c ± S.E.	1.3 ± 0.012	1.04 ± 0.0042

When these conditions are met, the median becomes a measure of group behavior which owes its validity not its conventional significance as a measure of central tendency of a normally distributed population, but to the fortuitous circumstance that the data obtained in this test happens to have an exponential distribution and in this context only, and under the restricted circumstances stated above, the median STL happens to be numerically close to $T_{1/2}$.

However, in many publications the median of the test STLS has the value of the cut-off time. This reduces the validity of this measure compared with $T_{1/2}$ which can be extrapolated from the regression line. In contrast to the median, $T_{1/2}$ is not a measure of central tendency; it is a concise quantitative description of the $F_R(t)$ distribution.

Regarding the influence of cut-off values on the analysis: in the usual way of reducing the data to a mean, the arbitrary cut-off values contribute spuriously in greater or smaller measure to the value of the mean. It is one of the advantages of the new approach we describe here, that the cut-off population contributes legitimately to the definition of the regression line from which the population step-through latency is derived.

As mentioned in the introduction, almost half of the authors sampled from Bammer's review reduced their STL data to a mean. This is not an appropriate way to treat the results since there is no central tendency and the exponential distribution of STLs causes the value of the mean to be unduly influenced by the larger STL values. (1/k is sometimes referred to as the "mean" of an exponential distribution [3], however, its value is often quite different from the value of the mean as usually calculated. As the K value decreases, the difference between the two "means" becomes larger. In our case the 1/k of the test data presented in Fig. 3 is 116 sec whereas the statistical mean is 96 sec.)

The change in the step-through rate constants from training to test indicates that learning has occurred. We consider the change in k or $T_{1/2}$ to be the best qualitative measure of this learning.

Since the effect of a treatment can be assessed by comparison of the slopes of treated vs. non-treated populations there is no need to adopt an arbitrary criterion for amnesia.

The most frequently used test of the significance of differences between groups in publications of passive-avoidance

data is the non-parametric Mann-Whitney U-test. Since in our analysis a k value is sufficient to describe the population behavior a test of significance based on k values alone is called for. The likelihood ratio parametric test or Cox F test may satisfy this requirement, however various non-parametric tests which have been used in survival analysis for data with an exponential distribution may also be applicable. These tests include (1) The Desu test and (2) Gehan's nonparametric test. For a description of these tests see [3] and [4]. Thus the data treatment described here enables the use of various tests of significance which cannot be used with the conventional method of data treatment.

There is no theoretical basis that we know of to support the use of an exponential function to describe the data points. The only justification is the fact that it fits the highly-skewed population data very well and therefore provides a concise, accurate and convenient metric for the description of the behavior of a population in this paradigm. The fact that the logplots display all of the data points and are easily made with readily available computer programs such as SPSS are further points in favour of their use.

In the absence of any convincing theory to support it, the use of a more complicated function to fit the data even more closely would not seem to be warranted, since the aim of data analysis is to quantify the test and training behavior of control and experimental groups for comparison.

There are three main ways in which drug treatments or other experimental procedures might alter the population distribution. They might change the value of the step-through rate constant, they may change the distribution function itself to some other distribution function or they may partition the population into two (or more) sub-groups with different step-through rate constants. It is a further advantage of our method of analysis that all three effects can be detected from the data. In the last case, the different sub-populations may in some cases be resolved by fitting an exponential expression with two (or more) terms to the data using graphical or computerized curve-fitting methods.

REFERENCES

1. Bammer, G. Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: A review and some new results. *Neurosci Biobehav Rev* **6**: 247–296, 1982.
2. Frieder, B. and C. Allweis. Memory consolidation: Further evidence for the four-phase model from the time-courses of diethyldithiocarbamate and ethacrinic acid amnesias. *Physiol Behav* **29**: 1071–1075, 1982.
3. Gross, A. J. and V. A. Clark. *Survival Distributions: Reliability Applications in the Biomedical Sciences*. New York: Wiley, 1975.
4. Lee, E. and M. Desu. A computer program for computing k samples with right censored data. *Comput Programs Biomed* **2**: 315–321, 1972.