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Computer-aided Calculation of Dose Response Curves in Behavioral Pharmacology by Using a Nonlinear Regression Procedure

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MORGENSTERN, R., H. FINK AND R. BLUTH. *Computer-aided calculation of dose response curves in behavioral pharmacology by using a nonlinear regression procedure.* PHARMAC. BIOCHEM. BEHAV. 15(6) 987–991, 1981.—In a variety of behavioral pharmacological experiments drug induced graded responses can be recorded even if one animal can be tested only once. In this case the analysis of dose response relationships will be accompanied with theoretical and practical problems additional to those known for dose response curves in single subjects as well as for the all-or-none type of responses. An experimental design was considered where one quantitatively measurable response of each animal tested contributed to an average dose response relationship. Use was made of a four parameter model capable of fitting s-shaped dose response curves over the whole feasible dose range for solving this nonlinear regression problem. Two examples, the dose dependent increased locomotor activity induced by apomorphine and the inhibited locomotor activity after pimozide treatment, were given to demonstrate the use of the method described and to direct the reader's attention to the wide range of its possible applications.

Dose response curves Nonlinear regression Open field test

MANY practical and theoretical problems arise when attempts are made to study drug-receptor interactions indirectly by observing responses of living systems to drugs. The simplest system would consist of a drug acting on a single cell [8]. As the structure of the living system becomes more complex, the quantitative interpretation of experimental results becomes increasingly difficult. In whole animals the situation is extremely complicated: the final overall response may result from various responses at the cellular level and from pharmacokinetic factors (see [8]).

Nevertheless, the dose response curves are well known to be hyperbolic or sigmoid, and there are well elaborated methods for fitting dose-percent effect curves of data of the all-or-none type [7, 9, 12, 15] as well as dose response curves of graded responses of single subjects [11, 14,16]. Additional problems arise when dose response curves are produced to study drug effects under the following suppositions: (1) each animal can be used only once and (2) contrary to the all-or-none type of responses the registered response is of the graded type.

This means that a single subject produces only one individual response in an experiment. Moreover, in the case of drug treatment, nothing is known about the individual zero level of activation, the individual maximum value of response, and the reactivity and sensitivity of the tested animal. Consequently, groups of animals must be used to obtain information about a dose response relationship, quite similar to the design of quantal biological assays [7,15]. However, in addition to the prerequisites of the quantal assay, the average minimum and maximum responses have to be determined. In this case, the individual quantitative response of each single subject contributes to an average dose response relationship which is influenced by biological variance [1]. When plotted against the logarithm of the dose, the values of the response will probably lie on a sigmoid curve.

Various functions having different characteristics of a generally sigmoid shape have been used empirically to fit dose response curves [1–3, 5, 6, 12, 17]. We have chosen to work with the function $y = A\emptyset + A1 \cdot \tanh c(x-k)$, since it has been demonstrated to be useful in fitting sigmoid curves [13]. This function is of manageable form, admits variation in slope, and the shape of the curve is directly indicated by the parameters of the function. The minimum is determined by $A\emptyset - A1$, the point of inflexion is given by the coordinates k and $A\emptyset$, and c is a measure of the slope. Consequently, k is the logarithm of the ED_{50}.

With the availability of modern desk-top computers it seems to be appropriate to use nonlinear regression for calculating dose response curves. In this paper we will demonstrate the handling of graded data obtained from the behav-

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ioral open field test. Locomotor activity will be used as an example of a wide range of possible applications of the method described.

METHOD

The experiments were carried out on male Wistar rats (VEB Versuchstierproduktion Schonwalde), weighing 150±20 g. The rats were housed in groups of 10 animals per cage at a room temperature between 20 and 24°C and with a 12 hours light/dark schedule (6:00 a.m.–6:00 p.m.). The animals received standard food and water ad lib prior to the experiment. Each animal was used only once.

We used a white wooden open field cage consisting of a 1 m × 1 m area divided into 36 equal squares and surrounded by a wall 40 cm high. The floor was diffusely lightened by a white 40 W fluorescent tube placed 2 m above the center of the cage. The open field test was carried out between 8:00 a.m. and 11:00 a.m. and between 2:00 p.m. and 4:00 p.m. in a soundproof room. After administration of the drug the rat was returned to its home cage. At the time of maximum response (apomorphine 7 min, pimozide 30 min) the rat was put into the middle of the open field cage. Locomotor activity was recorded by an observer for a period of 5 min as the number of crossed squares.

The drugs (apomorphine-HCl, SPOFA; pimozide, ORION) were injected intraperitoneally in a volume of 1 ml per 100 g body weight.

Fitting the function $y = A\emptyset + A1 \cdot \tanh c(x - k)$ is a nonlinear regression. In nonlinear procedures initial estimates of the parameters are provided and then adjusted in a series of iterations until the sum of squares of residuals reaches a minimum. We used the Gauss-Newton iterative technique with the linear approximation of the Taylor series expansion of the function of the squares of residuals. The initial estimates of the parameters were evaluated by Internal Least Squares. The flow diagram of the program is described in the Appendix. A listing of the HPL program for users of a Hewlett-Packard 9825 desk-top computer is available from the authors.

Measured Data

Saline treated animals crossed 38.5 ± 2.2 (n = 101) squares per 5 min. (The values are expressed as the mean ± standard error of the mean). The histogram (Fig. 1) reveals an acceptable bell-shaped distribution. Using the Chi^2 test we did not detect a significant departure from the normal distribution (Chi^2 8.13, d.f. 10, $p = 0.62$). The effects induced by increasing doses of drugs, i.e. stimulation after apomorphine and inhibition after pimozide, are shown in Table 1 and Figs. 2 and 3.

Fitting the Dose Response Curves

The two sets of dose response data which were used for the fitting process are given in Table 1: (1) Increase of locomotor activity induced by doses of apomorphine between 0.125 and 4 mg/kg and (2) Decrease of locomotor activity following the administration of pimozide (between 0.0075 and 0.5 mg/kg).

The Internal Least Squares technique gave initial estimates of the parameters Aø, A1, c, and k, as shown in Table 1. Stable final values of the 4 parameters of the dose response curves of apomorphine or pimozide were obtained after 13 or 8 iteration cycles, respectively. This coincided

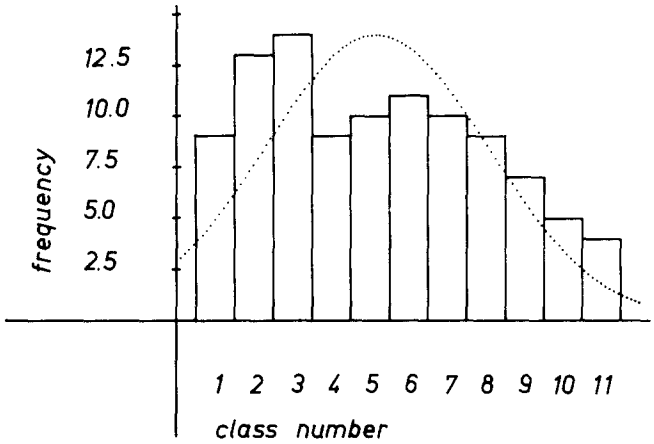


FIG. 1. Histogram for open field motility. The dotted line represents the theoretical standard normal distribution.

with reasonable stable minimum values of the sum of squares of residuals (Sr). The final value of Sr can be used as a measure of the goodness of fit (see Table 1). The calculated dose response curves prior to and after the iterative improvement are demonstrated in Figs. 2 and 3.

DISCUSSION

Nonlinear regression has apparently not been used very much in bioassays with graded responses measured quantitatively [14]. But, in many tests which have been performed in behavioral pharmacology graded responses to a given dose of drug can be observed [4,10]. Fitting of such dose response curves is a nonlinear regression problem.

It is common to simplify the problem by converting the data into those of the all-or-none type and then to work with methods which were developed for quantal responses. This technique may be performed by a transformation of the scale of response from quantitatively graded responses to the frequency of occurrence of an arbitrarily fixed effect. This procedure results in a loss of information; moreover, the ED_{50} value determined will then obviously depend on the scientist's definition of the all-or-none response.

Several other methods have been described in the literature which are likely to fit dose response curves over a limited but not the whole dose range [14]. In bioessays where there is a considerable drug-to-drug variation in ED_{50} it is not possible a priori to delimit the dose range with the steepest slope. In this case doses must be used which are distributed over a wide range and many experiments have to be carried out only for finding the range of the steepest slope.

However, the special problem of calculating dose response curves which has been demonstrated in the present paper can only satisfactorily be solved by nonlinear regression, using a dose response model which is capable of fitting the dose response curve over the whole feasible dose range. It does not seem to be necessary to point out the benefit of using complete dose response curves for the analysis of drug interactions (synergism, antagonism, potentiation etc.) in detail and to stress the more illustrative character of curves.

Different models have been used for fitting s-shaped curves and it has been shown that it might be advantageous to use a four parameter model [12,14]. We have chosen to work with the function $y = A\emptyset + A1 \cdot \tanh c(x - k)$ since it has been demonstrated to be useful in fitting s-shaped curves

TABLE 1

INITIAL ESTIMATES AND FINAL PARAMETERS OF APOMORPHINE (a) AND PIMOZIDE (b) DOSE RESPONSE CURVES FOR LOCOMOTOR ACTIVITY

Dose (mg/kg)	Response (squares)	Standard-Error	Initial Estimates	Final Parameters (after iterat. 13)
Apomorphine				
0.125	21.6	1.9	A∅=60.614562	A∅=59.144384
0.25	32.9	3.3	A1=38.607692	A1=33.918130
0.5	24.2	2.7	c=3.013336	c=5.224697
0.707	50.5	3.5	k=−0.150524	k=−0.072435
1.0	69.2	7.2	Sr=636.995109	Sr=138.908294
2.0	92.5	10.0	R=0.941591	R=0.987558
4.0	92.9	7.7	t-value=4.843	t-value=10.88
			$d.f.$=3	$d.f.$=3
				p=0.001663
log ED_{50}=−0.072435			ED_{50}=0.846 mg/kg apomorphine-HCl	
				(after iterat. 8)
Pimozide				
0.0075	37.8	5.5	A∅=28.547505	A∅=28.790578
0.015	35.1	7.2	A1=−8.134088	A1=−8.211434
0.03	34.3	5.7	c=2.582435	c=2.900539
0.06	27.6	3.8	k=−1.214251	k=−1.272012
0.125	22.0	2.2	Sr=5.198026	Sr=2.977977
0.25	21.4	5.8	R=0.991912	R=0.995374
0.5	20.4	2.7	t-value=13.54	t-value=17.95
			$d.f.$=3	$d.f.$=3
				p=0.000377
log ED_{50}=−1.272012			ED_{50}=0.053 mg/kg pimozide	

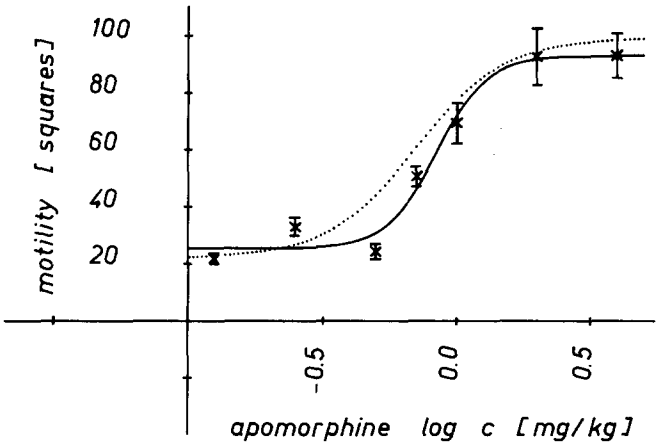


FIG. 2. Dose response curve of locomotor hyperactivity induced by increasing doses of apomorphine-HCl. The points represent mean values (± standard error of the mean) of, at least, 10 animals. The dotted line was plotted using the initial estimates after Internal Least Squares, and the full line represents the final dose response curve after 13 iteration cycles of Gauss-Newton iteration. (For data see Table 1a)

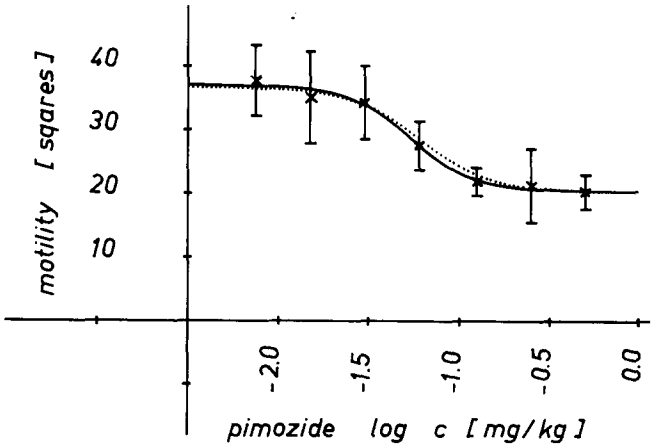
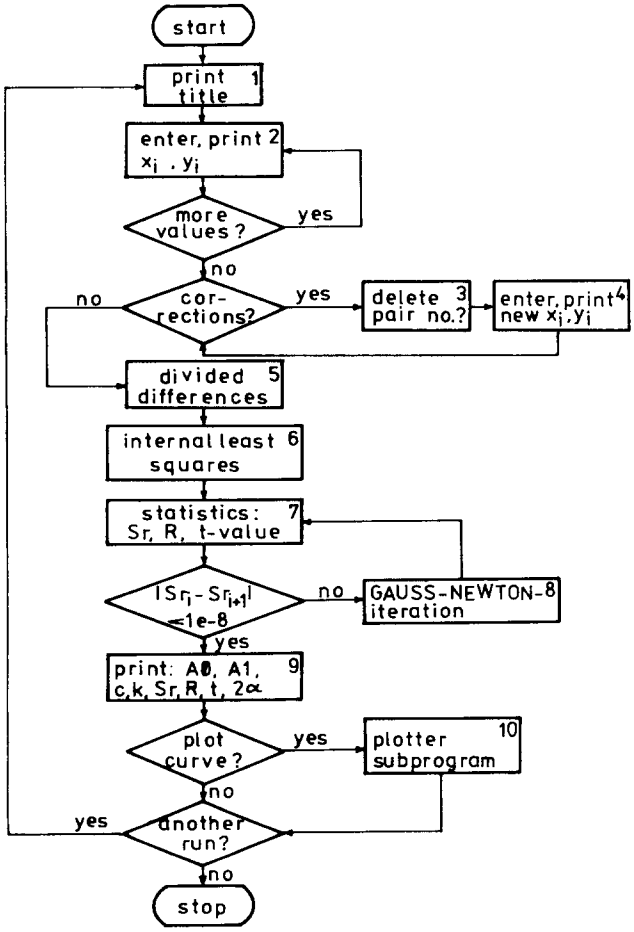


FIG. 3. Dose response curve of locomotor activity inhibited by increasing doses of pimozide. (For description see Fig. 2, for data see Table 1b)

[13]. It is easy to handle and the parameters of the funtion are in a direct relationship with the shape of the fitted curve. The calculated parameters provide a clear idea of the dose response curve. An evaluation of the goodness of the fit is provided by the nonlinear correlation coefficient R (according to Pearson) and the magnitude of the sum of squares of residuals.

Another point which has not been mentioned so far is that it has been assumed that the variance of responses would be independent of the magnitude of the response (i.e. the responses are homoscedastic). This prerequisite has been extensively discussed by other authors [9,11]. They have pointed out that small deviations from homoscedasticity are unlikely to modify the results obtained to any appreciable extent. This problem seems to be of little practical importance.

It should be added that the main aim of the present authors was, when preparing the fitting program, to make it functional. Very little attention has been paid to the efficiency of the program in terms of computation time. As a rough guide the calculation of each of the two examples shown in Table 1 will require about 10 sec. The program has already been used successfully on a 9825 A (Opt. 001) desktop computer (Hewlett-Packard) for more than 4 years.



Description of the program for calculating dose response curves by using Internal Least Squares and Gauss-Newton iteration with the four parameter function $y = A\emptyset + A \cdot \tanh c(x-k)$.

APPENDIX

The program consists of two main parts:

(1) Calculation of the initial estimates of the parameters Aø, A1, c, and k (by using Internal Least Squares) (Steps 5 and 6)

(2) Gradual improvement of the parameters until the sum of squares of residuals reaches a minimum ($Sr_i - Sr_{i+1} < 10^{-8}$) by using Gauss-Newton iterative technique. (Steps 7 and 8)

Description of the steps:

2: Enter pairs of data (x_i; y_i)

3 and 4: Corrections

5 and 6: The pairs of data must not essentially be equidistant. Internal Least Squares procedure is performed by using Divided Differences. Step 6 gives the initial estimates of Aø, A1, c, and k.

7: Goodness of fit test:
Sum of squares of residuals (Sr)

$$\text{Correlation coefficient (R); } R = + \sqrt{1 - \frac{Sr}{S_{yy}}}$$

$$S_{yy} = \sum y_i^2 - \frac{(\sum y_i)^2}{n}$$

$$t\text{-value; } t = \frac{R \cdot \sqrt{d.f.}}{\sqrt{1 - R^2}}; \; d.f. = n-4$$

8: Gauss-Newton iterative technique

9: Print out of final results:
Aø, A1, c, k, Sr, R, t-value, $p_{t\text{-value; } d.f.}$

10: Plotter subprogram:
Plots the initially entered pairs of data and the calculated dose response curve.

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