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ANALYSIS OF PHARMACOLOGICAL CONCENTRATION-RESPONSE CURVES WITH A BBC MICROCOMPUTER

A. D. Michel and R. L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

The logistic function has proved highly adept at describing the CRC for many pharmacological agents (Waud, 1981). However, the non linear nature of this relationship complicates data analysis and has resulted in the use of possibly inadequate linearising transforms and highly subjective "by eye" methods for parameter determination. While the development of computer-aided non linear regression methods for the analysis of CRC has provided a statistically more sound approach to data analysis, there has not been a widespread utilisation of these techniques. This may possibly be attributed to limited access to adequate computing facilities or to lack of "user friendliness" of available programmes. Furthermore the iterative nature of many available curve fitting programmes requires the user to provide initial estimates (IE) for each parameter to be calculated. When faced with data in a tabulated format selection of IE can be difficult, especially for abstract parameters such as the slope of a CRC.

To circumvent some of these problems, the present demonstration describes a disc-file based combined graphical/analytical method which enables CRC to be analysed using iterative curve fitting techniques but which uses the high resolution graphics of the BBC microcomputer to provide a simple graphical method for selecting parameter IE and for detecting incorrectly entered data points.

The analysis described consists of a suite of programmes accessed through a central menu. Data are entered in terms of a series of concentration and effect values and are stored as a disc based data file which can be accessed by each of the other analysis programmes.

After data entry, a graphical prefitting programme is entered which automatically selects axes and displays the data in a log concentration-effect format. An initial curve based upon the logistic function (Parker & Waud, 1971) is superimposed upon the data and, by means of a series of simple to use graphical aids, the slope, maximum and ED_{50} parameters that best describe the CRC are adjusted to produce the best graphical fit to the data. Each time one of the parameters is changed a new curve is drawn over the data points and the sum of the squares associated with the fit is calculated to provide a crude "goodness of fit" index.

When a reasonable graphical fit to the data has been achieved, an iterative curve fitting routine as described by Parker & Waud (1971) is entered. This utilises the best fit parameter estimates for the ED_{50}, maximum and slope of the CRC derived graphically, as IE. After completing the iterative curve fitting process the final parameter values are displayed graphically and a hard copy of the fit may be obtained using a screen dump facility designed for an Epson printer. After completing the analysis the best fit parameters are stored on the data file and can be recalled later if required. An additional editing programme that can directly access the data file at any stage in the analysis is also provided in order to edit erroneous or incorrectly entered data points.

Parker, R.B. & Waud, D.R. (1971) J.Pharmac.exp.Ther., 177., 13-24.
Waud, D.R. (1981) TIPS, 18, 52-55.

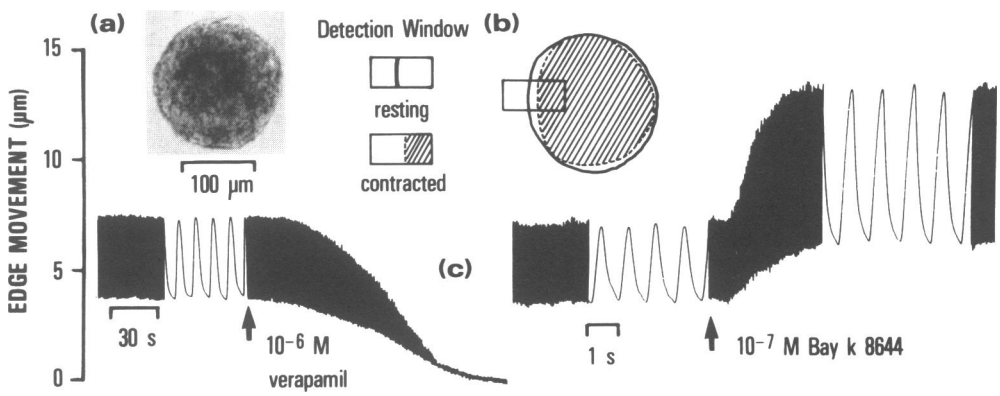
MEASUREMENT OF INOTROPIC RESPONSES OF CULTURED HEART CELLS USING A VIDEO TRACKING DEVICE

L. Patmore and R.L. Whiting,
Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

The inhibition of contraction of cultured embryonic chick heart cells can be used as a test for calcium antagonist activity (Clarke et al, 1984). Recent interest in compounds which facilitate calcium entry led us to develop a system for measuring both positive and negative inotropic responses from myocycte cultures.

Primary cultures were prepared from 11-day old chicken embryos as described by Clusin (1981). Aggregates of 100 - 300 μm dia. were formed in 3 - 4 days and transferred to Lux SAS plates. A phase contrast photomicrograph of an aggregate of approximately 1000 cells is shown in Figure 1a. The aggregates display automaticity and, on contraction, adopt a smaller area (shaded, Figure 1b). The video image of a 40x magnification of the aggregate (National Panasonic CCTV camera, Nikon Diaphot microscope) is fed into a motion detector (Tephcotronics Ltd., Edinburgh). This device samples video lines within an adjustable window positioned over the edge of the aggregate. A video threshold is set to define the edge where the image changes from light to dark. The threshold position relative to the width of the window is measured for between 20 and 30 raster lines (height of the window) and these values are integrated every 20 ms. The overall frequency response of the system is 10 Hz. The analogue signal output is proportional to the area of the aggregate in the detection window and changes on contraction.

Figure 1 Measurement of inotropic responses



Aggregates were continuously superfused with a salt solution based on the ionic composition of the culture media (253 mosmol, pH 7.3, 37°C). Superfusion with 1 x 10^{-6} M verapamil caused a rapid decrease in contractility whilst application of the calcium facilitator Bay k 8644 (1 x 10^{-7} M) was positively inotropic (Figure 1c). This system allows the rapid assessment of positive or negative inotropic activity and offers an alternative to the use of classical in vitro or in vivo preparations.

Clarke, B. et al (1984) Br.J.Pharmac., 83, 365P.
Clusin, W.T. (1981) J.Physiol., 320, 149-174.

AN INTERACTIVE COMPUTER PROGRAMME FOR DATA MANAGEMENT AND PARAMETRIC AND NONPARAMETRIC STATISTICAL ANALYSIS

P.R. Saxena
Department of Pharmacology, Erasmus University, Rotterdam, The Netherlands.

In biological experiments one obtains data belonging to several groups, for example, after different doses of a drug. It is often desirable to arrange such data, as they become available, according to groups and experiments and subject them to a parametric or nonparametric analysis of variance, followed by an appropriate test to discern a statistically significant difference between means of two groups. The present computer programme VARANL, which is written in FORTRAN and runs on a PDP 11/70 or Digital Professional microcomputer (Digital Equipment Corporation), has been developed for the above purpose.

At the start of the programme, VARANL presents a menu for selecting one of the several possibilities which include: (i) input of new data from the terminal into a user-named file (maximum: 20 groups, 100 experiments/group, and 1000 total data population); (ii) correction of eventual mistakes in the existing data; (iii) rearrangement of groups in a different sequence; (iv) deletion of experiments from the existing data; (v) addition of new data from terminal into the existing data; (vi) combination of data present in two files, according to groups or experiments; (vii) normalization of data as % of those in another group; and (viii) derivation of data as differences between (absolute or percent), or additions of data belonging to any two groups. The modified data, if needed, can be stored on the disk in a file named by the user.

Subsequently, one can perform one- and/or two-way analysis of variance using parametric (Steel & Torrie, 1980) and nonparametric (Kruskal-Wallis or Friedman's tests; Siegel, 1956) statistical procedures. Thereafter, the changes and percent changes between the values in the different groups are calculated. The significance of the differences in the different group means are evaluated by the use of unpaired or paired t test, Students-Neumans-Keuls test and Duncan's new multiple range test (parametric statistics), and of Mann-Whitney U test (one-way nonparametric statistics) or Wilcoxon's matched-pairs signed-ranked test (two-way nonparametric statistics). The calculated statistical values are compared with relevant values in appropriate tables (stored in the computer) and the significance of each test at $P<0.05$ is indicated by a symbol. The results are presented on the terminal or, in addition, can be printed on a line printer.

The programme is completely interactive and, I believe, it can be used by investigators with little knowledge of computers.

SIEGEL, S. (1956). ***Nonparametric statistics for behavioral sciences***, McGraw-Hill Kogakusha, Tokyo.

STEEL, R.G.D. & TORRIE, J.H. (1980). ***Principles and procedures of statistics, a biomedical approach***, McGraw-Hill Kogakusha, Tokyo.

A POOR MAN'S MOLECULAR GRAPHICS

R. Barlow, Department of Pharmacology, Bristol University.

This demonstration shows how you can use a microcomputer, such as a Commodore 64, to obtain access to the structures of compounds published in the crystallographic literature. Precise calculations can be made of bond lengths, interatomic distances, bond angles and torsion angles and the structures can be displayed on a video screen or drawn on paper. Examples at present available range from acetazolamide to yohimbine.