A MICROCOMPUTER METHOD TO AID IN VITRO SCREENING OF NOVEL COMPOUNDS FOR PHARMACOLOGICAL ACTIVITY

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We have developed an inexpensive, easy to use method, based on the BBC microcomputer, to semi-automate the in vitro screening of novel compounds for pharmacological activity using the electrically stimulated rat anococcygeus muscle. The system can collect data from up to six tissue baths, control the emptying and refilling of the baths and initiate electrical stimulation. On completion of the experiment the same computer will collate and analyse the data.

Data collection is carried out by a suite of programmes written partly in BASIC and partly in 6502 assembly language. Isometric tension from up to six strain gauge transducers is digitised to 12-bit resolution at a sampling frequency of 100Hz by an analogue-to-digital converter contained in an external interface unit. This unit is attached to the BBC microcomputer via the 1MHz bus and also contains an extra 16K of battery backed CMOS memory and four VIA chips to afford 64 lines which can be configured individually as inputs or outputs. These input/output lines are used to initiate stimulation by triggering a stimulator and to switch relays which operate solenoid valves to empty or to fill the baths. On start-up the programmes check the floppy disks for space and then produce the main working screen which gives information on the status of the experiment. The operator chooses from a range of actions by single keystrokes; unrecognised requests are ignored. In the treatment mode the computer initiates a preset number of control responses to short trains of electrical stimuli and then, with a short bleep, instructs the operator to administer a drug dose after which it collects a preset number of post treatment responses. On completion of this sequence, the peak responses to each period of stimulation are stored on floppy disk and the baths washed to await the next treatment. The peak changes in tension produced by each stimulus and any changes in baseline are plotted on the screen in numeric and graphic forms.

A second suite of programmes carries out data analysis. Usually a dose response curve (DRC) will be constructed to agonists and, if antagonists are being investigated DRCs will be repeated in the presence of several doses of the antagonist. First the raw data is collated and analysed to extract the peak effect of each dose of agonist and then drug doses and names are inserted. This is done during analysis rather than collection to avoid distraction during the experiment. Linear fits are then carried out on the log dose response data by the method of least squares. If more than one DRC is present for a tissue two fits are performed, One allowing different slopes and the other forcing the same slope. This allows statistical assessment of goodness of fits and parallelism from the analysis of variance. Log transformation of the dose does not linearise the extremes of the DRC and so the fit and the data points are plotted in graphical form on the screen and the operator is allowed to edit points at the extremes of the DRC and then repeat the fit. Once a 'satisfactory' fit has been achieved the statistics and either the ED_{50} or the dose ratios are printed.

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MICROCOMPUTER INTEGRATION OF TENSION DEVELOPMENT, A VERSATILE AID TO CONCENTRATION-RESPONSE DATA COLLECTION AND ANALYSIS

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Microcomputer software has been developed to enable inexpensive continuous integration of tension development in isolated smooth muscle preparations and subsequent rapid analysis for concentration:response parameters.

A Research Machines 380Z 56K microcomputer reads from a 16 channel Research Machines 10 bit analogue to digital converter with integral timer, the input of which is tapped directly (without special interfacing requirements) from the driver amplifier of a Grass Polygraph. Up to 16 channels are sequentially scanned and digitized at a selected high frequency in parallel with the analogue chart write-out. Integrals are computed over selected time periods and both printed and stored on diskette.

Modular program structure (Cope, 1981) allows menu selection of facilities in 3 groups of processes.

1) Initialization and continuous collection in memory and print out of integrals with a terminal write to diskette. Initialization comprises selection/declaration of diskette filename, number of channels (1-16), tension calibration (cm/g), galvanometer baseline position (-2.5 to 2.5 cm), digitizing frequency (61 to 538-3125 Hz), integral period and duration of experiment. A check is made that diskette space and memory requirements can be satisfied.

2) Read back data from diskette for editing and supplementation aided by VDU histogram representation of the current data set. Editing comprises deletion of redundant data, declaring any switched change in recorder sensitivity, addition of a constant, scaling to proportions of specified maxima and supplying concentration and time of agonist treatments. A backup copy of the original data file is made.

3) Analysis. Summary statistics of the concentration:response relationship are derived from regression analysis of log.concentration (over EC20-EC80 range): integral or log.concentration (EC5-EC95): probit integral data using the routines of Tallarida and Murray (1981).

All the modules are written in BASIC save the integrator which is in Zilog Z80 assembly language as a CALLed interruptible subroutine and an interrupt service routine.

The use of the system with phasic and tonic tension development in uterus will be illustrated.

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Cope, T. (1981) 'Design of programs' pp 205-217 in 'Computing using Basic : an interactive approach'. Ellis Horwood:Chichester

Tallarida, R.J. & Murray, R.B. (1981) 'Manual of pharmacologic calculations with computer programs'. Springer-Verlag:New York.

A SET OF COMPUTER PROGRAMS FOR ELECTROPHYSIOLOGICAL ANALYSIS OF END PLATE CURRENT CHARACTERISTICS

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A system of programs will be demonstrated for the routine computer analysis of data obtained from voltage clamp experiments on the neuromuscular junction. The programs make use of a CED 502 (Cambridge Electronic Design) high performance analogue-digital converter interfaced, via a SILO temporary storage memory, to a PDP11/23 (Digital Equipment Corp.) computer with a 10 Mbyte Winchester disc. The 4 us A/D conversion time of the CED 502, its capacity to store digitised data temporarily in the SILO, coupled with the fast data writing rate of the Winchester disc allow continuous sampling at the rate of 25000 samples per second, up to the capacity of the disc. Results are presented on a cathode ray tube (CRT) display and on a daisywheel printer. Analysis is performed off-line, from signals stored on FM magnetic tape, to make the most economical use of the computer.

The programs are written in the FORTRAN language with time critical subroutines written in assembler language. The user operates all programs in a similar manner, by selecting options from a series of menus and sub-menus. Three programs will be shown:

i) An end plate current (EPC) collection and analysis program. Series of nerve-evoked EPCs or spontaneous MEPCs can be collected, averaged, and double exponential curves fitted to their decay phases using a non-linear least squares method. The program continuously samples the signal from the tape recorder, using a pulse from a hardware spike detector to signify the presence of an EPC or MEPC, which is then copied on to the disc. By this means, pre-trigger information on spontaneous MEPCs can be acquired. The signals stored on disc can be viewed on a CRT and included in, or rejected from, further stages of analysis.

ii) A current fluctuation analysis program. Steady state currents through ACh channels can be collected, power spectra calculated, and single and double Lorentzian curves fitted.

iii) A single channel current analysis program. Channel open/close fluctuations can be collected and analysed with varying degrees of automation. Open/close state dwell time histograms can be compiled and exponential curves fitted.

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A MICROCOMPUTER-BASED IMAGE ANALYSER FOR RECEPTOR AUTORADIOGRAPHY

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Light microscopic autoradiography is widely used for visualising neurotransmitter receptors in brain sections with intact neuroanatomy (Young & Kuhar, 1979). Unfixed brain sections labelled with tritiated or iodinated ligands are exposed to film together with standards. The autoradiographs obtained have to be measured to obtain regional receptor densities. Measurements can be made with a microdensitometer (density measurements) or with a camera and a computer-based image analyser (grey level measurements). Image analysers allow the autoradiographs to be altered (e.g. thresholding, background subtraction) and are the method of choice (Goochee et al, 1983). The disadvantages of image analysers are the high cost and the fact that most are designed primarily for particle analysis - and much of the software is unnecessary for analysing autoradiographs.

This demonstration shows a relatively low-cost image analyser based on a 16-bit microcomputer that greatly facilitates the analysis of autoradiographs and standards. Autoradiographs are scanned with a video camera fitted with a macro-lens or a C-mount. The image is digitised and held in a frame store (Microeye II, Digithurst) with a resolution of 512 x 512. The image is read into the memory (512K) of an IBM PC microcomputer via an interface card.

Image analysis software written in machine code (Microscale II, Digithurst) allows the operator to measure and alter the images. The routines most applicable to receptor autoradiography include grey level measurements (64 grey levels) - made with a mouse-controlled cursor, and adjustment of the grey scales (thresholding) to display areas with the highest (or lowest) receptor densities. This is a valuable routine for revealing left-right differences produced by unilateral brain lesions. Another routine is image subtraction which is used to remove background or non-specific binding of ligands to brain sections

Goochee, C. et al (1983) Trends in Neurosci. 6, 256
Young, W.S. & Kuhar, M.J. (1979) Brain Res. 179, 255

A VERSATILE MICROCOMPUTER SYSTEM TO AUTOMATE IN VITRO PHARMACOLOGICAL EXPERIMENTS

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We have developed a versatile system, based on the BBC microcomputer, which can automate most (you still make up the KREBS!) aspects of in vitro pharmacological experiments.

The core of the system is a 6502 assembly language programme which controls an interface unit connected to the BBC microcomputer via the 1MHz bus. This interface unit contains a multiplexed 12-bit analogue to digital convertor to allow sampling from up to 8 transducers, 16K of battery backed CMOS memory (expandable to 60K in 4K steps) and a unit containing 4 VIA chips which give 64 TTL compatible logic lines which can be configured individually as inputs or outputs. The the maximum sampling rate of the ADC is 8Khz yielding 1Khz per channel when 8 channels are in use with pro rata increases for fewer channels. The lowest programmable rate is 15Hz and the actual rate can be freely adjusted between these limits to suit the biological application. The duration of sampling can also be preset but is limited by the amount of extra memory installed for data storage. The VIA input/output lines provide a means of controlling external devices such as, flow valves for bath washing and infusion pumps for drug additions. One of the VIA's, in conjunction with an extra timer board , generates variable duration trains of TTL pulses of programmable frequency and width to gate a separate high output square wave tissue stimulator. Facilities to control basal tension on isolated tissues are being developed. The assembly language program also contains a routine which will scroll portions of the VDU screen from right to left to simulate a chart recorder.

The facilities of the assembly language programme are accessed though a suite of chained BASIC programmes. Most of the programmes are concerned with routine 'housekeeping' functions common to all applications; such as setting default values, checking the current availability of disk storage space and setting up screen displays. A main programme determines the exact details of the particular application and is tailored to suit each experimental protocol. In practice, this programme seldom needs completely rewritten since most protocols have much in common.

At present we use the system to investigate the effect of drugs on the time course of the response of the rat vas deferens to single and dual pulse electrical field stimulatiom. The tissues are stimulated at 5 minute intervals. The mechanical responses to stimulation as monitored by isometric force transducers are digitised at a sampling rate of 100KHz for 2.5 second periods. On completion of collection the digitised data is written to floppy disk.

A further suite of BASIC programmes is available to analyse the data and present the results. The information from several experiments is collated into a single large random access file from which subgroups of responses representing different treatments can be extracted averaged and then compared with other subgroups. The results of the analyses can be printed in numerical form or plotted as graphs suitable for publication or photogaphy using a Hewlett Packard 7470A graph plotter.