VOLTAMMETRIC DETECTION OF DOPAMINE RELEASED BY STIMULATION OF THE MFB IN THE ANAESTHETISED RAT

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This demonstration is a video tape recording of an experiment in which the release of dopamine is monitored using fast cyclic voltammetry (Armstrong-James et al 1981, Stamford et al 1984). Rats weighing 250-350g, anaesthetised with chloral hydrate (400 mg/kg i.p), were fixed in a stereotaxic frame, and a carbon fibre microelectrode was placed carefully in the striatum. A platinum wire auxilary electrode, and a silver/silver chloride reference electrode were placed on the muscles of the head to allow voltammetric studies.

A concentric bipolar stimulating electrode was carefully lowered 6mm below the surface of the dura at co-ordinates A-2.2,L+1.6 (relative tobregma Pellegrino et al 1979), above the median forebrain bundle (MFB). The stimulating current (80-100 uA RMS, 50 Hz)was turned on, and the electrode lowered to between 8 and 9 mm below dura as necessary. During this phase, voltammetric scans were made 4/sec. Electrode lowering and stimulation were stopped, when a large increase in the signal was seen. Voltammograms were monitored on a digital storage oscilloscope (Nicolet Explorer II), and could be stored on disk. A sample and hold detector set at the dopamine oxidation peak, was used to plot out the dopamine current during electrical stimulation of the MFB.

The substance released was confirmed as being dopamine by means of in vitro calibrations of the electrode using standards (dopamine, DOPAC and ascorbic acid) , and by pharmacological manipulations.

Armstrong-James,M.A et al (1981) J.Neurosci.Methods 4 385-406
Pellegrino,L.J et al (1979) Appleton-Century-Crofts, New York
Stamford,J.A et al (1984) Neurosci.Lett. 51 133-138

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AN AUTOMATED BIOASSAY SYSTEM USING AN APPLE IIE MICROCOMPUTER

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The earliest automated bioassay systems used standard components from automatic telephone exchanges (Schild, 1946). More recently microcomputers have been introduced and these allow more precise and varied control and also data collection.

The system described uses one Apple IIE per organ bath. Either parallel line assays or assays based on full dose response curves (either sequential or cumulative) can be performed. The tissue response is displayed continually on the monitor and the positions at which readings are taken displayed (Baseline, response). An Imagewriter is used to output, continuously or periodically, the tissue responses (thus potentially replacing a flat bed recorder). At the end of the assay a Table is produced describing the assay and the readings made throughout the experiment.

The system allows varying degrees of control. In manual mode the agonist is added by hand at the indicated times. In semi-automatic mode the agonist is added from preloaded syringe pumps (Braun Perfusor VI), the sequence of doses being repeated until the operator intervenes. Automatic mode allows, for instance, when the tissue has settled down, for an antagonist to be added automatically and the onset of antagonism followed. The wash and agonist doses can either be added at preset times or their addition can be determined from the tissue response.

The data obtained in parallel line assays can be analysed using a program on a DEC VAX computer, the data being entered manually (Else, Roberts et al, 1984). Alternatively logistic curves can be fitted to full dose response curves using De Lean et al's (1978) ALLFIT program modified to run on the Apple IIE (Davies & Roberts, 1984). The data can either be entered by hand or from the results file.

De Lean, A. P. et al (1978). Am. J. Physiol. 235, E97 - 102
Davies, P. & Roberts, F. (1984). This meeting
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Schild H. O. (1946), Br. J. Pharmacol. 1, 135 - 138

A GENSTAT PROGRAM TO CALCULATE DOSE RATIOS FROM PARALLEL LINE ASSAYS AND/OR PA_2 VALUES FROM A SCHILD PLOT

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The calculations required to estimate a potency ratio or dose ratio from a parallel line assay are described by Finney (1978). A program has been written to do these calculations using GENSTAT, a widely available programmable statistical package. The program is run on a DEC VAX computer and the data is input from a menu. A hardcopy print of the entered data, the calculated values and the analysis of variance is obtained. The residuals are examined visually and plots can be obtained on the DEC printer. A plot of the data points and the fitted dose response lines can be obtained on the printer or a CALCOMP 81 plotter, if better definition is required.

If the dose ratios produced by a range of concentrations of an antagonist have been estimated a pA2 value can then be calculated using the method of Arunlakshana & Schild (1959). A hardcopy of the entered data and calculated pA2 value is produced. The confidence interval of the slope is also calculated. A hardcopy of the data points and best fit line, plotting log (Dose Ratio -1) against log (B), where B is the concentration of antagonist, can either be output on the DEC printer or on a CALCOMP 81 plotter.

The calculated pA2 value can be compared with values of -log(K), where K is the apparent dissociation constant. These values are calculated, as described for instance by Barlow et al (1967), from the formula:

$$(\text{Dose Ratio} - 1) = B / K$$

This equation is sometimes called the Schild Equation. The values of -log(K) are analogous to a pA2 value calculated if the slope of the Schild Plot is fixed equal to 1, as it might be expected to be if the antagonist is competitive.

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Barlow, R. B. et al (1967) Br. J. Pharmacol. 31, 188 - 196
Finney, D. J. Statistical Method in Biological Assay, Charles Griffin & Company Ltd., 1978

USE OF MICROPROCESSOR TECHNOLOGY IN A TEST BATTERY FOR PSYCHOPHARMACOLOGICAL VOLUNTEER STUDIES

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Computers and microprocessors are increasingly being used to present, record and analyse psychometric tests in human subjects. Current systems often have major drawbacks, for example: they perform a limited number or range of tests; amendment of the tests or statistical analyses is difficult; they require subjects to learn how to use a QWERTY keyboard; and they use expensive hardware. In an attempt to overcome many of these problems we have developed a system based on CUBE microprocessor units (Control Universal), combined with simple, inexpensive hardware, which are interfaced to an Acorn Atom microcomputer for data analysis and permanent storage.

Software for test presentation located in EPROM in the CUBE system, automatically consecutively presents the following tests:

1. Estimation of a 1 min period of elapsing time.
2. 16 different bipolar visual analogue scales, as developed by Bond & Lader (1974).
3. Two-choice visual reaction time, with an auditory cue at 2 different pitches, each pitch associated with the response button 80% of the time. A total of 100 stimuli are presented in a pseudorandom sequence with 50 responses on each button. Release and movement time are recorded separately.
4. Continuous performance, involving the pseudorandom presentation of 5 different letters of the alphabet in both upper and lower case (ie A B D E H) at 1 per sec on display for 0.1 sec. The subjects are required to respond to consecutive presentations of the same letter, irrespective of case. 500 presentations are made, with 20 repetitions of the same letter in the same case and 20 same letter different case.
5. Flash fusion threshold, based on the method of Venables (1963).
6. Timed motor manipulative task with small glass spheres.
7. Morse key tapping rate over 1 min.

Presentation of the test instructions and most of the tests is via a medium resolution monochrome monitor. A light emitting diode mounted at the end of a sealed oscilloscope viewing hood provides the light source for the flash fusion apparatus. All responses for all tests are recorded on the CUBE system solely via one box with 3 different coloured buttons. Light emitting diodes for the choice reaction time are also mounted on this box.

All data recorded during each session are temporarily stored on CUMEM (battery backed memory) and on command transferred to an Acorn Atom for permanent storage on a floppy disc for each individual subject. Data analysis and printout can be performed at a later date by the Atom.

We are currently operating 4 CUBE systems to the one Atom although expansion of the number of CUBES is theoretically infinite. This system is now being used in psychopharmacological studies in volunteers (McClelland & Raptopoulos 1984). Thus a microprocessor controlled system has been produced which is simple to use, relatively inexpensive, versatile and expandable and therefore well suited to use in the assessment of psychoactive drugs in normal subjects.

Bond, A. & Lader, M. (1974) Br. J. Med. Psychol., 47, 211-218.
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Venables, P.H. (1963) Arch. Gen. Psychiat., 9, 72-78.