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RUSSELL CHURCH, *Brown University, Presider*

On-line brain stimulation: Measurement of threshold of reinforcement

GERALDINE CASSENS, CLIFFORD SHAW, KATHRYN EIKE DUDDING, and A. WILLIAM MILLS
Tufts University, Medford, Massachusetts 02155

This program and interface for a small computer (PDP 8/e) performs the following operations: generates and delivers constant current stimulation to the brain of an animal, monitors electrical resistance at the stimulating electrode, and calculates the threshold of reinforcement based upon the frequency of characteristic pauses in the animal's responding.

Our research deals with the reinforcing properties of electrical stimulation of the brain. This on-line program and interface were devised to: (1) provide a versatile system for studies involving brain stimulation and monitoring, (2) provide instantaneous information about changes in electrical resistance in an animal's brain, and (3) measure threshold current for which an animal will reliably press a lever to stimulate his own brain, i.e., a threshold of intracranial reinforcement (ICR).

Previous methods for measuring a threshold of ICR are time consuming and open to ambiguous interpretation (Valenstein, 1964). One major defect with previous methods is that below a critical intensity of the electrical stimulus, the animal's rate of response falters, eventually extinguishes, and the experimenter loses control of the animal's behavior. Another difficulty is that methods utilizing rate of response fail to discriminate between the effects of an experimental treatment, e.g., a psychoactive drug, on motor response vs. its effect on threshold of reinforcement.

The procedure developed in this laboratory (Cassens & Mills, 1973; Huston & Mills, 1971) obtains a threshold that is independent of wide variations in the animal's rate of barpressing and maintains responding over the entire range of electrical stimuli presented. The animal is trained to respond on two concurrent schedules of self-stimulation. Reinforcement on one schedule simply maintains the animal's behavior throughout the experiment, while the other schedule is used to measure the reinforcing effects of a second stimulus. A fixed-ratio (FR) schedule is used to maintain behavior; the animal is reinforced for every *n*th response with an intracranial stimulus of constant intensity. A continuous reinforcement (CRF) schedule, one in which the animal is reinforced for every response, is superimposed on the FR schedule, and its intensity is varied to study changes in the animal's behavior. As the intensity of the CRF stimulation on the concurrent CRF-FR schedule is decreased, pauses in barpressing behavior begin to appear after each FR reinforcement. These are postreinforcement pauses (PRPs) and are characteristic of responding under an FR schedule (Ferster & Skinner, 1957) (see Figure 1). We define a pause in responding as an interresponse interval (IRI) 3 or more standard deviations above the mean IRI at a given current level.[1] As the intensity of the CRF stimulation is increased, PRPs begin to disappear. When the CRF stimulation is varied in descending and ascending series, keeping the FR reinforcement constant, PRPs reliably appear and disappear as a function of current level of the CRF stimulation. Thus, there is a current level at which control of the animal's responding switches from the FR schedule to the CRF-FR schedule as shown in Figure 1.

Once the animal is thoroughly trained to respond on a CRF-FR schedule, this method allows one to introduce, vary, or withdraw CRF stimulation without disrupting the sequence of responding typical of behavior on an FR schedule. This stability of response is especially desirable in studies in which drugs may alter response rate with or without affecting the threshold of intracranial reinforcement.

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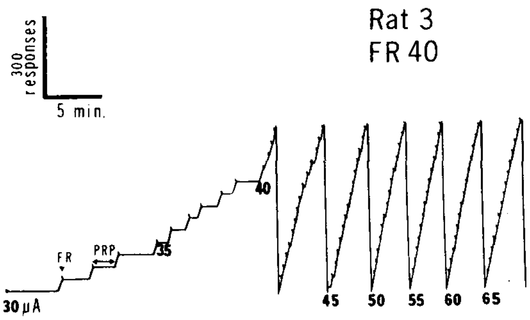

Figure 1. Cumulative record for a rat on a concurrent CRF/FR schedule. This rat received FR reinforcements of 120 microA on a schedule of 40:1. Changes in the CRF intensity are indicated along the base of the curve and are expressed in microamperes base to peak. The postreinforcement pauses (PRPs) are long and consistent when the CRF stimulation is low, such as 30 or 35 microA, but disappear when the CRF intensity is raised to 60 or 65 microA. When the PRPs disappear, the behavior is identical to that of a subject on a simple CRF schedule of equal reinforcements.

The threshold of intracranial reinforcement reported here is based upon the relative frequency of PRPs. As the current level of the CRF stimulation is systematically increased, the proportion of FR reinforcements that are followed by PRPs decreases. The number of PRPs per FR reinforcement (PRP/FR) is calculated at each current level. A least-squares regression analysis is applied to the resulting proportions as a function of current level to determine the best fitting line for the data. The threshold of ICR is derived from the computed best fitting line and is the current at which the proportion of PRP/FR is .5.

Manual control of this procedure was tedious, requiring continuous manipulation and observation by the experimenter. Consequently, the procedure was automated. An important consideration in our software development was to maximize the options for the experimenter for changing stimulus parameters and presentation. The computerized system performs the following operations: (1) sets the intensity of stimulation according to one or more reinforcement schedules determined by the experimenter, specifically a CRF schedule, any FR schedule, and/or a concurrent CRF-FR schedule; (2) accepts parameters specified by the experimenter concerning the range of current levels to be sampled, the size of the increments within the range, and sets the requisite level of current; (3) generates trains of 100-Hz bipolar square waves of .2 sec duration. (This duration can be varied.); (4) determines the voltage drop across the stimulating electrodes, computes, and prints out the electrical resistance of the animal's brain; (5) turns stimulation off and rings a bell if the resistance level is abnormally high or low (indicating an open or short circuit); (6) times the intervals between each of the animal's responses, and calculates the mean and standard deviation of the IRIs for each current level; (7) determines whether a pause has occurred, whether it is a postreinforcement pause (i.e., has occurred immediately after the FR reinforcement and is greater than 3 standard deviations above the mean IRI) and determines the ratio of PRPs per FR reinforcement at each current level; (8) After the program has sampled the predetermined number of current levels in a method of limits fashion, an equation for the best fitting line is determined by least-squares regression analysis utilizing the PRP/FR ratio as a function of current level. Finally, the program computes the threshold of ICR based upon the current level at which the proportion of PRP/FR is .5.

HARDWARE

The basic hardware consists of a Digital Equipment Corporation PDP 8/e. The program uses the following peripherals: an ASR-33 Teletype with low speed paper tape reader and punch; a 10-bit A/D converter (AD8/EA); a 10-bit point-plot display control (VC8/E) with VR 14 scope; a programmable real-time clock (DK8/ES); a 12-channel buffered digital I/O (DR8/EA); a DECtape system (TD8/EA); a 24-bit extended arithmetic element (KE8/E); and an additional 4,096 words of core memory. Neither the scope nor the DECtape units are essential to the functioning of the program.

The interface equipment between the computer and animal utilizes a digitally controlled constant current stimulator which: (a) converts the digital output from the computer to an analog signal to the animal via a D/A converter; (b) varies the voltage as a function of resistance to keep the current constant, using a modification of a design by Wayner, Peterson, and Florczyk (1972). The circuit holds current constant for loads of less than 2.25 mW; (c) allows simultaneous monitoring of current and voltage through the use of independent operational and FET instrumentational amplifiers.

The circuit diagram for this interface is shown in Figure 2, and the components for the circuit are listed in Table 1.

Behavioral Equipment

The experimental apparatus consists of a rectangular box 10 x 16 x 12 in. with Plexiglas top and sides, containing a Gerbrands lever. A closed-circuit television provides continuous observation of the animal.

SOFTWARE

The program software was written in PAL 8 and includes DEC's 23-bit extended arithmetic element floating point package (EAEFPP). The program fills approximately 3,000 memory locations and requires an additional 4,000-word buffer for data storage which permits asynchronous output of the experimental data. A brief description of the program flowchart (Figure 3) follows:

Program parameters are initially input by the

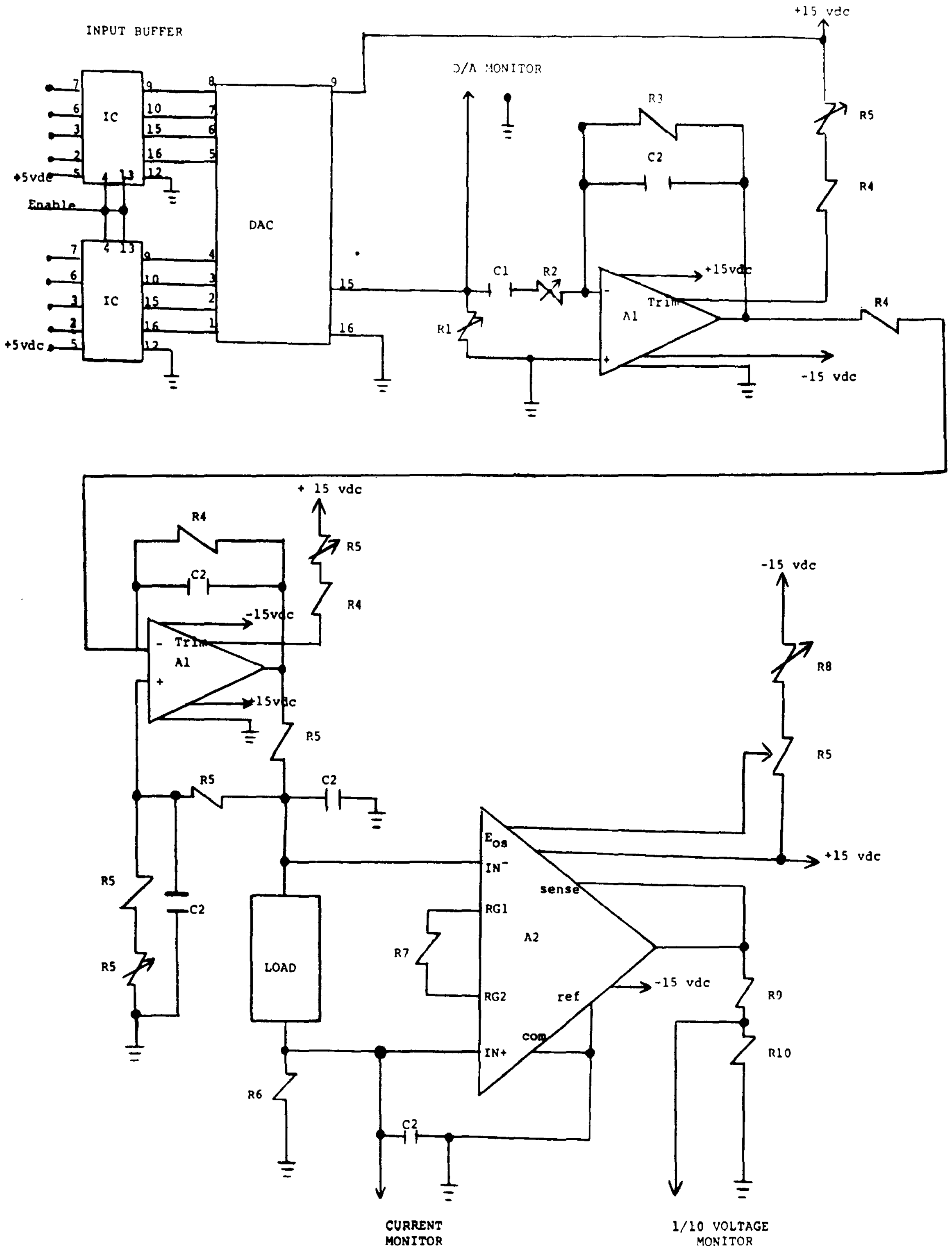

Figure 2. Circuit diagram of D/A constant current stimulator. Components are listed in Table 1.

experimenter from the Teletype (TTY) keyboard, including stimulus range, amplitudes, and ratio schedules. The actual experimental run begins following an initial barpress by the animal, or by the experimenter (as a priming stimulus) from the TTY.

Central to the program is a continuous loop which services the various peripherals: the A/D converter, the scope, the digital output and input device, the clock, and the TTY. An important portion of the loop is devoted to generation of the stimulus and to real-time monitoring of the voltage return from the intracranial electrodes. The program monitors voltage return via the A/D converter concurrently with stimulus generation. Approximately 1,000 voltage samples are read from the A/D converter, output to the scope, and summed during the stimulus train. This summing is accomplished by using a 24-bit arithmetic register rather than the relatively slower floating-point pseudoinstructions.

At the conclusion of the stimulus output, the mean A/D voltage and the current are converted to a resistance measurement (RESIST). When the stimulus is not being generated, the momentary voltage is monitored. Any resistance or voltage out of the limits set by the experimenter triggers an alarm which interrupts the program, prints out the reason for the interrupt, sets all stimulus output values to zero, and signals the experimenter by ringing the TTY bell (ALARM). A switch register option permits the experimenter to continue the program from the point of interrupt if the problem has been solved.

The continuous loop also allows output of the experimental data to a 10-character/sec printer. The TTY printer frequently lags behind program data output. However, the animal's frequent pauses offer ample time to output all the stored data to the printer and the paper tape punch.[2] The data can be accessed at a later time from the paper tape.

The final function of the loop is to determine the interresponse interval by reading a 1/10-sec software counter immediately following each barpress. This

Table 1
List of Components for D/A Constant Current Stimulator

A1	Philbrick/Nexus 1005	
A2	Philbrick/Nexus FET 4253	
C1	50 μF	
C2	.001 μF	
DAC	D/A 371-8 Bit, Hybrid Systems Corporation	
IC	Texas Instruments Quad Bistable Latch, SN 7475N	
R1	500Ω	
R2	1K	
R3	12K	1%
R4	20K	1%
R5	10K	
R6	2K	1%
R7	.5M	1%
R8	100K	
R9	900	1%
R10	100	1%

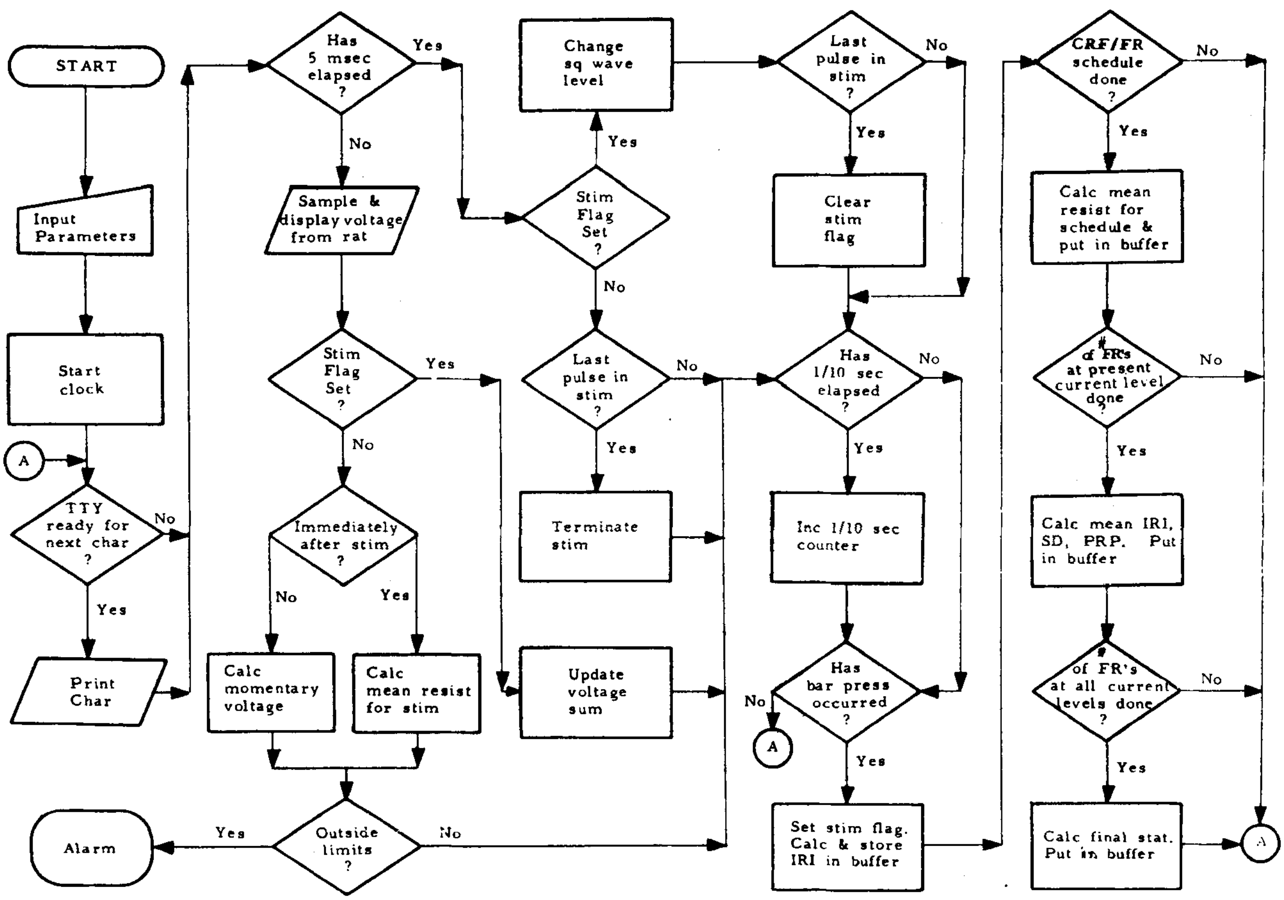

Figure 3. Flowchart of program. See text for discussion.

software counter, which is dependent on the real-time clock, may be easily set for a precision of up to 10 microsec. The accumulated clock counts for a single IRI are stored with previous IRIs as sums of IRI and IRI^2 for later statistical manipulation. The IRIs following each FR are stored individually as potential PRPs.

At the conclusion of the requisite number of FRs at a given current level, the program uses the stored sums of IRI and IRI^2 to determine whether any of the individually stored pauses (potential PRPs) are sufficiently deviant to constitute a PRP (i.e., greater than 3 SD above the mean). The number of PRPs, mean IRI, and standard deviation of the IRIs are output to the software TTY buffer followed by the PRP/FR ratio and

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GRUMPY  THRESHOLD MEASUREMENT AUGUST 23, 1974

FR INTENSITY IN UA = 83
CRF INTENSITY 1 IN UA = 30
CRF INTENSITY 2 IN UA = 48
CRF-FR SCHEDULE = 20
# OF FR'S PER CURRENT LEVEL = 30
# OF STEPS FROM CURRENT 1 TO 2 = 4
# OF DIRECTION CHANGES = 2

BEGINNING OF MAIN RUN:

  90   21   21  239   29   13   13   16   11   10
  12   10   11   14   10   11   11   10   11   10
 A/D=  137 R=   68
  66   16   11   15   13   12   13   33   15   12
  14   25   12   10   10   15   32   11   13   14
 A/D=  137 R=   68
  58   16   11   32   18   10   15   11   14   13
  12   13   17   15   10   12   11   12   12   13
 A/D=  137 R=   68
  93   14   14   13   17   67   65   14   14   15
  17   12   13   13   11   11   12   18   16   10
 A/D=  137 R=   69
  99  320   10   35   11   10   13   10   21   14
  13    8   10   24   13   17   10   14   16   13
 A/D=  136 R=   68
  61   12   14   47   13   14   13   12   10   11
  11   15   27   11   12   11   [illegible]   16   11   11
 A/D=  1[illegible] R=   68
  62             14   14                  [illegible]   12
  11                  21                       [illegible]
 A/[illegible]

       21                       59   10
       13   15             13   32   10   16
   D=  137 R=   69
  66   13   12   14   12   12   12   16   13   13
  14   14   16   14    9   14    9   10   10   16
 A/D=  137 R=   68
 154   10   20   10   14   15   11   11   14   11
  12   88   15   12   15   17   11   14   11   41
 A/D=  138 R=   69
 132   14   16   15   18   14   11   12   11   15
  10   36   10   14   10   11   24   10    9    9
 A/D=  138 R=   69
 131   12   12   12   19   17   35   11   17   15
  13   23   11   10   10   13   10   13   10   10
 A/D=  137 R=   69

/ TOTPRP= 28     MEAN IRI= 9      3SD=7
PRP RATIO=  0.933 AT  36.000 UA

R= -0.875

Y= -.055X + 2.652

THRESHOLD= 39.121 UA
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Figure 4. Segment of printout of experimental results. The IRIs are expressed in tenths of a second. R = resistance in K ohms, M= mean IRI, 3 SD = 3 standard deviations above the mean IRI. Input from the experimenter is underlined on the printout.

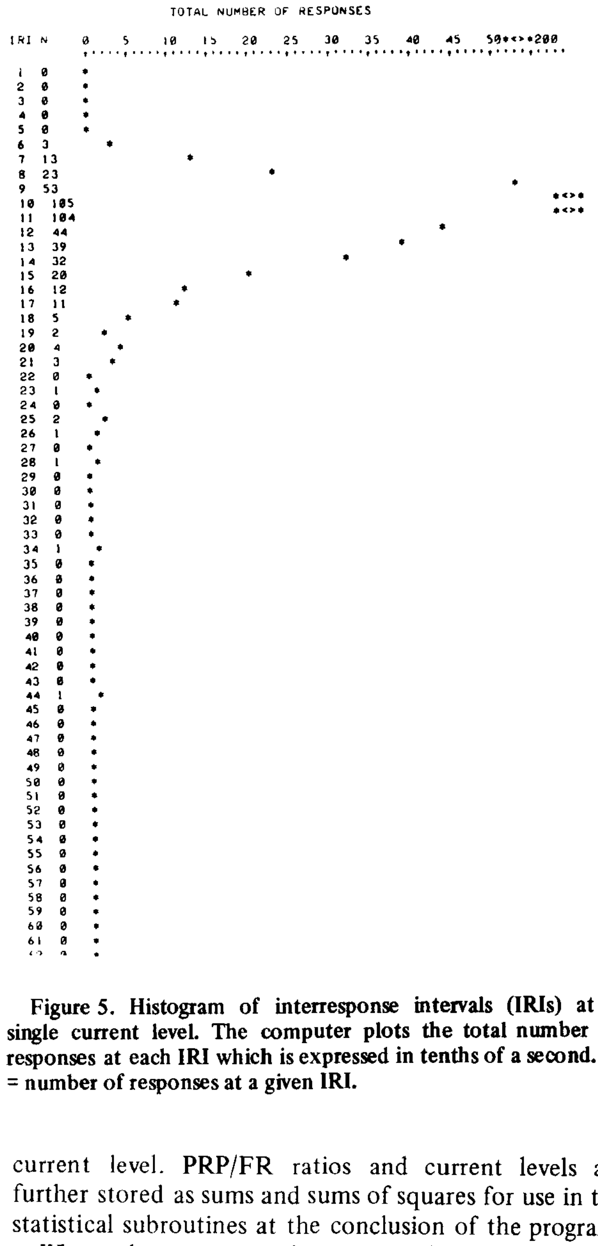

Figure 5. Histogram of interresponse intervals (IRIs) at a single current level. The computer plots the total number of responses at each IRI which is expressed in tenths of a second. N = number of responses at a given IRI.

current level. PRP/FR ratios and current levels are further stored as sums and sums of squares for use in the statistical subroutines at the conclusion of the program.

When the program determines that the required number of FRs have been completed at all current levels, the stored sums and sums of squares are used to calculate the correlation coefficient between current levels and the standard deviates of the PRP/FR ratios. The threshold current is determined from the regression equation and output to the software TTY buffer. A segment of the printout is shown in Figure 4.

At the conclusion of the experiment, all counters are reset to deliver a CRF/FR schedule of indeterminate length at the maximum designated current level. This extended run provides reinforcement until the animal is removed from the experimental chamber.

For the postexperimental analyses, all data are accessible on paper tape.[2] For example, a histogram of the interresponse intervals at a single current level is shown in Figure 5.

EXPERIMENTAL APPLICATIONS

This program and system have been used thus far to measure the thresholds of self-stimulation in the midbrain of rats. The brain site under investigation has been an ascending noradrenergic fiber tract which supports self-stimulation. Our research plans include measuring thresholds of ICR before and after administration of psychoactive drugs and lesions which affect the levels and/or metabolism of endogenous norepinephrine. Our experiences with on-line experimentation have been rewarding. We anticipate further application of this behavioral system to the study of the neural mechanisms underlying reinforcement.

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NOTES

1. A previous publication (Cassens & Mills, 1973) arbitrarily defined a PRP as an interval exceeding 7 sec, because without a computer we were unable to calculate the mean and standard deviation of responses in real time.

2. An additional routine, currently in preparation, will permit direct output of the experimental data in ASCII format to DECtape, while preserving the TTY printout. The ASCII format will be compatible with OS/8 BASIC.