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A microcomputer-controlled system for measuring reactivity in small animals

ROBERT W. SILVERMAN, ANN S. CHANG, and ROGER W. RUSSELL
School of Medicine and the Brain Research Institute
University of California, Los Angeles, California

A microcomputer-controlled system has been designed for measuring reactivity in small laboratory animals. This system uses a microcomputer to trigger an acoustic signal, which is fed to a loudspeaker through an audio power amplifier. An animal's acoustic startle response is recorded using a moving-coil loudspeaker as a movement transducer. The transducer output is coupled to a peak-hold circuit that records the maximum voltage generated by the animal's response. After conversion to digital form, the data for each stimulus presentation are stored and then printed when all trials have been completed.

A microcomputer-controlled system has been developed for measuring reactivity in experiments involving small laboratory animals. Levels of behavioral reactivity of living organisms are often very sensitive to changes in the stimulus environment. Reactivity has been shown to be altered by disease processes and by exposure to chemicals in the external environment. Furthermore, reactivity has been manipulated experimentally by morphological lesions of the brain and by pharmacological intervention in neurochemical events in the central nervous system. We describe below the apparatus that we have developed to measure reactivity.

Instrumentation

The reactivity apparatus uses a microcomputer to trigger the stimulus and to record the intensity of the startle response from 2 subjects simultaneously (see Figure 1). We initially developed the system to work with a Radio Shack TRS-80 Model III microcomputer. However, we later modified the interface and program to work also with an IBM Model XT.

The experiments are performed under dim red illumination. Two tweeter loudspeakers, which present the acoustic stimulus simultaneously to 2 subjects, are mounted over two woofer loudspeakers, which function as the movement transducers. The interface, two peak detectors, and the tone generator are integrated into a single piece of equipment. The microcomputer collects the data from each trial and performs the necessary analysis of the information.

In our experiments we have chosen an acoustic signal for the stimulus, although other sensory modalities can be triggered in place of the tone oscillator. The critical parameters for the acoustic signal have recently been examined by Davis (1984) and by Pilz, Schnitzler, and Menne (1987). The optimal stimulus was found to be a 10-kHz tone with an intensity of greater than 90 dB (Pilz, Schnitzler, & Menne, 1987). In our system, the stimulus consists of a 10-kHz tone oscillator, which is gated *on* for periods of 0.15 sec and fed to a hard-cone tweeter loudspeaker through an audio power amplifier. The tweeter is mounted 42 cm above the nominal position of the rat's head when the animal is situated in the test environment. The sound intensity is adjusted to produce 100 dB measured at the position of the rat's head by a sound-level meter (Quest Electronics Model 215). The environment within which the animal reacts must be essentially isometric to avoid harmonics resulting from the reaction, which could provide undesirable feedback stimuli.

The apparatus is based upon a transducer that was used earlier to analyze tremor (Silverman & Jenden, 1970). The tremor analyzer used a loudspeaker as an electromechanical transducer that provided an electric signal that could be amplified, filtered, rectified, and integrated to provide a continuous minute-to-minute record of tremor amplitude. The use of a phonocartridge with voltage-to-frequency conversion for digital recording took advantage of this approach for isometric recording of the startle response (Cunningham, Crowell, Eaton, & Brown, 1973). In the present system, the animal's response to the stimulus is recorded using a moving-coil loudspeaker with a cone diameter of 30 cm (Quam-Nichols Co. 12C10PAX) as a movement transducer. This particular

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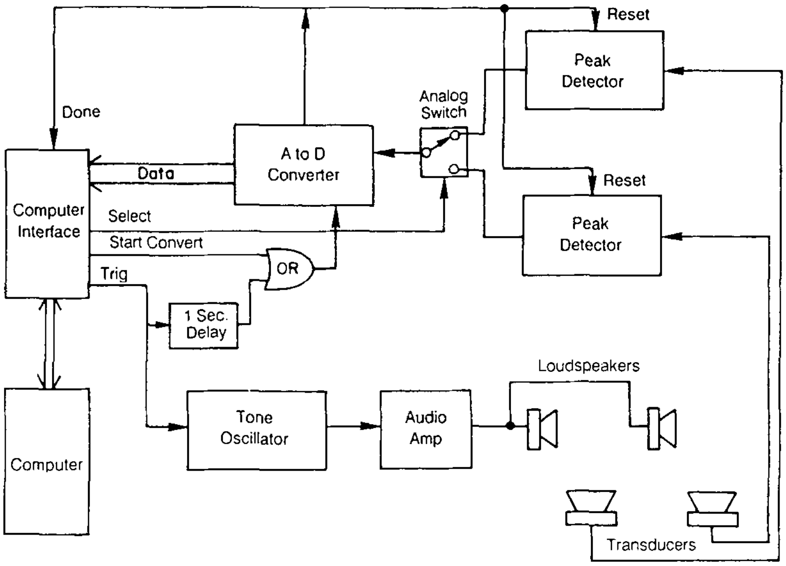

Figure 1. Block diagram of the system for measuring reactivity. One animal is placed on each transducer at the start of each group of trials.

woofer was selected because of the stiffness of its paper cone, which can support the weight of a large rat (about 500 g) and still have sufficient voice coil-movement range to pick up the startle response. The woofer is placed on a table with its cone facing upward on four 12-cm-long legs attached to the rim of the speaker frame. A 3-mm-thick, 25.5-cm-diam acrylic plastic disk rests freely on the upper portion of the cone to serve as the subject platform. A bead of silicone rubber is placed on the periphery of the disk to retain any excretory fluids. The animal's horizontal movements are confined by a 46-cm-high acrylic plastic tube with a wall that is 3 mm thick and 29 cm in outside diameter. The tweeter, which presents the stimulus tone, is mounted in the center of an acrylic plastic disk situated at the top of the plastic tube (see Figure 2).

The response of the system (transducers, peak detectors, and analog-to-digital converter) was measured by dropping various masses of sand contained in small plastic bags from a height of 30 cm onto the disk platform of each transducer. The masses ranged from 0.3 to 60 g and were selected to produce an output signal range similar to that generated by 200–500-g rats. The response of the system was independent of position of impact on the disk. The resulting voltage signal from each of the transducers is linearly dependent on the mass dropped with a correlation coefficient of greater than .99.

The transducer output is coupled to a peak-hold circuit (see Figure 3) that records the maximum voltage generated from the animal's downward pressure on the platform as a response to the stimulus. After the presentation of the acoustic stimulus, a 1-msec reset pulse is applied to field-effect transistors Q1 and Q2, and a delay of nominally 1 sec, which allows ample time for the

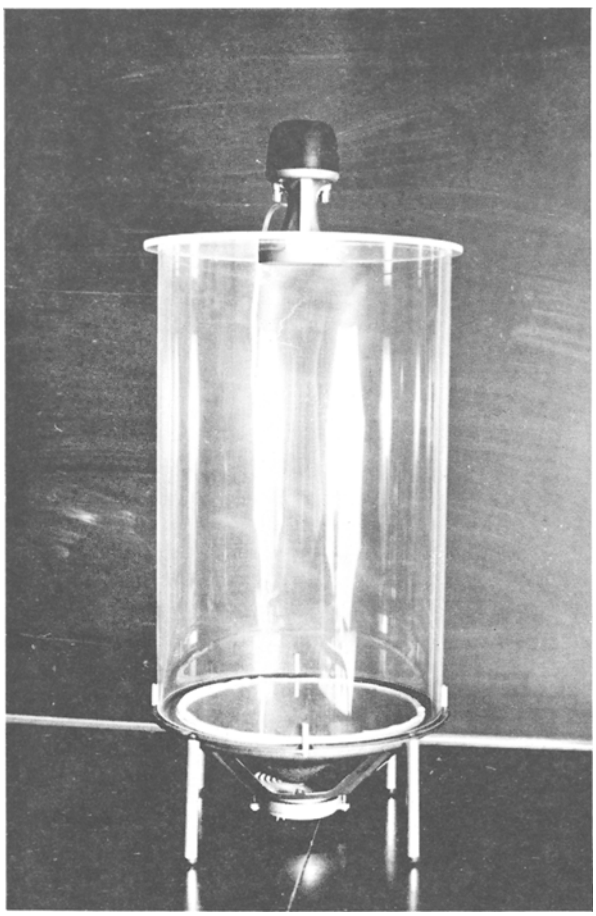

Figure 2. Photograph of one of two reactivity monitor transducers fabricated from two woofers.

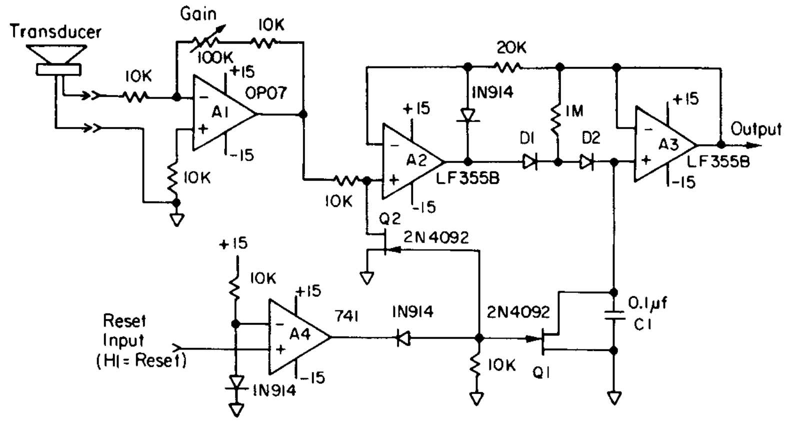

Figure 3. Schematic diagram of one of two identical peak-hold circuits coupled to the transducer outputs to record the maximum voltage generated by an animal's reaction to the acoustic signal. All resistors are expressed in ohms. D1 and D2 are Type IN459A.

animals to respond, is initiated. The signal, which is of the order of a 10^{-2}-V peak, is amplified by amplifier A1 and passes through diodes D1 and D2 to charge capacitor C1 to the most positive signal level. This value is read by buffer amplifier A3. To achieve satisfactory linearity and stability, unit voltage-gain negative feedback is provided around amplifiers A2 and A3. The peak detector is reset by discharging capacitor C1 with the field-effect transistor Q1, which is driven by amplifier A4, and prepared to record the next event. Transistor Q2 is used to shunt any input signal present during a reset cycle. The response from the first channel, which is recorded in one peak-detector circuit, is converted into digital form using an 8-bit analog-to-digital converter (Analog Devices AD673JD). After the conversion, a flag bit is set to indicate to the computer program that data are ready. After the data from the first peak detector are read, the computer outputs a command to an analog switch (Siliconix DG301BP), which connects the input of the analog-to-digital converter to the second peak-detector circuit. The computer initiates a second conversion and, after the flag bit is set, reads the data from the second channel. An 8255 interface controller accepts information from the computer's data bus and provides control signals to the electronics. Data returned from the apparatus are coupled to the computer bus through the same 8255 controller. An 8255 controller-based input/output board to provide these functions for the IBM microcomputer can be purchased from numerous manufacturers (e.g., Industrial Computer Source, San Diego, California, Model PC-DI024) or can be constructed by the investigator (see Sargent & Shoemaker, 1984).

Software

A BASIC program controls each experimental run on the reactivity apparatus. A run consists of up to 20 trials. In each trial, the program waits for the intertrial interval, triggers the acoustic stimulus, and then reads and records the startle response. The first step in the program is to initialize the 8255 interface controller. Next, the program checks the number of animals to be tested (1 or 2) and prompts the user to enter the weight, subject number, and identification label for each animal. Then, the computer prompts the user to enter the number of trials in the run and to indicate whether the intertrial intervals will be of fixed or random duration. If fixed intertrial intervals are specified, the interval length must also be entered.

When random intertrial intervals are used, they are generated so that the interval lengths are orthogonal (statistically independent) to the trial numbers scaled with mean zero. Thus, after an experimental run, startle response can be regressed on trial number (to get a measure of habituation) and on intertrial interval (to see if interval and response are related) without concern for joint effects of trial number and interval length on the response. Note that, because the time from the last trial is unknown, the interval before the first trial is for all practical purposes undefined. In the program, this first interval will be set to some arbitrary value and the random values will be used starting with Trial 2. To get the desired random intervals, random numbers between 0 and 1 are regressed against the scaled trial numbers. The residuals of this regression form a set of numbers orthogonal to the scaled trial numbers, which are scaled to produce intertrial intervals with a mean length of 30 sec and a range of 10 to 50 sec.

After waiting for the user to signal that the equipment and animals are ready, the program enters a loop to collect data for each trial. After the intertrial interval, the program triggers the acoustic stimulus, waits until the data-ready flag bit is set by the analog-to-digital converter, and then reads the response for the first animal. If there is a second animal, the program reads its response after the data-ready flag is set. The responses are then displayed on the video monitor. The data-collection loop is exited after all the trials have been completed.

When the run is over, statistics are computed and results are printed. The startle response amplitude is regressed on trial number and, for random intertrial intervals, on interval duration (starting with Trial 2). The slope of the regression line of response on trial number gives an indication of how the response changes during the course of the experiment and thus is of interest as a possible measure of habituation. For each animal, the program produces a separate printout containing the following information (see Figure 4): weight, identification label, number of trials, average response and its standard error, slope and standard error of the regression of response on trial number, slope and standard error of the linear regression of response on interval if random intertrial intervals were used, standard error for the average response, taking into account the error from the effect of the response on trial number (and interval, for random intervals), and finally, a table of response and interval length for each trial. After the printout is finished, the program is ready for the next experimental run.

Figure 5 is a plot of the data derived from the program printout, which shows a rapid decrease from the level of reactivity in Trial 1 until (with the single exception of Trial 13) an asymptote was reached at a mean amplitude of 2.35±0.353 bits (1 bit ≅ .04 V). Such trends in behavior during repetition of the same stimulus conditions have been labeled *habituation* and described as "primitive forms of learning" (Peeke & Petrinovich, 1984). The present system enables investigation of intrasession as well as intersession habituation, the former requiring multiple trials within one experimental session and the latter requiring multiple trials within repeated sessions.

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REACTIVITY        12/11/87 01:09:56

Subject #:  4
     Weight (grams):  485
     ID label       : CON2

     Number of trials:  20
     Average response:  4.5
          S.E.:              1.29878
     Regression of response on trial #
          Slope = -.338346
          S.E.  =  .220502
     Regression of response on interval   (Trial #1 omitted)
          Slope =  .0749575
          S.E.  =  .109294
     S.E. for response after regression:  1.27148

     Trial #      Response      Interval
       1            23             7.0
       2            10            25.2
       3             1            30.7
       4             2            12.5
       5             0            32.8
       6             3            24.5
       7             3            43.8
       8             5            47.1
       9             4            47.1
      10             1            16.7
      11             4            50.0
      12             3            20.2
      13            17            33.4
      14             2            22.7
      15             2            13.0
      16             2            26.2
      17             3            29.2
      18             0            14.9
      19             1            33.4
      20             4            46.8
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Figure 4. Reproduction of a printout of measures of reactivity in a control subject. Responses are measured in bits. Intertrial intervals are measured in seconds.

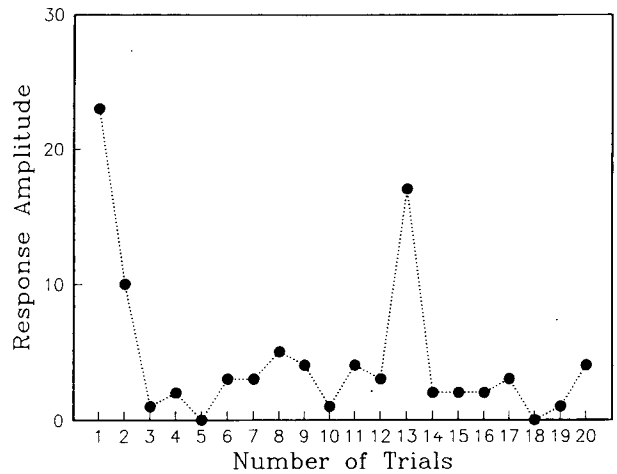

Figure 5. Graph showing reactivity to acoustic stimulation. Circles show response amplitudes on each of 20 trials for control animals. Response amplitudes are measured in bits.

Availability. A copy of the computer program is available for the IBM or Radio Shack microcomputer by sending a formatted diskette to the authors.

REFERENCES

Cunningham, C., Crowell, C. R., Eaton, N. K., & Brown, J. S. (1973). A digital system for recording startle responses in small animals. *Behavior Research Methods & Instrumentation*, **5**, 1-3.

Davis, M. (1984). The mammalian startle response. In R. C. Eaton (Ed.), *Neural mechanisms of startle behavior* (pp. 287-351). New York: Plenum Press.

Peeke, H. V. S., & Petrinovich, L. (1984). *Habituation, sensitization and behavior*. New York: Academic Press.

Pilz, P. K. D., Schnitzler, H.-U., & Menne, D. (1987). Acoustic startle threshold of the albino rat (*Rattus norvegicus*). *Journal of Comparative Psychology*, **101**, 67-72.

Sargent, M., III, & Shoemaker, R. L. (1984). *The IBM Personal Computer from the inside out*. Reading, MA: Addison-Wesley.

Silverman, R. W., & Jenden, D. J. (1970). Tremor analyzer for small laboratory animals. *Journal of Applied Physiology*, **28**, 513-514.

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