Apple II/FIRST system control of electrical brain stimulation in the rabbit

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An Apple II/FIRST system has been developed to control classical conditioning experiments, collect analog data, and extract dependent variable measures of conditioning from uniphasic (Scandrett & Gormezano, 1980) and multiphasic (Johnson, 1981) response signals. The present paper details the development of an electrical brain stimulation system as a peripheral device to the Apple II/FIRST system. In addition, data are presented that document the capabilities of the EBS system to concurrently stimulate up to eight animals while values along the dimension of each of four stimulation parameters are manipulated by a program written in FIRST. In our judgment, the EBS system has a number of advantages over currently available commercial stimulators, including cost, number of sites that can be independently stimulated, and availability of complete software control.

The purpose of the present paper is to report our development of an electrical brain stimulation (EBS) system that functions as a peripheral device to an Apple II/FIRST system interfaced to a classical conditioning laboratory (Scandrett & Gormezano, 1980). In addition, we provide an illustrative set of data for the purpose of documenting the potential of the EBS system to: (1) map the anatomical pathways of the rabbit nictitating membrane reflex (NMR), and (2) determine those portions of the NMR pathway at which stimulation may serve as a conditioned stimulus (CS) or an unconditioned stimulus (UCS).

The Apple II/FIRST system of which the EBS system is a peripheral device has been described in detail by Scandrett and Gormezano (1980). Briefly, the Apple II/FIRST system has been independently interfaced to each of our three conditioning laboratories. More specifically, each laboratory consists of an Apple II with 64 KB of memory, a floating-point processor, a video monitor, two programmable peripheral interface chips, each providing a 24-bit digital I/O buffer, an 8253 programmable counter/timer providing a real-time clock, and a 16-channel 8-bit A/D converter yielding 16 A/D counts for each millimeter of nictitating membrane extension. The software system, FIRST, resident in 13,800 bytes in the Apple II computer, is an expandable dictionary-based language that is tailored for the most efficient use of the 64-KB Apple II system to provide great flexibility and very high processing speed. The set of available (defined) words in the system is stored in a dictionary resident in memory. The word

definitions composing a particular program involve creating, in effect, the most efficient new language that allows the Apple II microcomputer to carry out the control and computational elements of the program.

The experiments in each of our conditioning laboratories have been run under a generalized classical conditioning program, written in FIRST, that has provisions for the presentation of CSs and UCSs, with the onset and offset times specified as a variable. Typically, a tone, a light, or both have been used as CSs; an air puff to the cornea or paraorbital shock have been used as UCSs. The analog response data for all experiments are routinely logged on a 20-MB hard-disk system (Corvus) after on-line processing by the generalized classical conditioning program. The timing of stimuli and the multiplexed A/D conversion of NMR signals are implemented by a programmable timer that contains three independent 16-bit pulse counters. Stimulus control is carried out by storing or writing output control bits into programmable peripheral interfaces. As an addition to our stimulus control system, we developed a device peripheral to the Apple II/FIRST system for the delivery of constant-current EBS.

Our development of the EBS system was primarily for the purpose of examining the neural basis of learning with the rabbit NMR preparation. Although there are a number of physiological techniques that have been used to study the neural processes of learning (e.g., histochemical labeling, autoradiography), for our initial efforts, directed at delineating the neural pathways of the rabbit NMR, we selected EBS. The use of EBS permits the delivery of a discrete, well defined stimulus to a localizable group of cells in an intact, behaving animal. Furthermore, use of the rabbit NMR preparation offers the benefits of a well understood behavioral response system in which a number of neural pathways, including the final common pathway, have been detailed

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(Cegavske, Thompson, Patterson, & Gormezano, 1976; Gray, McMaster, Harvey, & Gormezano, 1981). Accordingly, EBS can be used to activate identifiable groups of cell bodies along the anatomical pathways of the rabbit NMR and, thus, has the potential to determine whether such stimulation is sufficient to: (1) elicit the NMR and (2) serve as UCS to support conditioning of the NMR. Accordingly, by using EBS we are in a position to more precisely determine the UCS-UCR pathway of the rabbit's NMR. Moreover, by employing EBS as a UCS, we can identify specific neural structures that are involved in conditioning.

In order to determine the characteristics of EBS that would best serve as a UCS, we wanted to delineate the relationship between the parameters of stimulation and the topographical characteristics of the reflex (e.g., latency, amplitude, area). In particular, we wanted to permute the values of as many as four different parameters (pulse amplitude, width, frequency, and train duration) and present them in a randomized sequence over a short interstimulus interval to a large number of subjects. Of course, such sequences could be presented manually but would be tedious and potentially error prone; the two problems, however, could be overcome by computer control. In addition to the problems associated with manual stimulus presentation, in a typical EBS experiment, variations in the placement of electrodes from the designated site make the accumulation of data inherently inefficient. Consequently, the sample sizes typically generated by EBS experiments are small and the resultant data bases lack robustness. In the context of the inherent less than perfect hit rate of experiments employing stereotaxic coordinates, the use of multiple subjects maximizes the ability to generate a robust data base. Accordingly, we designed and constructed a 16-channel EBS system as a peripheral device to one of our Apple II/FIRST systems in order that the parameters of pulse width, pulse amplitude, interpulse interval, and pulse train duration could be independently programmed and simultaneously presented to eight animals.

THE EBS SYSTEM

For expository purposes, the EBS system, under control of a program written in FIRST, can be broken down into three functional components: (1) control, (2) timing, and (3) isolation and constant current. The FIRST program is responsible for the selection and operation of components of the EBS system (e.g., timers, D/A converters) that control the timing and amplitude of the individual pulses of electrical stimulation. The control component is a buffered device for generating chip-select, data, and address signals to each of up to 16 individual pulse-generating units. The timing component is composed of eight programmable timer chips (MC6840), each of which can determine the duration of two timing pulses. Further, the isolation component receives the timing component-generated wave-

form and, via a D/A converter (AM6080), receives computer-controlled specification of pulse amplitude. The isolation component then produces either a monophasic or biphasic pulse train that is transmitted to the constant-current component. To minimize signal distortion, the constant-current component is mounted 10 mm above the skull of the animal. Specifically, the animal has an electrode assembly cemented to the skull upon which a Plexiglas platform can be attached. The Plexiglas platform, in turn, serves to hold both the constant-current component and an NMR movement transducer.

Programming

In order to operate the EBS system, a program was written in FIRST that would initialize and load binary values for pulse timing and pulse amplitude into the timer counters and D/A converters, respectively. Pulse timing was accomplished by repeated operation of the timers and D/A converters within a timing loop. To control the interpulse interval (frequency) and duration of the pulse train, an appropriate delay was included in the timing loop and the loop was then repeated a sufficient number of times to provide the required pulse train duration. A separate timing loop for each of the 16 potential stimulation sites, together with the separately addressable D/A converters and timers, allowed for independent control of the pulse characteristics at each site.

Control

Panels a and b of Figure 1 are block diagrams of the control and timing components, respectively. An inspection of Panel a shows that a program-generated signal from the Apple II computer, sent along the address lines to a 4-to-16 address decoder (74LS154), permits selection of any one of 16 pulse amplitude-controlling D/A converters. Panel a also shows that all the address and data lines to the EBS system are buffered and that all data (i.e., timing signals and pulse amplitude values) are sent via the data bus to the timing and isolation components. In fact, because the Apple II/FIRST program permits individual programmable timers (MC6840) and D/A converters to be selected, whatever data are on the data bus will be loaded into the selected timer and converter. Consequently, with each chip-

Figure 1. Block diagram of the control and timing components.

select signal, different data can be loaded onto the data bus and be sent to whichever device is selected.

Timing

Inspection of Panel b of Figure 1 shows that the timing component of the EBS system is based on eight independently programmable timers (MC6840P). Each timer consists of three 16-bit binary counters that, together with gating logic, are capable of generating two timing signals (e.g., TIM1, TIM1). Timing of the EBS pulses originates from a 1-MHz clock on the Apple II (θ_0) that is fed into a divide-by-10 counter (CD4017) and subsequently split into three clock outputs (CLK0, CLK1, CLK2) responsible for the rate at which three counters on the programmable timer are decremented. In turn, the three counters produce either a single square wave or a continuous square wave, depending upon the program-generated timing values loaded into the timer. Furthermore, the duration of a single or continuous square wave is also determined by program-generated values supplied to the timer.

Isolation and Constant Current

Figure 2 shows a block diagram of both the isolation and constant-current components of the EBS system. The isolation component serves to electrically isolate the stimulation sites from the rest of the EBS circuitry. Specifically, electrical isolation is accomplished using two optical isolator devices (6N135) that are driven by the D/A converters with current proportional to the value of the pulse amplitude previously loaded into the converters. Activation of the optical isolators is controlled via the D/A converters by the signal outputs of the timing component (e.g., TIM1, TIM1). In particular, during a positive cycle of the timing signal, the first optical isolator is turned on while the second is turned off; during the negative cycle, the first optical isolator is turned off and the second is turned on. The output of each optical isolator is fed to the inverting and noninverting inputs of an operational amplifier (LM138), producing a biphasic signal that, in turn, is fed into the noninverting input of another operational amplifier (LM318). This final operational amplifier forms the basis

of the constant-current portion of the EBS system. The input to the inverting terminal of the operational amplifier comes from a 100-kohm "sense" resistor that provides feedback of potential variations in impedance at the stimulation site. When there is an impedance change, the resultant variation in voltage across the sense resistor is applied to the inverting terminal of the operational amplifier so that the signal is in opposite phase to the isolation component input voltage. Consequently, the detected impedance changes are followed by the operational amplifier whose output becomes the stimulating current at the electrode site.

RESULTS

Although stimulators are commercially available, they are usually expensive (e.g., Ortec \$2,200), designed to be used for a single stimulation site, and engineered so that a change in stimulation values must be made manually (e.g., 10-turn potentiometer). In contrast, our constant-current EBS system is relatively inexpensive, capable of independently stimulating up to eight animals, and, most important, completely computer controlled. Thus, in terms of cost, we have been able to take advantage of the cost benefits of integrated-circuit technology so that the components of the EBS system can be purchased for about \$500. In addition, the modular nature of the system, with separate control, timing, and isolation components, makes construction and maintenance relatively simple. Moreover, the EBS circuitry has been designed so that 16 independently controlled channels are available. The 16 available channels can be arranged to stimulate 2 brain sites in eight animals or any other combination up to 16 different sites in one animal. Finally, values of stimulus intensity, pulse width, pulse frequency (interpulse interval), and pulse train duration for each site of stimulation are all under software control. Accordingly, once programmed and tested, the system is virtually error free.

To provide an indication of the capabilities of our EBS system, we now present data obtained from eight subjects with electrodes aimed at the sensory nucleus of the trigeminal spinal tract (TRIG). We chose to stimulate TRIG on the basis of anatomical and histochemical evidence that suggested that the trigeminal sensory system receives inputs from the face (Hiroaka & Shimamura, 1977) and then projects to abducens and accessory abducens motoneurons controlling the NMR. Accordingly, stimulation of TRIG was expected to activate abducens and accessory abducens motoneurons that, in turn, would activate the retractor bulbi muscle, resulting in an NMR.

In order to determine the parameters of EBS that would reliably elicit the rabbit NMR, we selected a range of values from each of the parameters provided by our EBS system. In particular, we manipulated pulse amplitude (intensity) (range = 25-250 microA), pulse width (range = .0 msec-length of interpulse interval), pulse

Figure 2. Block diagram of the isolation and constant-current components.

Figure 3. Panel a presents NMR amplitude as a function of intensity and train duration at a .1-msec pulse width and 1.0-msec interpulse interval. Panels b and c present NMR amplitude for intensities of 35 and 70 microA, respectively, as a function of frequency and pulse width.

train duration (range = .0 msec-length of trial), and interpulse interval (range = .0 msec-length of trial). For each NMR elicited by EBS, the Apple II/FIRST system determined a number of dependent variables (e.g., amplitude, area, frequency, peak latency). For present purposes, we restrict our discussion to response amplitude.

Panel a of Figure 3 shows NMR amplitude to be a monotonic increasing function of intensity (25, 35, 50, 60, 70, 80, 90, 100 microA) and pulse train duration (10, 20, 40 msec) under a .1-msec pulse width and 1.0-msec interpulse interval. Moreover, Panels b and c of Figure 3 show, for intensities of 35 and 70 microA, respectively, that at a 20-msec pulse train duration, NMR amplitude is a monotonic increasing function of frequency (250, 500, 1,000 Hz) and pulse width (.1, .2, .4 msec). Accordingly, our results indicate that the NMR varied systematically over an extensive range of each of the manipulated EBS pulse characteristics. Moreover, the fact that the EBS system was effective in eliciting UCRs suggests that, consistent with anatomical evidence (Harvey, Note 1), TRIG may be a part of, or at least have inputs to, the reflex pathway of the rabbit NMR. Moreover, since stimulation of the trigeminal sensory area was capable of eliciting an NMR, the present data suggest that such stimulation may serve as a candidate for a UCS that would support conditioning of the NMR. However, there is still a need to establish the efficacy of electrical stimulation to the trigeminal sensory area as a UCS, and to that end, we have begun investigating the UCS properties of the

TRIG stimulation using parameter values selected on the basis of the present results. In fact, preliminary results indicate that TRIG stimulation produces CR acquisition rates comparable to peripheral shock when peripheral shock and EBS are matched for amplitude and frequency of UCR elicitation.

CONCLUSION

The Apple II/FIRST system was developed to control classical conditioning experiments, collect analog data, and extract dependent variable measures from the response signals (Scandrett & Gormezano, 1980). The addition of a peripheral device for the delivery of electrical brain stimulation constitutes an expansion of the stimulus delivery capabilities of the system. Moreover, the development of the EBS system reported here represents a substantial advance over commercially available EBS devices. Specifically, our constant-current EBS system can be constructed at a fraction of the cost of commercial devices. More important, our EBS system is capable of independently stimulating up to two sites in each of eight animals, whereas commercially available devices are capable of stimulating only one or two sites in a single animal. Finally, the single most outstanding advantage our EBS system has over other available systems is the ability to specify and control all the parameters of stimulation using our Apple II/FIRST system.

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