SESSION XIV GENERAL APPLICATIONS II

Adam Reed, *Presider*

STUDENT COMPETITION AWARD

The analysis of multiphasic signals with the Apple II/FIRST microprocessor system

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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 response signals (Scandrett & Gormezano, 1980). The present paper covers an expansion in the capabilities of the system by the development of a multiphasic signal analysis algorithm written in FIRST, an interactive, high-level, dictionary-based language. The program provides unprecedented efficiency, sensitivity, and flexibility in the analysis of multiphasic analog response signals in classical conditioning experiments. Moreover, the program has the potential to analyze even more complex signal patterns.

In classical conditioning experiments, the subject is presented a pattern of stimulation involving an unconditioned stimulus (UCS), which reliably produces an unconditioned response (UCR), and one or more conditioned stimuli (CS), which are shown not originally to produce the UCR. The CS and UCS are then presented to the organism in a specified order and temporal spacing, and the experimenter examines responding during a specified observation interval. Early in training, only the UCR occurs. However, as seen, for example, from the analog response signal depicted in Figure 1 for the rabbit's nictitating membrane response (NMR), as conditioning progresses a conditioned response (CR) develops to the CS and results in the uniphasic UCR's gradually becoming a multiphasic response with two peaks. Accordingly, in order to analyze the total topography of only the CR, test trials, in which the CS is presented with no reinforcing UCS, are randomly inserted into the conditioning sequence. A program capable of analyzing the multiphasic responses arising from overlapping CR-UCR occurrences on CS-UCS trials would yield a significant increment in the understanding of fundamen-

tal mechanisms of conditioning (see Gormezano & Kehoe, 1981). Moreover, in complex conditioning situations involving compound serial CSs (e.g., Kehoe, Gibbs, Garcia, & Gormezano, 1979) and/or multiple mixed CS-UCS intervals (e.g., Millenson, Kehoe, & Gormezano, 1977), as well as in systems employing responses that are inherently multiphasic (e.g., the jaw movement reflex in rabbits; see Gormezano, 1972), the response signals on test trials (as well as reinforced trials) are typically multiphasic. Hence, a determination of process in these cases is critically dependent upon the ability to extract quantitative measures of the multiphasic response signals. Until now, the Apple II/FIRST signal analysis programs used in the three independent

Figure 1. An oscillograph record taken at a paper speed of 200 mm/sec of CS and UCS occurrence and topography of a nictitating membrane CR merging into a UCR.

The preparation of the manuscript and the software development was supported, in part, by NSF Grant BNS 80-05907, NIMH Grant MH 15773, and NIDA Grant DA 01759. The author is presently a college senior in the Department of Physics, Washington University, St. Louis, Missouri 63130. Requests for reprints should be addressed to I. Gormezano, Department of Psychology, University of Iowa, Iowa City, Iowa 52242.

facilities composing the classical conditioning laboratories at the University of Iowa have been developed to analyze only uniphasic response signals from the 8-12 stations in each laboratory (Scandrett & Gormezano, 1980). Accordingly, the present paper reports the development of a multiphasic signal analysis algorithm written on the Apple II/FIRST system.

THE OPERATING SYSTEM

Apple II/FIRST System

Scandrett and Gormezano (1980) have described in detail the hardware configuration, system capabilities, software (FIRST) and its dictionary-based language structure, and the control hardware and software composing the three Apple II/FIRST systems independently interfaced to each of the three conditioning laboratories. In brief, each laboratory consists of an Apple II with 48K of memory, a floating-point processor, an Apple II disk drive, 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. FIRST is tailored for the most efficient use of a 48K-byte 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 suitable new language to carry out the control and computational elements of the program. Stimulus control is carried out by storing or writing output control bits into programmable peripheral interfaces, and the timing of stimuli and the multiplexed A/D conversion of NMR signals are implemented by a programmable interval timer that contains three independent 16-bit pulse counters.

Classical Conditioning

In classical conditioning of the rabbit's NMR, the experiments are run under a generalized classical conditioning program that has provisions for the presentation of CSs and UCSs, with onset and offset times specified as variables. Typically, a tone, light, or both have been used as CSs; an air puff to the cornea or a paraorbital shock have been used as UCSs. The monitoring of membrane movement is achieved by connecting a small hook to a nylon loop sutured into the rabbit's nictitating membrane. The hook is connected by a thread to the arm of a transducer assembly that provides a linear analog output of membrane extension. The analog response data for all experiments are routinely logged on floppy disks for later signal processing by the generalized classical conditioning program. The program records all sampled A/D values for all subjects and displays the

digitized NMR topographies for each subject, successively, during the intertrial interval of the experiment, along with NMR topography measures extracted by the signal analysis program. Next, the salient response measures are stored in a memory buffer until filled, at which point the data are moved to disk files reserved for data storage. In practice, subsequent to an experimental run, the digitized NMRs are copied from the disk file onto a sequential library disk, which holds all the data for a sequence of runs. This library disk then serves as the input to programs that (1) classify each subject's response as a UCR or CR, (2) perform ANOVAs and other statistical analyses on CR percentages and NMR topography measures extracted by the signal analysis program, and (3) plot and display summaries of the preceding measures.

The Uniphasic Analysis

Analysis of the uniphasic signal by the Apple II/FIRST system has few complexities (Scandrett & Gormezano, 1980). The analog signal is read at a sampling rate (e.g., 4 msec) controlled by computer software. The resulting digitized signal is prepared for computer analysis by finding the baseline through the averaging of the signal level for 200 msec immediately prior to CS onset. A specified root mean square (RMS) value of the baseline is used to discard trials in which a stable baseline is not found. The discarding of trials due to an unstable baseline occurs in about 2% of the trials and is primarily attributable to a spurious (base-rate) membrane extension (Gormezano, 1966). The next step in the analysis is the determination of the peak of the response. The computer searches the data to find the absolute maximum, with the time from CS onset to the response peak referenced as the peak latency. The maximum amplitude of the response (the UCR or, if it occurs, the CR) is determined by finding the A/D level at which the response peak occurs and subtracting from it the A/D level of the baseline. On the other hand, response onset (latency) is determined by finding the first point that attains a criterion deviation from baseline (eight A/D counts or .5 mm of membrane extension) and then following the signal back down to the first point on baseline and determining the time from CS onset. Moreover, the area of the response is calculated by numerically integrating the amplitude of each digitized point in the signal from CS onset to the end of the sampling period. In summary, the uniphasic signal analysis program extracts (1) an RMS estimate of the quality of the baseline signal, (2) CR or UCR latency, (3) CR or UCR amplitude, (4) peak CR or UCR latency, and (5) area under the CR or UCR topographies.

The Multiphasic Analysis

The complexities presented by the need for a multiphasic analysis algorithm in classical conditioning research are far from trivial, since multiphasic responses

include an unlimited spectrum of possible shapes to which the computer must respond with an acceptably low error rate. Prior to the implementation of the present program, the method of scoring multiphasic responses consisted of ruler measurement of the amplitudes of pen deflections recorded on a high-speed (200- to 250-mm/sec) oscillograph recorder. The detection of multiple responses relied on a sequence of amplitude criteria. For example, one sequence of amplitude criteria used to detect double responses consisted of three distinct measurements. Specifically, double response peaks were defined by (1) an initial upward pen deflection in excess of 2 mm above baseline (1 mm of membrane extension), (2) a downward pen deflection in excess of 2 mm, and (3) a final upward pen deflection in excess of 2 mm. After the detection of a double response, it was necessary to make precise ruler measurements of the amplitudes and temporal placements of both responses. Since hand scoring even double responses for a restricted set of ruler measurements was time-consuming, tedious, and error prone, the detailed analysis of more complex response patterns has not been undertaken. Yet, in our judgment, a fundamental understanding of the laws governing conditioning to simple and complex (sequential) stimuli waits upon the ability to routinely analyze multiphasic response patterns.

Number of responses. Figure 2 shows the digitized topography of a multiphasic NMR to a serial CS as it is displayed on the video monitor. In subjecting such a multiphasic signal to analysis, the first determination to be made is the number of responses present in the signal. Originally, the determination of what constitutes a response was approached by examining the slope at each point in the signal. However, the slope method was eventually replaced by a displacement (amplitude) criterion specifying an increase in the signal of eight A/D counts (.5 mm of membrane extension). This was found to be just as sensitive as the slope method to responses with rapid rise times, and it had the additional advantages of being more sensitive to gradual rise times, being simpler to program, and having closer correspondence with scoring methods previously used. On the other hand, the determination of where the response ended retained a slope criterion requiring that the difference in amplitudes of two points spaced five points apart be less than a set value. Determining where a response ends is probably more important than determining the presence of a response, because the end of a response tells the computer to start looking for another response. If the criterion for determining the end of a response is too weak, spurious responses will be introduced into the analysis. If the criterion is too strong, important information will be missing. The slope method, of course, has the advantage of being able to assess the dynamics or motion of the response system. In practice, the computer runs through the data, from the beginning

Figure 2. The digitized topography of a multiphasic signal arising from NMRs to a serial CS. The full-length vertical lines at the far left and right indicate the limits of the observation interval; those in between indicate the first CS onset, the second CS onset, and the UCS onset, respectively. The second horizontal line from the bottom specifies the response baseline. The short vertical lines extending to the signal specify the positions of the response peaks; the short vertical lines extending below the bottom line specify the response onsets. Note the presence of several subcriterion bumps, which, accordingly, fail to count as separate responses.

of the signal to its termination, setting flags wherever the criterion for a response is met.

Amplitudes. Once the number of responses was determined and the approximate positions of the responses were known (through the positions of the flags), the data were ready to be analyzed for the maximum amplitude of each response. However, the task was formidable, because most such multiphasic signals (cf. Figure 2) do not contain one or more "bumps" (shoulders) that stand out as obvious maxima. Rather, the signals typically have several bumps that do not meet the response amplitude criterion (.5 mm of membrane extension). Thus, the question arises as to which response a subcriterion bump belongs to, since it may be considered to be the maximum of one response or simply part of the following response. The last response in a signal is the only one that avoids this ambiguity. The solution to the problem of determining which bump is the local maximum is discussed below. A further complication in the analysis of maxima was the small amount of noise coming from the response system and transducer assembly, which occasionally caused the A/D converter to repeatedly jump between two adjacent A/D levels (e.g., 190-191). The problem was remedied by finding the maximum amplitude of the response and averaging the positions of all points at which the maximum amplitude occurred. The program stopped testing for a maximum when a point was found whose amplitude was a set number of A/D counts below the maximum amplitude. As judged by investigators experienced in ruler measurement ($n = 7$), the results obtained with

such a simple technique were impressively accurate.

Latencies. The determination of response onset latency was the last major complexity in the analysis of multiphasic signals. The onset latency of the first response involved the same calculations as in the uniphase analysis program, but in order to determine the onset latency of responses following the first response, several methods of analysis were attempted. A criterion based on the inflection (second derivative) of the signal was used, on the assumption that onset latency would be indicated by a change in curvature of the signal as the signal peeled away from the shape it would have had if only one response had been present. The inflection criterion did not prove satisfactory, possibly because a second derivative, which exaggerates signal noise, contained too little information to yield good results. However, a method that simply involved examining the minimum between two responses gave excellent results, as revealed by comparisons with the ruler measurements of experienced investigators. Specifically, any rise above the local minimum found by the same averaging technique used in determining the maximum of a response was regarded as being due to the onset of the latter response. The number-of-points criterion was needed because comparison of the slopes of the leading and trailing edges of a response indicated that a very broad minimum represented a continuation of the trailing edge of the former response.

Local maxima. Having presented the methods of determining the positions of the peaks and onsets of responses, the major problem of determining to which response a subcriticon bump belongs remains. For instance, on a generally rising signal, a subcriticon bump occurring between two responses can be considered the maximum of the former response or merely part of the latter response. In such a case, the placement of the peak of the former response and the onset of the latter response are both dependent upon the decision made. Accordingly, the algorithm contained extensive rules for making accurate decisions regarding the placement of onsets and peaks of responses. Two novel features were incorporated into the algorithm. First, the concurrent determinations of the peak of one response and the onset of the subsequent response were determined in a pairwise fashion in order to markedly increase the accuracy of evaluation. Second, to facilitate the concurrent determination, the data were examined in a reverse fashion (i.e., from the termination of the signal to its beginning). The algorithm for analyzing the multiphasic signal consisted of the following sequence.

(1) The maximum of the last response was determined first. Since no ambiguities arise if several subcriticon bumps were present, the program found the absolute maximum in the region from the last response flag to the termination of the signal.

(2) The data were examined in reverse order for minimum-maximum (onset-peak) pairs between each pair of consecutive flags indicating the approximate position of responses (e.g., the last flag and the next-to-last flag) until the first response flag was reached. When more than one minimum-maximum pair arises in the region between two consecutive flags as the data are being traced through from the latter flag (e.g., last flag) to the former (e.g., next-to-last flag), the onset of a response is taken to be the local minimum of lowest amplitude in the region. Similarly, the peak of a response is taken to be the local maximum of highest amplitude between the former flag and the onset to the latter response. The following rules were then employed for determining onset and peak positions: (a) The program started at the latter flag and searched for a local minimum, and upon finding one, the location of the local minimum and its amplitude were stored. (b) The program searched for a local maximum, and when a data point dropped a set number of points (e.g., two) below the maximum amplitude, the program stored its amplitude and position. (c) The program searched for another minimum-maximum pair, and if one was found in which the amplitude of the new minimum was less than that of the previous minimum, the amplitudes and positions of the new minimum-maximum pair were stored in place of the old values. (d) If the new minimum was of greater amplitude than the previous one, the amplitude of the new maximum was then checked to determine whether it was of greater amplitude than the previous one, and if so, its position and amplitude were stored in place of the old values. (e) The program searched for another minimum-maximum pair, and if one was found, Steps c and d were repeated until the former flag was reached. (f) When the former flag was reached, the computer stopped searching for minimum-maximum pairs, retained the position and amplitude of the currently stored pair, and regarded the maximum to be the peak of the former response and the minimum to be the onset of the latter response. However, a minimum occurring at the former flag was not counted as such because no maximum was associated with it.

(3) When the first response flag was reached, no more response peaks could have occurred, so the signal dropped to baseline and the previously described technique for determining the onset of the first response was used.

(4) Peak amplitudes were obtained by storing the amplitude, referenced to baseline, at which the peak of each response occurs. The total area between the signal and baseline was calculated as in the uniphase analysis.

(5) The results of the multiphasic analysis were stored on disk in a library format similar to the library generated by the uniphase analysis, except that variable-length records were used because of the large variance in number of responses in a signal (e.g., 0 to 12).

CONCLUSION

The Apple II/FIRST system was developed to control classical conditioning experiments, collect analog data, and extract dependent variable measures of conditioning from uniphasic response signals (Scandrett & Gormezano, 1980). The multiphasic analysis program reported here constitutes a substantial expansion in the analyzing capabilities of the system. The results indicate that, in addition to speed and reliability, the multiphasic analysis program has the advantages over human scoring of greater sensitivity to small changes in the signal and flexibility to make the analysis more or less sensitive, depending on characteristics of the signal. Accordingly, the potential of the program to analyze present and future complex patterns is impressive. In our judgment, the program, in conjunction with the Apple II/FIRST system, provides unprecedented efficiency, sensitivity, and flexibility in the conducting and analysis of classical conditioning experiments.

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