COMPUTER TECHNOLOGY

Ruler vs. the Apple II/FIRST system analysis of analog signals in classical conditioning

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In our judgment, the Apple II/FIRST system (Scandrett & Gormezano, 1980) is an efficient and versatile system for experimental control and data acquisition in classical conditioning experiments. However, these attributes would be of limited value if the system did not extract measures from our analog signals with a high degree of correspondence with our ruler measurement procedures. Accordingly, we determined the system's validity in extracting measures of CR occurrence and CR latency in three conditioning experiments. Pearson product-moment correlation coefficients indicated a very satisfactory degree of agreement on measurements made by the Apple II/FIRST system and ruler. Moreover, intraclass correlations and analysis of variance procedures applied to percent CRs and CR latency revealed several small, but divergent, differences between ruler and computer measurement of CR latency across the three experiments. However, subsequent analyses of variance revealed that the number and pattern of significant sources of variation for ruler or computer measurements were virtually identical. Accordingly, we have concluded that our system can successfully replace our traditional method of ruler measurement.

Recently, Scandrett and Gormezano (1980) reported the development of an Apple II/FIRST system to control classical conditioning experiments, collect analog data, and extract dependent variable measures of conditioning. With the selection of the Apple II microprocessor, we have been able to establish independent Apple II/FIRST systems for each of the three facilities composing the Iowa Conditioning Laboratories at a fraction of the cost of our previous DEC PDP-8/e system (Millenson, Kehoe, Tait, & Gormezano, 1973). Specifically, the three classical conditioning laboratories are controlled by individual Apple II/FIRST systems consisting of an Apple II with 48 KB of memory, AM9511 floating-point processor, Apple II disk drive, Sanyo 9-in. video monitor, two 8255 programmable peripheral interface (PPI) chips, each providing a 24-bit digital input/output (I/O) buffer, an 8253 programmable counter/timer configured to provide a real-time clock, and a 16-channel 8-bit A/D converter (ADC0816CCN)

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calibrated to yield, for each subject, 16 A/D counts for each millimeter of movement. Moreover, our software system, FIRST, an adaptation of FORTH (Moore, 1974), is an interactive, high-level, dictionary-based language that provides a programming and control system whose very high processing speed, flexibility, and ease of programming far exceeds that experienced with our DEC PDP-8/e system. For example, FIRST is typically 10 times faster than Apple integer BASIC and 12-70 times faster than APPLESOFT floating-point BASIC (cf. Scandrett & Gormezano, 1980).

Several years of experience with the Apple II/FIRST system has revealed that it carries out stimulus control and A/D recording functions with gratifying reliability. In our judgment, the system provides efficiency and versatility in the control and collection of analog data in classical conditioning experiments. However, the major impetus for the application of computer technology to the classical conditioning preparations from the Iowa Conditioning Laboratories has been the desire to have the computer relieve the investigator from the tedious and time-consuming task of determining the frequency and latency of CRs through the ruler measurement of analog signals. Accordingly, the system would be of very limited use if it did not also extract dependent variable measures from the digitized analog signals with a high degree of correspondence with our ruler measurement (i.e., criterion validity). Although a preliminary assess-

ment indicated a good qualitative correspondence between ruler and computer measurement of CR frequency and latency, a thorough quantitative comparison was also deemed necessary. In particular, over an extended period of time, we simultaneously collected both computer and ruler measurements from divergent treatments and conditions employing a number of different highly trained human scorers. Therefore, in the present paper, we report the results of determining the validity of our Apple II/FIRST system's measurement of the frequency and latency of CRs in three widely divergent experiments involving classical conditioning of the rabbit's nictitating membrane response (NMR).

COMPUTER VS. RULER

Ruler Measurement

In 20 years of classical conditioning research with the rabbit NMR preparation, our ruler measurements of analog signals have provided a robust body of empirical findings (cf. Gormezano, Kehoe, & Marshall, *in press*) that have permitted it to serve, for example, as a model preparation for the study of the neural substrates of learning (e.g., Thompson, 1976), as a laboratory model of associative learning (e.g., Gormezano & Kehoe, 1981), and as a source of axioms for CR-mediational theories of goal-directed activity (e.g., Gormezano, 1980; Gormezano & Kehoe, 1975). Specifically, from the oscillograph records of the rabbit's NMR on a single conditioning trial presented in Figure 1, investigators have been trained to reliably extract the following information: (1) the presence or absence of a CR, identified by an upward deflection in the analog signal to a criterion amplitude of at least 1 mm from baseline (representing .5 mm of membrane extension) initiated in the interval between CS and UCS onset, and (2) the latency of CRs through the measurement of the distance between onset of the CS and the tangent point at which the upward deflection appears to begin and, subsequently, conversion of such ruler measurements to time (latency) by the appropriate transform based upon the paper speed of the oscillograph (e.g., 200 mm/sec). From time to time, other dependent variable measurements, as portrayed in Figure 1 (e.g., CR peak latency, CR peak amplitude, area under the CR), were determined by ruler measurements from the analog record, transcribed by hand to data sheets, and subsequently keypunched on cards for statistical treatment. However, in spite of the extensive training of human scorers designed to guarantee high inter- and intrascorer reliability, the amount of data involved made the collection of such additional dependent variable measures an exceedingly laborious and time-consuming task. In fact, the task has been so formidable that, despite our theoretical interest in being able to specify precisely the changes in CR topography, of almost 100 investigations, only 2 (Coleman &

Figure 1. An oscillograph record of CS and US occurrence and the topography of a nictitating membrane CR taken at a paper speed of 200 mm/sec.

Gormezano, 1971; Smith, 1968) have involved the ruler measurement of these additional variables. Hence, our interest in obtaining CR topography measures provided an additional reason for wishing to apply computer technology to conditioning preparations.

Computer Measurement

The details of our Apple II/FIRST system's experimental control, data acquisition, signal analysis, data display and storage, and statistical processing have been discussed (Johnson, 1981; Scandrett & Gormezano, 1980). Briefly, in regard to data acquisition, the Apple II/FIRST system takes the analog NMR signal for each subject and digitizes the signal using a 16-channel A/D converter. The digitized output of each A/D converter channel has a range of 256 units, where 16 units are calibrated to be equivalent to 1 mm of nictitating membrane movement. The output of the A/D converter channel for every subject can be sampled and recorded as frequently as once every 2 msec, but the typical sampling rate is once every 4 or 5 msec. Currently, the signal analysis program extracts the following measures from the digitized NMR topographies: (1) a root mean square (RMS) estimate of the quality of the baseline signal prior to CS onset, (2) CR (or UCR) latency, (3) CR (or UCR) amplitude, (4) peak CR (or UCR) latency, and (5) total area under the CR (or UCR) topographies. The program first determines if the peak amplitude of the NMR is greater than the (.5-mm) criterion. Second, the program averages A/D counts for 200 msec preceding the CS to obtain a baseline measure and calculates the RMS of the baseline. The baseline serves as a reference for amplitude measures, and the RMS is used as a measure of baseline quality for the latency-finding algorithm. The detection of NMR latency (CR or UCR) is determined by searching the digitized response to the right, from CS onset, until a displacement from baseline exceeds .5 mm, and then sampling to the left until the displacement (found by successive averaging of a specific data point with the values of the neighboring point on either side) is reduced to 1/16 mm, which is approximately 5 standard devia-

tions above the baseline noise level. Algorithms also exist for extracting the other dependent variable measures (cf. Johnson, 1981).

Assessing Ruler-Computer Correspondence

The Apple II/FIRST system's manifest reliability and sensitivity (i.e., 1/16-mm amplitude and 2- to 5-msec temporal resolution) would appear to make it ideally suited for replacing our ruler method of measurement. However, for a computer system to become a viable alternative to established laboratory procedures, it must be empirically demonstrated to provide measures highly correlated with the existing measurement procedures (i.e., criterion validity). Accordingly, for three experiments with a total of 14 groups and three experimenters, we assessed, in four ways, the extent to which the computer measured what the ruler measured. First, we determined the validity of the computer-scored measures by the traditional procedure of calculating Pearson product-moment correlations between ruler- and computer-measured CR occurrence and CR latency for each group. However, although providing a good estimate of how well two variables are related, the Pearson product-moment correlation is indifferent to systematic differences. Second, we, therefore, obtained intraclass correlations (Haggard, 1958), which are sensitive to the absolute differences that may exist between ruler- and computer-measured CR occurrence and CR latency. The method of "double entry" (Fisher, 1946) was employed such that each ruler and computer measure was entered twice, with the second entry occurring in the reverse order of the first. With the double-entry method, the ruler method was compared to the computer and the computer method was compared to the ruler with the assumption that the mean and variance of both measurement techniques was the same. Accordingly, rather than simply assessing the presence of a linear relationship between the two measurement techniques, as in the case of the Pearson product-moment correlation, the intraclass correlation provides an assessment of the identity of measures. Third, for each experiment, an analysis of variance (ANOVA) was performed separately on percent CRs and CR latency, with the computer vs. ruler measurement technique as a source of variation. These ANOVAs not only permitted a determination of whether or not measurement method yielded significantly different percent CRs and CR latency but also provided an efficient and powerful means of determining the possible interaction of measurement method with training trial blocks, as well as with the variety of independent variables characterizing each experiment. Finally, ANOVAs were conducted separately for computer- and ruler-determined measures of percent CRs and CR latency for each experiment. These separate ANOVAs were conducted to determine if there would be differences in the number and pattern of significant F values obtained and, therefore, differ-

ences in the conclusions drawn about an experiment when measured by computer or ruler.

Experimental protocols. Briefly, Experiment 1 investigated the effects of CS1 and CS2 durations on differential conditioning to two CS1-CS2 serial compounds, only one of which was followed by a 3-mA, 100-msec shock UCS. Six groups ($n = 12$) were formed from the orthogonal combination of three CS1 durations (400, 600, and 800 msec) and two CS2 durations (100 and 400 msec), with the interstimulus interval between CS2 and the UCS fixed at 400 msec and the CS1-CS2 interval at 900 msec. Each of 20 acquisition sessions included 30 reinforced CS1(+)-CS2 and 30 nonreinforced CS1(-)-CS2 serial compound trials, plus 6 nonreinforced test trials. Experiment 2 examined the effects of amount of prior conditioning to Stimulus A on the "blocking" (cf. Kamin, 1968) of conditioning to stimulus X in an AX compound. During Stage 1, three experimental groups ($n = 8$) received 60 daily conditioning trials to a 300-msec tone CS (A) at a CS-UCS interval of 400 msec for one (Group B1), two (Group B2), or three (Group B3) successive days, while three corresponding control groups (Groups BC1, BC2, and BC3; $n = 8$) were restrained in their experimental chambers for comparable daily periods of time. For all groups, State 2 consisted of 54 conditioning trials to a tone (A) and light (X) compound and 6 nonreinforced test trials for 6 successive days. Experiment 3 determined the effects of LSD on the acquisition of CRs. The experimental group ($n = 12$) received LSD (30 nmoles/kg iv) immediately prior to each conditioning session, and the control group ($n = 12$) received injections of an equivalent volume of distilled water. The first 2 days of training consisted of 60 daily CS-UCS trials, and the 3rd day, of 40 CS-UCS trials, all at a CS-UCS interval of 200 msec and with a 4-mA shock UCS.

RESULTS AND DISCUSSION

Validity

Table 1 presents the Pearson product-moment and intraclass correlation coefficients of ruler- and computer-measured CR occurrence and CR latency, the corresponding SEMs, and the number of subjects for each of the 14 groups in the three experiments. Examination of the table reveals that, for Experiments 1, 2, and 3, Pearson product-moment correlation coefficients for the measurement of CR occurrence ranged from .74 to .87, from .80 to .92, and from .91 to .93, respectively, whereas those for CR latency varied from .80 to .88, from .67 to .87, and at .81, respectively. Although these values of the Pearson product-moment coefficients indicate that the computer's measurements closely corresponded with those of the ruler, as noted earlier; they do not necessarily indicate that the two methods are equivalent in yielding the same absolute percent CRs and CR latencies. For example, if the computer tended

Table 1
Pearson Product-Moment Correlation Coefficients and Intraclass Correlation Coefficients
of Ruler and Computer Measurements of CR Occurrence and CR Latency

Group	CR Occurrence				CR Latency				n
	Pearson		Intraclass		Pearson		Intraclass		
	r	SE	r	SE	r	SE	r	SE	
Experiment 1									
1	.84	.03	.85	.08	.88	.02	.86	.02	12
2	.86	.01	.86	.05	.86	.02	.83	.03	12
3	.87	.03	.88	.08	.83	.02	.80	.03	12
4	.84	.02	.85	.05	.80	.02	.80	.02	11
5	.85	.02	.86	.09	.85	.02	.84	.02	12
6	.74	.03	.74	.06	.82	.02	.80	.03	12
Experiment 2									
B1	.81	.03	.82	.09	.86	.02	.79	.10	8
B2	.81	.05	.84	.16	.85	.02	.81	.09	8
B3	.82	.04	.84	.12	.67	.04	.46	.13	8
BC1	.91	.02	.92	.14	.86	.03	.82	.12	8
BC2	.92	.02	.93	.11	.87	.02	.81	.11	8
BC3	.80	.03	.81	.08	.82	.02	.77	.12	8
Experiment 3									
LSD	.91	.01	.91	.06	.81	.02	.40	.10	11
Control	.93	.01	.94	.07	.81	.02	.33	.08	12

to consistently report latencies 50 msec earlier (or later) than the ruler method, the Pearson product-moment correlation between the two methods would still be high and positive. Accordingly, intraclass correlation coefficients were calculated to provide, in effect, an assessment of the degree of correspondence between ruler and computer in their absolute values of measurement of CR occurrence and CR latency. Examination of Table 1 reveals that in the measurement of CR occurrences for each of the 14 groups in Experiments 1-3, each of the intraclass coefficients was virtually identical to the corresponding, high and positive, Pearson product-moment correlation coefficients. Clearly, these intraclass coefficients suggest there was high agreement between ruler and computer in the measurement of CR occurrence across all three experiments. On the other hand, while for CR latency measurement the intraclass coefficients were, on the average, only slightly lower than their corresponding Pearson product-moment coefficients, there were three groups revealing substantial departures. Specifically, in Experiment 2, Group B3 and, in Experiment 3, Groups LSD and control, relative to their Pearson product-moment coefficients, revealed markedly lower coefficients of .46, .40, and .33, respectively, and thereby indicate a small absolute difference between ruler- and computer-scored CR latencies.

Ruler-Computer ANOVAs

If the ruler-computer differences revealed by the intraclass correlations were found to be consistent, the computer algorithm could be finely tuned to reduce or eliminate the absolute difference. However, one would

first have to obtain some estimate of the magnitude and reliability of the ruler-computer differences. Accordingly, we applied ANOVAs to each of the experiments, with each analysis involving the inclusion of measurement method (ruler, computer) as one source of variation. Moreover, these ANOVAs, by retaining the various experimental factors within each experiment as separate sources of variation, permitted an assessment of their possible interaction with measurement method.

In Experiment 1, the ANOVAs were conducted with the factors of measurement method (ruler, computer), CS1 duration (400, 600, 800 msec), CS2 duration (100, 400 msec), trial type (reinforced and nonreinforced serial compound), and 4-day blocks of trials. Both the CR latency and percent CRs analyses revealed significant effects of trial type, blocks, Trial Type by Blocks, and various interactions involving CS1 and CS2 duration, but, importantly, there were no significant main or interactive effects of measurement method. In Experiment 2, the ANOVAs were conducted only on the 6 days of compound conditioning (Stage 2), due to the low percentage of CRs during pretraining (Stage 1). The ANOVAs included the factors of measurement method (ruler, computer), pretraining level (1, 2, or 3 days), condition (experimental, control), and 2-day blocks. The analyses of both CR latency and percent CRs indicated significant effects of level, condition, and blocks, and, although the percent CRs analysis revealed no significant main or interactive effects of measurement method, the latency analysis revealed a significant effect of measurement method [$F(1,84) = 5.64$, $p < .05$]. A plot portraying the mean CR latency in 2-day blocks, shown in Panel a of Figure 2, reveals that

Figure 2. Panel a presents the mean latency of CRs in 2-day blocks for Experiment 2, and Panel b, in 20-trial blocks for Experiment 3, as a function of ruler or computer measurement.

computer measurement yielded latencies consistently 15-20 msec shorter than ruler measurement. Although the magnitude of the ruler-computer differences in CR latency measurement was relatively small, the ANOVA indicates the difference was characteristic of all groups. In contrast, the intraclass correlations for CR latency measurement in Experiment 2 were more restrictive in indicating that ruler-computer differences were mainly localized to a single group (Group B3). Finally, for Experiment 3, the ANOVAs were conducted with the factors of measurement method (ruler, computer), drug condition (LSD, control), and 20-trial blocks. The ANOVAs revealed a significant effect of blocks for both CR latency and percent CRs and a significant effect of measurement method for CR latency [$F(1,44) = 17.30$, $p < .01$]. A plot of mean CR latency in 20-trial blocks, displayed in Panel b of Figure 2, reveals that, in direct contrast to Experiment 2, the computer measurements of latencies were consistently 20-25 msec longer than were ruler measurements. The significant effect of measurement method for CR latency is consistent with the low intraclass correlation coefficients obtained for ruler-computer measurement of CR latency in Experiment 3.

Separate ANOVAs on Ruler and Computer

The small or no differences in ruler-computer measurements revealed by the above ANOVAs should not be surprising, since the development of the computer's CR detection and CR latency algorithms involved the successive modification of these algorithms over several other smaller sets of data until a "reasonable" correspondence was observed between computer and ruler measurements. With this iterative procedure, we assumed, of course, that our ruler measurement provides for the "true" measurement of our dependent variables. Logically, however, the source of the small but reliable ruler-computer differences in CR latency observed in

Experiments 2 and 3 could have been due to a measurement bias in the computer, ruler, or both. Nevertheless, if the pattern of ruler-computer differences in CR latency had been consistent across all three experiments, rather than disparate, it would have been possible to decrease the differences by a "fine tuning" of the computer's latency-finding algorithm to produce an even closer correspondence to ruler measurement. Recall that the CR latency-finding algorithm currently employed first checks for a criterion displacement from baseline that exceeds .5 mm. If this response displacement criterion is satisfied, data points are sampled to the left (backward in time) and CR latency is scored at the point at which a successive averaging procedure indicates displacement from baseline is reduced to 1/16 mm. Accordingly, a fine tuning of the CR latency-finding algorithm would involve changing the criterion displacement from baseline, at which the CR latency is scored, to some value above or below 1/16 mm. Given the small but disparate ruler-computer differences in CR latency, it became important to determine whether or not the differences would result in different experimental conclusions. Hence, ANOVAs were conducted separately on the ruler- and computer-determined measures of percent CRs and CR latency for each of the three experiments.

Examination of Table 2, which presents the summary of the ANOVAs performed separately on the ruler and computer measures, reveals that the number and pattern of significant F values for ruler and computer measurement of percent CRs and CR latency were virtually identical across all three experiments. The only exception occurs in Experiment 1, where for the analysis of percent CRs, the ruler method indicated a significant effect of CS2 duration (Line 2), whereas the computer method revealed an interaction of Type by CS2 Duration (Line 7). Moreover, for the analysis of CR latency, the ruler method indicated a significant interaction of Type by CS1 Duration (Line 6), whereas the computer method revealed a significant interaction of Type by Blocks by CS1 Duration (Line 16). Thus, despite our previous ruler-computer ANOVAs revealing small but reliable ruler-computer differences in CR latency in Experiments 2 and 3, these differences were not reflected in any differences in the number and pattern of significant sources of variation between computer and ruler measurement. Rather, any differences that did appear were restricted to Experiment 1, in which the few small differences, possibly attributable to a Type I error, would not have affected the conclusions to be drawn from the experiment. Accordingly, in a judgment that we do not make lightly, the pattern of results portrayed in Table 2 and the results of our previous analyses lead us to conclude that our computer system can now replace our ruler method of measurement of CR occurrence and CR latency.

Table 2
Analyses of Variance for Ruler and Computer Measurements

Source of Variation	df	F Ratio			
		Percent CRs		CR Latency	
		Ruler	Computer	Ruler	Computer
Experiment 1					
CS1 Duration	2	.44	.14	.09	.02
CS2 Duration	1	4.24*	.47	.08	.35
CS1 Duration by CS2 Duration	2	1.10	.05	7.23**	7.44**
Between Error	65	.28	.23†	367,457.22†	421,549.72†
Trial Type	1	189.03**	218.81**	42.29**	15.70**
Type by CS1 Duration	2	1.46	1.98	4.42*	.04
Type by CS2 Duration	1	3.65	3.98*	.04	.05
Type by CS1 by CS2	2	.20	.03	.46	.94
Error Within	65	.06†	.06†	14,489.24†	14,262.06†
Blocks	4	99.18**	122.46**	21.96**	47.70**
Blocks by CS1 Duration	8	2.46*	2.26*	1.63	1.92
Blocks by CS2 Duration	4	.43	.23	.37	2.04
Blocks by CS1 Duration by CS2 Duration	8	.58	1.14	3.20**	2.80**
Error Within	260	.05†	.04†	39,024.32†	35,940.11†
Type by Blocks	4	71.93**	64.03**	18.25**	19.75**
Type by Blocks by CS1 Duration	8	1.36	1.64	.84	2.99**
Type by Blocks by CS2 Duration	4	1.59	1.27	1.67	.83
Type by Blocks by CS1 Duration by CS2 Duration	8	.39	.54	.98	1.07
Within Error	260	.01†	.01†	4,762.88†	7,490.39†
Experiment 2					
Level	2	2.28	1.63	.33	.69
Condition	1	73.37**	56.00**	11.75**	17.61**
Condition by Level	2	.26	.45	5.42*	5.12*
Between Error	42	.02†	.03†	6,517.54†	4,554.31†
Blocks	5	149.03**	136.38**	79.84**	57.46**
Blocks by Level	10	2.04	1.46	1.04	1.60
Blocks by Condition	5	75.96**	70.13**	20.09**	14.15**
Condition by Level by Blocks	10	1.23	.07	.30	.34
Within Error	210	.01†	.01†	448.33†	483.12†
Experiment 3					
Drug Condition	1	.24	.13	2.85	1.50
Between Error	22	.24†	.24†	670.87†	543.79†
Blocks	7	109.66**	113.13**	36.73*	42.88*
Blocks by Condition	7	.83	.93	2.06	1.36
Within Error	154	.03†	.03†	128.03†	85.99†

* $p = .05$. ** $p = .01$. †Error mean square.

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