COMPUTER CORRELATION ANALYSIS OF INTRACELLULAR NEURONAL RESPONSES*

F. F. Hiltz

Applied Physics Laboratory
The Johns Hopkins University
Silver Spring, Maryland

INTRODUCTION

The contribution of large general-purpose digital computers to the bio-medical discipline has been considerable. However, as great as the contribution has been, the full potential has yet to be realized.

Increasingly less is the bio-medical investigator confined to spend large segments of his efforts reducing raw data manually. Increased instrumentation capabilities and the utilization of both analog and digital computers are being profitably employed to semiautomatically or automatically reduce large portions of raw data.

These procedures have their Pandora’s boxes, however. Not infrequently, the large amount of raw data confronting an experimenter has been replaced by an equally large, or larger, amount of reduced data; leaving the experimenter with the dilemma that he knows less about a good deal more.

In evolving a digital computer program1,2 to recognize intracellular neuronal responses in a time scale of approximately six man-months to one machine-hour, the Applied Physics Laboratory, The Johns Hopkins University was instrumental in replacing the first stack of data with the second for investigators of neuronal responses. Having accomplished this replacement, the problem arose of substituting a third amount of data for the second; the third being an informative reduction, through various forms of analyses, of the second.

This paper is a description of a digital computer program which will receive the data (of the second form) from the neuronal response recognition program, analyze the data in terms of various statistical procedures, and present the results in a condensed, and hopefully profitable, form.

The questions asked by the analysis program are simple and basic. Whether the answers will be adequate remains for the future to determine. If they are not, other perhaps more complex questions and techniques will have to be evolved. The questions asked by this program are: (a) What are the time interval histograms of the recognized excitatory postsynaptic potentials (EPSP’s), the recognized inhibitory postsynaptic potentials (IPSP’s), the action potentials (spikes),? (b) What is the likelihood that given one particular type of event response out of the previous three, that a second particular type of event response will occur within a given delay-

---

*This work was supported by the Bureau of Naval Weapons, Department of the Navy, under Contract NOw 62-0604-c.
time class interval, independent of whether the event responses are contiguous, as with the histograms? (c) What are the amplitude histograms of the recognized EPSP's and the IPSP's? and (d) What is the probability that given a particular type of event response of a given amplitude class, a particular type of event response of the same, or different, amplitude class will occur within a given delay-time class interval?

**EVENT RECOGNITION**

In order to appreciate better the results of the computer program to be discussed, some background in the preceding collection and preliminary processing of the neuronal data is necessary. Figure 1 illustrates in block diagram form the data handling from initial collection through the event recognition program, and finally, analysis.

The experimenter monitors the transmembrane electrical response of a neuron via his probe. The electrical signal is amplified and bifurcated into high and low gain channels. The high gain channel is devoted to subthreshold responses comprised of excitatory and inhibitory postsynaptic potentials (EPSP's and IPSP's respectively). Suprathreshold activity (spikes) are clipped in this channel. The low gain channel, however, contains the entire signal. Both channels of information are placed on magnetic tape in the form of an FM signal. This separation of the signal is important for signal-to-noise reasons, as well as ease and economy of automatic reduction.

In the second area, the preprocessing data reduction is in the form of a conversion of the analog FM signal to digital form. The two channels, high and low gain, are retained in the digitizing process through a multiplexing or interlacing process. The digital form of the neuronal response is in turn placed on magnetic tape. In addition, a time code is placed on the digital magnetic tape in order to ascertain the time of occurrence of recognized responses.

The third step in the process is the insertion of the digital information into the digital computer under control of the program for recognizing the neuronal events viz., the EPSP's, IPSP's and spikes. Figure 2 represents a segment of typical neuronal data which has been processed through the first three techniques outlined in Fig. 1. In this illustration, the symbol E on the graphical portion denotes the initiation of an EPSP. The * in the rise time (DELTA T) column signifies an event which was interrupted in its rising phase by a second event, in this case, another EPSP. The event recognition program is able to recognize these responses with an accuracy of approximately 95 percent. One form of output from this program is a tabular listing—as shown in Fig. 2—as listing the type of event, the time of its initiation, the rise time and its amplitude excursion. If the recognized event was a spike, not shown in this illustration, the tabular printout listing also includes its duration.

To date, the minimum event amplitude which may be reliably detected is 250 microvolts. The dynamic range of measurable activity between sub- and suprathreshold events may be in excess of 100 millivolts. To operate over so large a dynamic range in a recognition mode is one basis for the previously mentioned split of the data into two channels.

One additional output from the event recognition program is recorded on digital magnetic tape. This is the same data contained in the tabular printout. The purpose of this output is to enable statistical
analyses to be performed on the digital computer, rather than manually.

HISTOGRAM GENERATION

A common form of preliminary statistical analysis, associated with intracellular neuronal response data, is to count the number of times a given variation occurs within a sample. These discrete probability distributions, or histograms, are divided into class increments of the variation. For neuronal data, two types of variation are of interest. In one type, the variable is amplitude, thereby generating an amplitude histogram. For variations with time, there is the second type, a time interval histogram.

The production of either or both of these two main types of histograms may be performed during the event recognition program, or from the reduced data contained on magnetic tape.

Amplitude Histogram

In examining the neuronal data, only two types of events are considered for inclusion in the amplitude histogram category. They are the EPSP's and the IPSP's. Spikes are not included in this form of histogram. In addition, only those events which have definitely attained their response peak prior to initiation of another event are included. The events which are interrupted—those marked with an * in Fig. 2—by the onset of another event are not included since no accurate determination of their peak value may be made.

The procedure governing assignment of an event to a particular amplitude class interval, or amplitude 'box,' is depicted in a very simplified flow chart shown in Fig. 3. There are separate amplitude histograms for the EPSP's and the IPSP's.

In Fig. 3, the symbol $\Delta A$ represents the basic amplitude class interval. To avoid truncation errors, which may be quite misleading, $\Delta A$ (an input variable to the program in millivolts) must be an integer multiple of the digitizing amplitude resolution. N is an integer representing the assigned class interval and ranges from 1 to 200.

Time Interval Histogram

There are three separate time interval histograms: one each for the EPSP's, the IPSP's and the spikes. The requirement on the EPSP's and IPSP's that they display a discernible peak (i.e., be unin-
interrupted on the rising phase), as in the case for the amplitude histograms, is absent. All that is required in the form of an amplitude criterion is that their amplitude be at least as large as the minimum acceptable at the time of the peak, or of their interruption by a supervening event.

The class interval for these histograms is $\Delta T$ seconds, an input variable to the program. The measured times between contiguous like events is taken as the time difference between initiation of one event and initiation of the next like event. The first is termed the reference event, while the second is the crosreference event. Their times are labeled as $T_1$ and $T_2$ respectively. Similar to the amplitude class width $N$, $M$ is an integer denoting the assigned time class interval or 'box,' and its value ranges from a present minimum value $M_{MIN}$ to $M_{MIN} + 200$. Since the time intervals between adjacent like events may exceed $200\Delta T$, counts indicating that two events separated by $200\Delta T$ or more are all placed in the $200^{th}$ box.

Histogram Readout

As was mentioned previously, the histogram may be formed concurrently with the operation of the event recognition program, or in subsequent operations involving the reduced data on magnetic tape. In either case, selection of particular histograms is an input variable. That is, a choice may be made, prior to program operation, of which histograms are to be printed out. Another program input variable is the periodicity of histogram printout. This option will supply the investigator with a time history of the histogram formation if he so desires every $\Delta T$ seconds. The time history is supplied in two forms: (a)
the total of the $\Delta T_j$th time of printout and $(b)$ the difference between the histograms at the times associated with $\Delta T_j$ and $\Delta T_j - 1$.

Printout of all histograms may be in either of two forms, tabular or graphical, or both. Collateral with the tabular printout is the total number of counts entered into each particular histogram at the time of printout. Figures 4 and 5 are representative samples of the graphical plots of an EPSP amplitude histogram and time interval histogram respectively. The original data was obtained from a motoneuron in the spinal cord of a cat.*

![Graphical plot of EPSP amplitude and time interval histograms.]

**Figure 4. Amplitude histogram for excitatory postsynaptic potentials (EPSP's) obtained from a motoneuron in a cat spinal cord. Only EPSP's which were uninterrupted during the rising phase are included. Minimum acceptance amplitude was 250 microvolts.**

**TIME CORRELATION OF EVENTS**

The program described here is employed primarily with the data reduced by the event recognition program and stored on magnetic tape. However, it may be used on other data in card form in the correct format. Due to storage limitations of the digital computer, it cannot be performed concurrently with the event recognition program.

In essence, the time correlation of neuronal events is an extension of the frequency distribution...

---

*Obtained from experimental data supplied by Dr. T. G. Smith, Spinal Cord Section, Laboratory of Neurophysiology, NINDB, National Institutes of Health, Bethesda, Md.
analysis just described. Whereas the time interval histograms were involved with contiguous and like events, this form of analysis is not so limited. This program, referred to as the correlation program, measures the probability (or correlates) that, given a particular type of event (EPSP, IPSP or spike), there will be another event of a particular type within a given delay time class interval. The two events, reference and cross-reference, need not be adjacent; nor need they be of the same type.

This type of analysis is felt to be much more meaningful than the normal frequency distribution, or histogram, form. The observed EPSP’s and IPSP’s are usually each from multiple input pathways to the single neuron being monitored. Because the various signals on the multiple pathways may be asynchronous, indications of underlying signal processing or event relationships in time by histogram may be undiscernible.

There are two main types of time interval correlations performed. One type, designated type-A, is independent of the amplitude of either the reference or the cross-reference event. All events included in the time interval histograms are included in this type. The second type of correlation, designated type-B, is performed with a condition of amplitude imposed. Only subthreshold events (EPSP’s and IPSP’s) which meet the requirements for inclusion in the amplitude histograms are eligible for this type of correlation. All spikes may be included in either type.

Type-A Correlation

The type-A time interval correlations are in turn divided into six categories as a function of the reference and cross-reference event types. These six categories are referred to as the IPAIR’s. Figure 6 lists the various A-types and their collateral ref-
Figure 6. Classification of time interval correlation types.

Type-A includes all events meeting the minimum amplitude criterion. Type-B performed for a particular type-A, and in addition, has conditions of peak amplitude imposed.

Associated with each type-A correlation are M class intervals of time. The minimum (MIN) and maximum (MAX) values of the class intervals are input parameters to the program. The maximum difference allowed between MAX and MIN for any single processing run is 200. The values of M must be positive and consecutive integers. The width, AT in milliseconds, associated with each delay time class interval is also an input parameter to the program.

Figure 7 is a simplified program flow chart for the correlation program for the type-A. Data from the magnetic tape is read into a buffer storage. Contained in the storage is data designating the type of event, its initiation time and its amplitude between initiation and peak; or if the event was interrupted, an equivalent of the * (Fig. 2) indicating an interruption in place of amplitude.

The data is examined until an event initiation time is found which corresponds to an input start time.

The time of the event is set equal to \( t_i \). This time is checked against the input stop time and the print time criterion. If \( t_i \) is equal to or greater than either of these, the PRINT routine is entered. This will be discussed later.

The type of event (I) is determined (i.e., EPSP, IPSP or spike) and a count is entered in the appropriate reference event counter \( KE(I) \) for that type of event. Next, the amplitude is checked to see if it was a completed event or not.

If the event was completed (no *), its type is checked against the type of reference event to be used for the type-B correlation; that is, an ICLASS input corresponding to one of the PAIRS. Assume that the reference event is the type to be used for the type-B correlations. The event amplitude class interval is determined, \( LT \). The value of \( LT \) is checked against 16 input amplitude classes. If the event’s value of \( LT \) corresponds to one of these classes, a count is entered into the appropriate type-B reference event counter \( KEB(L) \).

After such preliminary processing of the reference event, the computer advances to the next event in
storage. This event is the first cross-reference event. A check is made (compute the M-class interval) to ascertain if the difference in the initiation times of the reference and cross-reference events lies between \((M_{\text{MAX}}) \times (\Delta T)\) and \((M_{\text{MIN}}) \times (\Delta T)\). If the time difference is in excess of \((M_{\text{MAX}}) \times (\Delta T)\) and the reference event was interrupted in amplitude, a new reference event is chosen. A time difference less than \((M_{\text{MIN}}) \times (\Delta T)\) calls for a new cross-reference event. Assume that neither of these conditions was encountered.

At this point, a determination of the cross-reference event type is made (\(\text{IPAIR} \) determined), and a count in the appropriate M-class interval for the corresponding type-A correlation is made. The reference event is checked again at this point to see if it is a completed or interrupted event. If it is an interrupted event, no entry is made into the type-B correlation routine, and the program then examines the next data point as a cross-reference event, and makes the previously mentioned time checks, etc. If, in this portion of the loop, the determined class interval falls outside of either the minimum or maximum values of class interval, entry into the type-B correlation is made if the reference event is uninterrupted, and is the proper amplitude class for a type-B reference event \((L \neq 0)\).
Type-B Correlations

Collaterally with the six type-A correlations, time analysis on conditions of amplitude may be performed to a limited extent. Due to storage limitations in the digital computer, only a limited number of combinatorial conditions of event type, conditional amplitude class interval and delay time class intervals may be imposed. For any one processing run, the type-B correlations may correspond to only one particular type-A IPAIR; e.g., EPSP's correlated with EPSP's, where both the reference and cross-reference events have an amplitude condition imposed. The type chosen is an input parameter labeled ICLASS, where the ICLASS equals one of the six allowed IPAIRS. Referring to Fig. 8, the processing procedure for the type-B is given in the following (it will be assumed here that the reference event had a measurable amplitude so that entry into this portion of the program was made).

The first check in this portion of the routine is to determine if the combination of reference—cross-reference events is the chosen ICLASS.

Following this, a check is made upon the amplitude of the cross-reference event to determine if it is an uninterrupted event or not. Assume for discussion purposes that the ICLASS = IPAIR and that the cross-reference event had a measurable amplitude. The amplitude class corresponding to the cross-reference event (NT) is then determined. This class is adjusted to the class interval of the reference event by forming N = NT − LT.

The allowable class intervals for the cross-reference events are designated by the symbol N, which is also an input to the program. N may vary positively or negatively, and is an integer. The maximum num-

![Flowchart](image-url)

Figure 8. Simplified program flow chart for the type-B correlation mode.
ber of values for \( N \) for a particular processing is ten. For each value of \( L \), a conditional correlation is possible for each value of \( N \). The function of \( N \) is to adjust \( \Delta A \) for the cross-reference events. For positive values of \( N \), the maximum allowed cross-reference amplitude class for each value of \( L \) is \( L(\Delta A) - (N-1)\Delta A \). For negative values of \( N \), the minimum allowed class is \( L(\Delta A) - |N|\Delta A \).

The actual allowed cross-reference amplitude classes are determined by imposing the condition that the determined value of \( N \) must equal one of the \( L \) values. This condition is imposed in order to obtain conveniently the number of cross-reference events employed in any amplitude-conditional correlation. The \( L \) inputs may number 16, and the \( N \) inputs 10.

Assume that the value of \( N \) corresponds to one of the input values, and that this value was \( N = 1 \). A check is made \((K = 0?)\) to determine if the time difference already made is equal to an allowable MD class interval. If it is, a count is made into the appropriate array element for type-B \((MD, 1, L)\), where this array has the same 200 delay class intervals as the type-A arrays.

The next step is to determine if the time difference is one of the 50 class intervals of the type-B array, where these 50 class intervals do not necessarily have to correspond to any of the 200 class intervals of the type-A's or the \( B(MD, 1, L) \)'s. The new class interval is determined using the input delay class width \( \Delta T_2 \), and a check is made to determine if this class interval, \( MC \), is allowed. If it is, a count is made in the appropriate array element for the type-B \((MC, N, L)\). Following this, either a new reference event or cross-reference event is obtained, and the processing continued until a reference event's time is found which equals or exceeds the input stop time.

### Printout

The results of the correlation processing may be printed as often as desired. A \( \Delta T_k \) input to the program determines the frequency of print in real (experimental) time, not machine time. The time of each reference event is examined. If its time is \( \geq \) to the start time plus \( \Delta T_k \) \((k = 1, 2, 3, \ldots)\), the results of the correlation as of the time of that event are printed, contingent upon meeting certain print criteria.

Since the processing is slowed every time the digital computer has to print results, it is economical to print only when it is felt the results may have meaning. Hence, there are print criteria included in the program. At present, there are three print options from which one may choose. Fig. 9 lists these in the form of a simplified flow chart.

Upon entry into the print routine, certain values are computed for each array. These values are the number of counts, or correlations, expected if one assumes that the events are Poisson-distributed in time. The expected number of correlation counts per class interval is found by forming the product of the number of reference events for the particular array being examined \((N_1)\) and the number of cross-reference events possible for that array \((N_2; N_2\) may equal \( N_1 \) for certain array types as shown in Fig. 9). This product in turn is multiplied by the individual delay class interval width of the array being examined. This product is divided by the time difference between the time of the first reference event employed in the correlation and the time of the last reference event corresponding to the print time. The value so determined, \( X \), is the average number of correlation counts expected if the process being examined has a Poisson distribution of event times. One standard deviation is determined by calculating the square root of \( X \).

In the first option, in order to determine if a particular correlation array is to be printed, the counts in all of the class intervals of that array are summed. The sum is divided by the square root of the product of the number of reference events and the number of cross-reference events. This quotient, \( P_e \), is compared to an input criteria number. If it is larger, the array is printed out.

The second option employs the previously computed value of \( X \). \( X \) is multiplied by the number of delay class intervals to form a value \( Y \). \( Y \) is the expected average number of counts for the entire array, and \( \sqrt{Y} \) the standard deviation. If the sum of the correlation counts in the array exceeds \( P_e = Y \pm z\sqrt{Y} \), where \( z \) is an input parameter, then the array is printed out.

The third option consecutively examines each element in the array. As soon as an array element (where the count \( \neq 0 \)) is found which exceeds \( X \pm z\sqrt{X} \), the array is printed. Again, \( z \) is an input parameter.

In all options, if the print criteria is not met, the array type, the value of \( X \) and \( \sqrt{X} \) for that array, and the value of \( N_1 \) (the number of reference events for that array) are printed. In addition: for options
ANALYSIS OF INTRACELLULAR NEURONAL RESPONSES

**Printout to date is in tabular form only. To obtain a graphical plot, an auxiliary program is employed where the count values (to a scale normalized to 1000 maximum) are consecutively entered onto cards as a function of the delay class interval.**

**RESULTS**

In addition to control test runs, experimental data from several motoneurons in the spinal cord of cats has been processed through the entire set of programs herein described. The results have been quite fruitful.* Complete histograms and type-A and B correlations have been obtained for a total of over 55,000 events. Computation time for event recognition, histogram generation and the various correlations (e.g., type-A's from 0 to 2000 ms in 1 ms delay class intervals) totaled less than 20 hours. The reduction in time compared to processing the same data by hand is fairly obvious.

Fig. 10 illustrates one particular result obtained from the just mentioned processing. The plot is correlation counts on the ordinate and delay class intervals along the abscissa. The reference and cross-reference events were both EPSP's. The plot indicates that there is a preference for EPSP's to occur fairly close together. This was borne out by recalling the EPSP time interval histogram previously seen (Fig. 5). The remaining portion of the correlogram would indicate that the activity is more or less a Poisson process. The next illustration (Fig. 11) is a time interval histogram from the same experiment but at a later time. The input ac-

---

*These results will be detailed and the possible anatomical and physiological mechanisms underlying them will be discussed in a subsequent paper.

---

Figure 9. Simplified program flow chart for the Correlation Program's *print* routine.
Figure 10. Time interval correlation (auto-) results for the EPSP's used for the generation of Fig. 5. The time listed in the legend is the duration of the experimental data analyzed. X is the expected average number of correlation counts assuming a Poisson distribution.

Activity to the monitored neuron had increased considerably. This time interval histogram shows the same preference of EPSP's to occur close together. In addition, there is a suggestion of a preferred spacing in the vicinity of 40 to 50 ms, which might be overlooked.

The next figure (Fig. 12) is a correlation plot similar to that in Fig. 10. It encompasses the same data as the immediately preceding histogram. The preference of EPSP's to occur at spacings of 50 ms is not obscured in this plot as it was in the histogram. In the histogram, the peak occurs around 40 ms; whereas in the correlogram, the peak is at 50 ms. The difference between these two peaks is accounted for by the high preference of closely spaced EPSP's pairs obscuring the real peak in the histogram, but not in the correlogram. Furthermore, examination of the next illustration, Fig. 13, shows a relationship between the EPSP's and IPSP's, which occurred between the times encompassed by Fig. 10 and Fig. 12, and which would not be discernible from examination of histograms.

The correlograms processed to date have uncovered activity relationships between neuronal responses which were not anticipated by monitoring during the original experiment or by histograms. They were not anticipated for two main reasons. First, standard histograms are not overly sensitive to relationships of responses due to numerous sources mixed together, and prior to this, the majority of analyses of the time relationships between neuronal events has been in the form of time interval histograms. Secondly, detailed analysis of several thousand events obtained in a single experiment has been a long time-consuming process which few experimenters have been willing or able to undertake.
Figure 11. Time interval histogram of data from the same experiment as the previous figures. However, this histogram is for a longer duration. Note the suggestion of a periodicity at 40 ms.

REFERENCES


Figure 12. Time interval correlation (auto-) results for the EPSP's in the same experiment. The data was for a slightly longer duration than that of Figure 11. Note the pronounced periodicities, and at a different period than indicated by the histogram in Fig. 11.
Figure 13. Time interval correlation (cross-) results for the EPSP's and the IPSP's occurring during the same experimental time encompassed by Fig. 12. Separation of the correlation peaks is nominally 50 ms, with the first peak occurring at 45 ms, compared to the 50 ms peak in Fig. 12.