Partition of Gross Peripheral Nerve Activity into Single Unit Responses by Correlation Techniques

Abstract. *The neural activity in the medial articular nerve is partitioned on the basis of conduction velocity by correlation techniques. Because of the small number of cat knee joint units (two to ten) responding under a prescribed set of rotational conditions, it is possible to partition and simultaneously study the units' responses to periodic joint articulation waveforms.*

It has long been a goal of neurophysiologists to be able to deduce and describe the total neural traffic involved in the mediation of a particular sensory or motor function. This hope has been realized most fully in studies of invertebrates, where the number of neurons is small compared to that in vertebrates. The target cells are often large, easily identified, and readily impaled by microelectrodes. By observing simultaneous recordings from critical groups of neurons and the accompanying behavioral manifestations, it may be possible to deduce the neural processing schemes of these simple organisms. Such an approach to the vertebrate nervous system has not been possible because of the hundreds to millions of neurons involved in a particular sensory or motor function. Consequently, the view through the single microelectrode or gross electrode has depended on analysis of many "single units" isolated successively in an experiment or series of experiments.

We have developed a method of multiple unit recording in the peripheral nervous system that allows us to observe virtually all of the afferent discharge in a small nerve, the medial articular nerve (MAN), which innervates the knee joint of the cat. This nerve carries approximately one-third of all the fibers that innervate the joint. The anatomy and physiology of the nerves and receptors associated with this joint have been extensively studied (*1*).

Cross correlation is a powerful technique that has found wide use in the study of biological systems. Segregation of axonal conduction delay groups by cross correlation has been reported (*2*). However, our technique allows one to follow the dynamic response of each neural unit, whereas previous techniques have yielded only conduction delay and average activity information. We believe our approach embodies several significant advances that make multiple unit recording practical. In this report we give the essence of our approach in descriptive rather than mathematical terms to reach the broad group of potential users of these techniques; details may be found in the literature (*2, 3*).

The operation of cross correlation involves point by point multiplication of two time records at a number of discrete points in time and summation of the products. This is repeated for a series of time shifts wherein one waveform remains fixed in time and the other waveform is shifted in time. The summated products obtained at each time shift form the desired cross correlogram when plotted against the series of discrete time shift values.

The processing steps from action potential recording on a nerve to a cross correlogram are illustrated in Fig. 1. To understand how cross correlation can partition nervous activity according to latency, suppose that an action potential passes the first recording site at time T. If the conduction latency for the fiber is t_0 the same action potential will pass the second site at time $T + t_0$. This will result in a binary correlation at one of the two discrete correlation time delays that bracket the fiber latency t_0. Cross correlating the discrete events instead of the undetected analog activity eliminates the high sensitivity to action potential shape inherent in earlier correlation approaches and results in greatly improved time resolution. In addition, the simple data structure allows computations of multiple, simultaneous correlograms at high speeds.

In the application discussed here 16 separate correlation subroutines average over 16 portions of the input cycle. This produces a cross correlogram for each of 16 successive 1/16 cycle time intervals within the period of a stimulus waveform and allows one to follow the dynamic response of each unit to the stimulus sinusoid (*4*). Figure 2A shows the 16 correlograms generated at successive intervals on the superim-

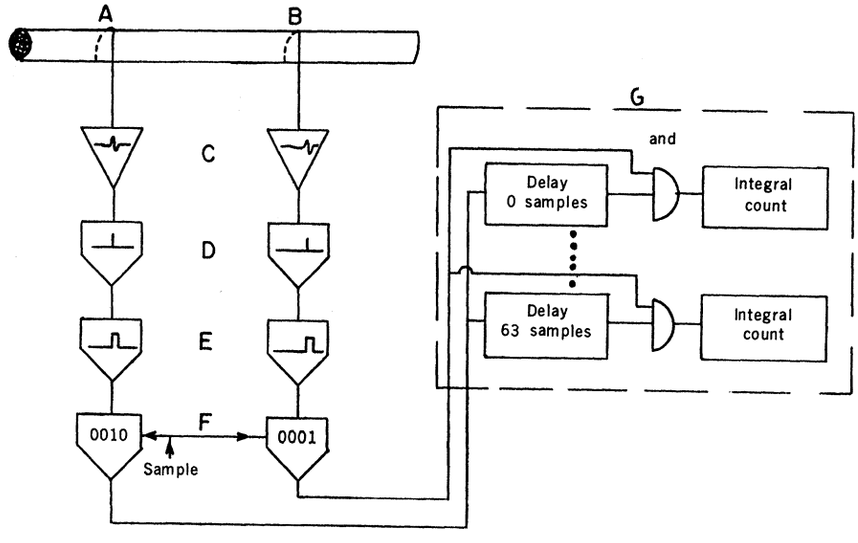

Fig. 1. Block diagram of correlation algorithm. Action potentials are recorded from a nerve at two points (*A* and *B*) separated by about 3 cm. The neural signals are amplified (*C*) and detected (*D*) by Schmitt trigger amplitude detectors. The output of the amplitude detector is converted into a pulse of unit height and of width equal to the sample interval (*E*). At *F* for each channel a 16-bit buffer fills with 0's and 1's. Each sample pulse causes the present state of *E* to be entered into the buffer at *F*. After 16 sample periods the twin buffers at *F* contain the past history of action potential occurrences on the two channels during that time, with a 0 denoting a nonoccurrence and a 1 denoting an occurrence (only four bits are shown). When filled, the buffers are transferred directly into the computer memory, one computer word representing each buffer. This permits a 16-fold increase in data input rate. Within the computer (*G*) the strings of 0's and 1's are "anded" for a succession of delays, and the integral count is incremented only if a 1 appears at both inputs to the and gate. A 64-lag-value correlogram spanning the conduction delays of interest results. An added complexity not shown here is the simultaneous 16-fold application of this technique at 16 points during the phase of an applied stimulus (see Fig. 2).

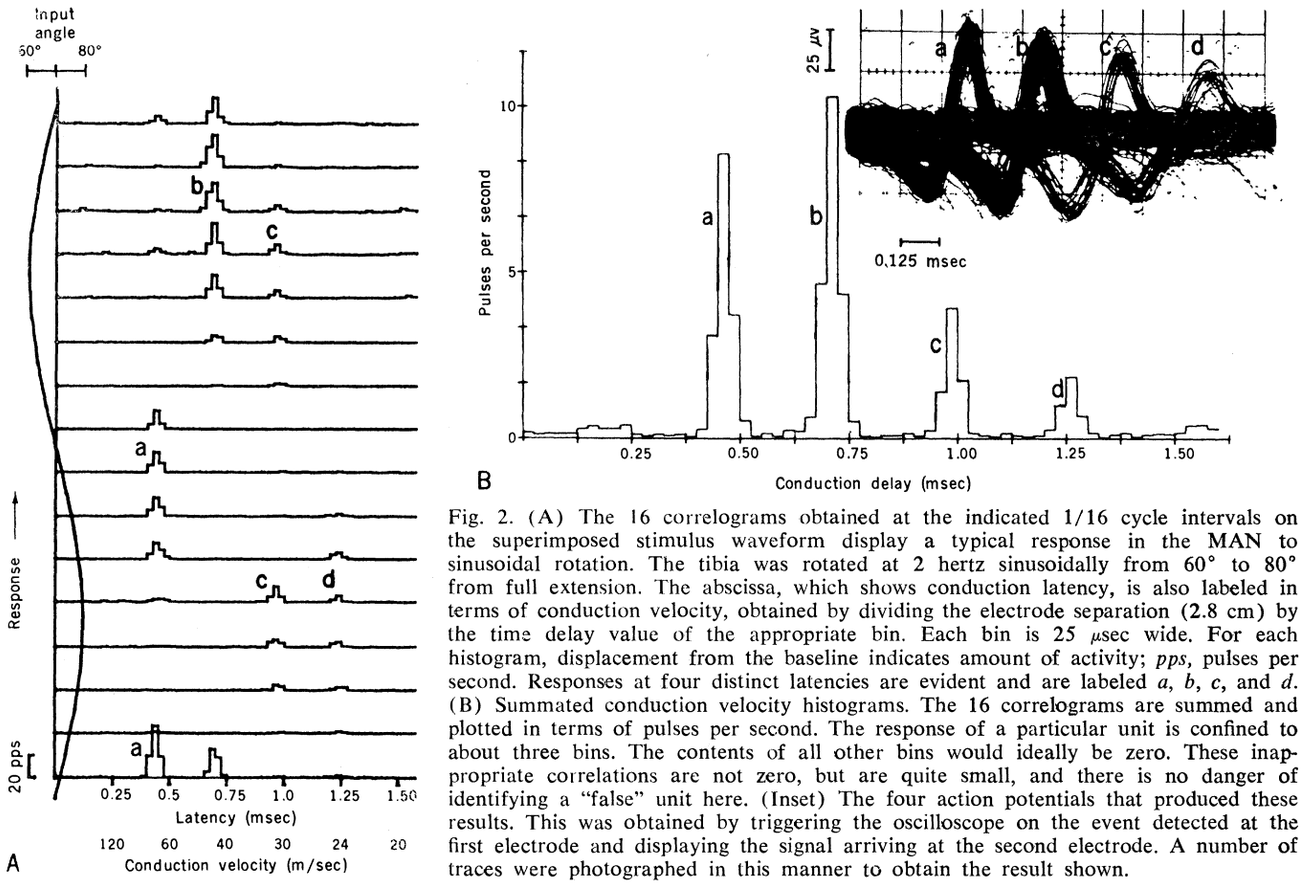

Fig. 2. (A) The 16 correlograms obtained at the indicated 1/16 cycle intervals on the superimposed stimulus waveform display a typical response in the MAN to sinusoidal rotation. The tibia was rotated at 2 hertz sinusoidally from 60° to 80° from full extension. The abscissa, which shows conduction latency, is also labeled in terms of conduction velocity, obtained by dividing the electrode separation (2.8 cm) by the time delay value of the appropriate bin. Each bin is 25 μsec wide. For each histogram, displacement from the baseline indicates amount of activity; *pps*, pulses per second. Responses at four distinct latencies are evident and are labeled *a*, *b*, *c*, and *d*. (B) Summated conduction velocity histograms. The 16 correlograms are summed and plotted in terms of pulses per second. The response of a particular unit is confined to about three bins. The contents of all other bins would ideally be zero. These inappropriate correlations are not zero, but are quite small, and there is no danger of identifying a "false" unit here. (Inset) The four action potentials that produced these results. This was obtained by triggering the oscilloscope on the event detected at the first electrode and displaying the signal arriving at the second electrode. A number of traces were photographed in this manner to obtain the result shown.

posed input stimulus sinusoid for a 1-minute data set. Four distinct "units" are active in this case.

One feature of this processing technique is its speed. At present we can on-line process and display 16 correlograms (each defined by 64 time shifts) at a rate of 10,000 samples per second. The resolution is improved when the data are sampled at 40,000 samples per second, so the tape-recorded data (Hewlett-Packard model 3690a) are replayed into the computer at one-quarter speed. The cross correlograms are stored in computer disk files for additional processing and plotting at a later time.

Normally, for the MAN, no more than six to ten units are active for a particular set of experimental conditions (joint bias angles; flexion-extension, medial-lateral and twist; frequency of the stimulus sinusoid, and so forth) (*5*). It is usually possible to resolve the half-dozen active units by virtue of the differences in conduction velocity. The four units shown in Fig. 2A represent a typical response at intermediate joint angles. A cycle histogram or poststimulus histogram for each active unit is readily derived from the data of Fig. 2A (*6*). As full extension is approached, more units are brought into activity and single units may not be easily resolved (*7*). In this case the correlograms at a particular delay may exhibit the summed response of a few units with similar conduction velocities (*8*).

The action potentials shown in Fig. 2B illustrate a way of judging the quality of the input to the processing system. The tape-recorded data can be reprocessed once the conduction delays have been determined, and the action potential of each unit can be displayed, delayed by its conduction time between the two electrodes. When two or more units possess conduction velocities so similar that they cannot be resolved on the basis of latency and action potential heights so similar that they cannot be resolved on that basis either, it is obviously impossible to separate the units with the present technique. Displaying the action potentials sometimes reveals two unresolved units at a particular delay.

We believe that this technique allows us to study the total population response of the units in the MAN under a wide variety of knee joint stimulus conditions. The approach is also potentially useful for the study of cutaneous receptor populations and for the simultaneous study of afferent and efferent muscle activity. The correlograms for the afferent and efferent activity would be segregated to opposite sides of the time shift zero point, so that receptor and motor units could be studied simultaneously. This technique does not assume or require linearity of the system under study, but could be used as a front end for powerful linear, quasilinear, or nonlinear analysis techniques based on transient analysis or system frequency response.

Since the nerve bundle studied by this technique can be left intact, it may be possible to use chronic, indwelling electrodes. This offers the possibility of studying behaving animals or eventually adapting the approach for use in rehabilitation in human medicine.

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➞ W. J. Williams, *Med. Biol. Eng.* **7**, 283 (1969); *IEEE (Inst. Electr. Electron. Eng.) Trans. Syst. Man Cybernet.* **2** (No. 1), 72 (19➞ ———, J. W. Gesink, M. M. Stern, *Med. Biol. Eng.* **10**, 609 (1972). The literature on radar range resolution is appropriate to this approach. We are preparing a rigorous mathematical treatment.
4. Phased correlation as described here requires, in addition to the computer inputs illustrated in Fig. 1, an input which marks the beginning of each stimulus cycle. Before the data are processed the period of the stimulus cycle is measured so that it can be divided into phase bins, just as for conventional single unit cycle histograms.
5. By focusing on the response at a particular conduction velocity under a number of stimulus conditions (such as static bias angle, frequency, or rate of rotation), one may deduce the repertoire of responses of a particular unit. We assume that the response of a given unit is principally within two or three bins, depending on signal to noise ratio. Since each bin is 25 μsec in width, the nominal resolution is 75 μsec. Initially we were surprised that such a small number of units were active under a given set of conditions. The viability of the MAN was tested by observing that units present at one electrode were also present at the other and by observing the size of a compound action potential generated at one site and recorded at the other. We conclude that only a small fraction of the units (perhaps 10 of 75) respond under a particular set of stimulus conditions.
6. Cycle histograms or poststimulus histograms may be obtained by collecting the contributions at a particular conduction velocity (two or three adjacent bins) and plotting the contribution against the time of occurrence within the stimulus period.
7. The velocity resolution obtainable is directly related to the signal to noise ratio and the electrode separation. Improved resolution could be obtained by increasing either of these factors. In the application described here it is usually possible to restrict the number of active units to a manageable size by controlling the stimulus conditions. Phasic units can be eliminated by using low-frequency stimuli, for example. Different groups of units are recruited at different static bias angle combinations. The total spatial-temporal response of the MAN can be deduced by observation and collation of the restricted data sets.
8. The results shown in the inset in Fig. 2B indicate that these are probably four distinct units. This is most clear in the case of *a* and *c*, where all traces seem to superimpose with one amplitude value. Responses *b* and *d* are less clear, but are thought to result from single units. Although these results are probably no more equivocal than most results obtained in single unit studies, we think this problem needs more work. On occasion we have observed pairs of tonic units that had similar conduction velocities and similar response patterns. The existence of two units with nearly identical conduction velocities and action potential shapes is revealed by careful examination of their responses under varying stimulus conditions. It is usually possible to selectively recruit only one unit of the pair by manipulation of the stimulus conditions. Under most conditions they seem to operate as a "functional unit." Improvement of the resolution of our technique may allow separation of such responses. A separate, but related, problem involves two or more action potentials triggering one event. When nearly simultaneous occurrence of action potentials at one of the electrodes takes place, information is lost. We have found this to be rare (1 in 10,000 for the data shown), and the effect is distributed uniformly over the units under study.

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HL-A Linked Antigen E Immune Response Genes: An Unproved Hypothesis

Levine *et al.* (*1*, *2*,) and Blumenthal *et al.* (*3*) have presented data from which they suggest that there is genetic linkage between HL-A and responsiveness to the ragweed pollen allergen, antigen E. We do not question the data of these authors but seriously question the interpretations. There are standard methods for linkage analysis in families (*4*, *5*) which were not used by either Levine *et al.* (*1*, *2*) or Blumenthal *et al.* (*3*). We wish to raise the following points.

Concerning the report of Levine *et al.* only one out of a total of 14 allergic families interviewed was reported in detail. Seven of the interviewed families were not studied because hay fever was manifested only in the propositus. A more detailed analysis of all families would have enabled the reader to evaluate all the evidence. Levine *et al.* believe that they have demonstrated linkage between IgE (immunoglobulin E) mediated skin sensitivity to antigen E and a particular familial HL-A haplotype in the seven families specially selected for "expressivity" of ragweed hay fever. Seven families, of a size sufficient for these authors to make a positive statement for linkage, should be amenable to conventional linkage analysis by a method such as that of Morton (*4*); this was not attempted.

Levine *et al.* discussed multifactorial inheritance of IgE mediated skin sensitivity to antigen E, but they did not fully consider how the presence of "unexpressed" Ir–antigen E (Ir-AgE) genes might affect linkage analysis in the seven families actually selected for study.

The statistical methods used to analyze for "linkage" (*1*, table 2) and to determine the prevalence of Ir-AgE genes (*1*, reference 13) are not stated clearly. It is not obvious how selected subjects (entering an allergy clinic) can be used to ascertain the population frequencies of Ir-AgE genes. Nevertheless, assuming that their estimate of 0.4 for the combined frequency of all such genes is approximately correct, that estimate must be used in the linkage analysis to calculate the probability that unaffected people possess an Ir-AgE gene (in the homozygous or heterozygous state) or that affected individuals are homozygous for the Ir-AgE gene.

In the family reported in detail by Levine *et al.*, the spouses of subjects 1, 3, 4, and 15 apparently were not studied. The data were not reported in table 1 (*1*) and were not further clarified in a subsequent publication (*2*). One cannot assume that a common trait such as possession of an Ir gene for antigen E might not be present in these people (see above). In this case, sensitivity to antigen E in their offspring, Nos. 5, 6, 7, and 16, might easily have been inherited from the unstudied parent whether or not he or she "expresses" specific allergy. On the one hand, there is the problem of the probable high frequency of Ir-AgE genes and, on the other, the differential expressivity in IgE mediated skin responsiveness in different family members. This problem is difficult to analyze if the mating types cannot be unequivocally identified, and analysis is impossible when only one parent is studied. For example, if subject 3 and her spouse both carry a single Ir gene, the mating is an intercross; only homozygous offspring from any intercrosses are informative for linkage. In this case, homozygous Ir-AgE positive individuals cannot be distinguished from heterozygotes; and, further, the homozygous negatives cannot be distinguished from unexpressed positive individuals. Consequently, this pedigree cannot be analyzed for linkage.

Subjects 3 and 8 (mother and daughter), who have the HL-A 1,8 haplotype