G. D. McCann

New Research Techniques for the Life Sciences

Abstract: A data collection and analysis system, using a high-speed, general-purpose digital computer, has been developed at the California Institute of Technology and applied to the study of visual processes of pattern recognition in living nervous systems. The experimental technique employs a rapid, flexible method of neural network modeling that permits an analysis of network functional behavior and a comparison with experimental data. Some details are given of research on neural activity in the visual system of the insect order *Diptera*.

Introduction

As modern research focuses its attention on new and important areas of the life and social sciences it becomes apparent that the conventional strategies used in the physical sciences are not adequate. The research techniques of the physicists and chemists traditionally limited each experiment to the investigation of a relatively small number of informational relationships and gradually structured a comprehensive understanding of a given subject area by careful correlations of the interrelations between a series of such experiments. Formal axiomatic mathematics proved a powerful aid to such conceptualization.

Many of the important areas of the life and social sciences, however, require the simultaneous investigation of much more varied and complex informational relationships. The dynamic, changing nature of a living nervous system or the behavior of a social group are typical examples. Furthermore, attempts to search the vast quantities of information required to understand these systems soon reveal the important fact that these relationships fail to obey in important aspects the postulates of today's existing formal mathematics.

A California Institute of Technology research program in Information Science has centered its attention on these fundamental problems. Three basic areas of research have been the result. One of these is in the area of mathematical linguistics and is concerned with the development of richer, more suitable languages for examining such data and structuring it into meaningful concepts. Another basic area has been the development of new research techniques to make possible more complex experiments that will elicit more simultaneous information. A third has been the development of new concepts for modeling theories for the relationship studied. This article describes a research environment for enhancing this enlarged concept of data acquisition and analysis in living nervous systems.

In 1962 the decision was made to concentrate on these fundamental problems through the development of more suitable combined research and analysis facilities that could extract much more meaningful information from each unit experiment. It was decided to start at the functional level of neural activity in visual systems of only modest complexity. The insect order *Diptera* (Fig. 1) was chosen which is characterized by a total of only 10^6 neurons as contrasted

Figure 1 The eyes of the insect *Musca domestica*. Black dots are rhabdomere light absorption images that permit the precise mapping of the field axes of all facets.

The author is a professor of Applied Science at the California Institute of Technology, Pasadena.

Figure 2 Simplified neural topology showing convergence properties of neuronal units under study in optic lobes and brain of Diptera.

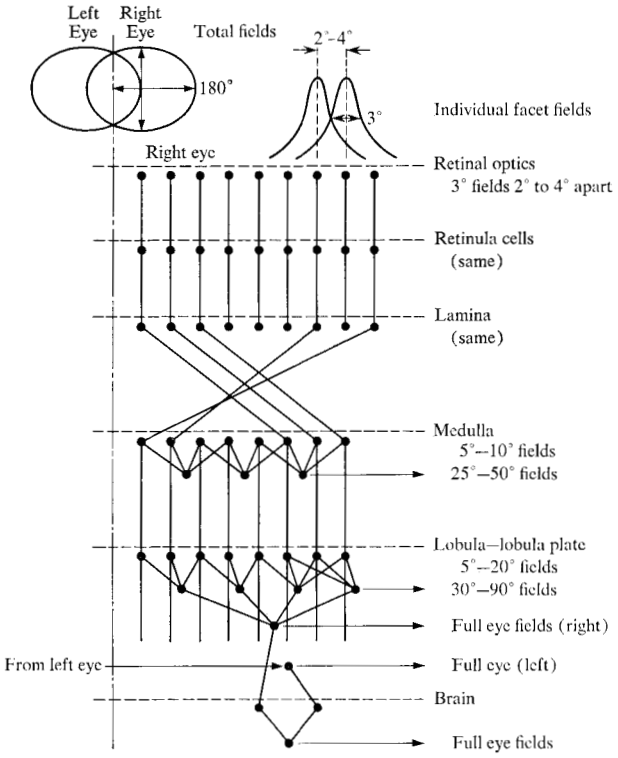


Table 1 General summary of optic lobe and brain unit classifications. (Referred to right lobe and right half of brain.) (Lobes and brain have mirror symmetry.

Contralateral Fields	*Ipsilateral Fields*
	Class Ia1, Ib1 ⟩ Gradient polarity insensitive
	Ia2 ⟩ Fields 15 to 40 degrees
IIa1	IIb1
IIa2	IIb2 Gradient polarity insensitive
IIa3	IIb3 Full and partial eye fields
IIa4	IIb4
Classes IIIa and IIIb are generally the same as IIa and IIb as described here	
Class IIIc1	

Definition of symbols

1.) Form perception- vertical edges- horizontal edges

2.) Selective motion detection with reverse motion inhibition — Horizontal inward toward proboscis Inhibition in reverse direction

3.) Selective motion detection no reverse motion inhibition

4.) Combination denotes both form form-motion detection

to 10^{10} in man. These systems possess important properties of pattern recognition basic to the future, more thorough understanding of higher order systems. Another important consideration in the choice of *Diptera* is the indication that little or no modifications of nervous function result from sensory experience. This makes *Diptera* more suitable for combined genetic and functional studies.

However, despite the relative simplicity of this nervous system, the logistic problems of obtaining and understanding the vast amounts of information are quite formidable and a special stimulus-control, data-acquisition, examination and analysis system was found to be indispensable.

Research procedures

The system requirements can be illustrated best by a description of typical desired research conditions. *Musca domestica*, the house fly and a member of the *Diptera* family, has about 3200 individual facets in each primary eye (Fig. 1). The facets have visual fields of about 3 degrees and their field axes are spaced at about 2 to 4 degrees (Fig. 2). This acuity is such that the relations of the individual facets can be mapped accurately and precise visual stimuli can be devised to test this visual system to the detailed limits of its acuity.[1] The generator potentials of the transducers (the retinula cells) can be measured accurately (Fig. 3).[2] Detailed extra-cellular microprobing is also quite practical in the three major areas of the optic lobes (the lamina, medulla and lobula-lobula plate) as well as in the central brain.[3] In fact, all areas of the nervous system can be probed quite readily as compared to most other nervous systems of general interest. Muscular activity and behavioral response studies (optokinetic)[4] are also fruitful.

The basic research problem will be illustrated with reference to micro-electrode probing studies of the optic lobes and brain and at a reference point in the course of a general investigation where the general knowledge of the system is indicated by Figs. 2 and 3. More than 50 distinctive classes of interneurons have been identified tentatively. They have visual fields (Fig. 2) ranging from that of the facet optics to successively greater areas (or convergence) until certain Class II units (Fig. 3 and Table 1) have the visual field of a total eye and certain Class III units have the total visual field of both eyes. Some 22 of these classes have been studied in considerable detail (Fig. 3). The

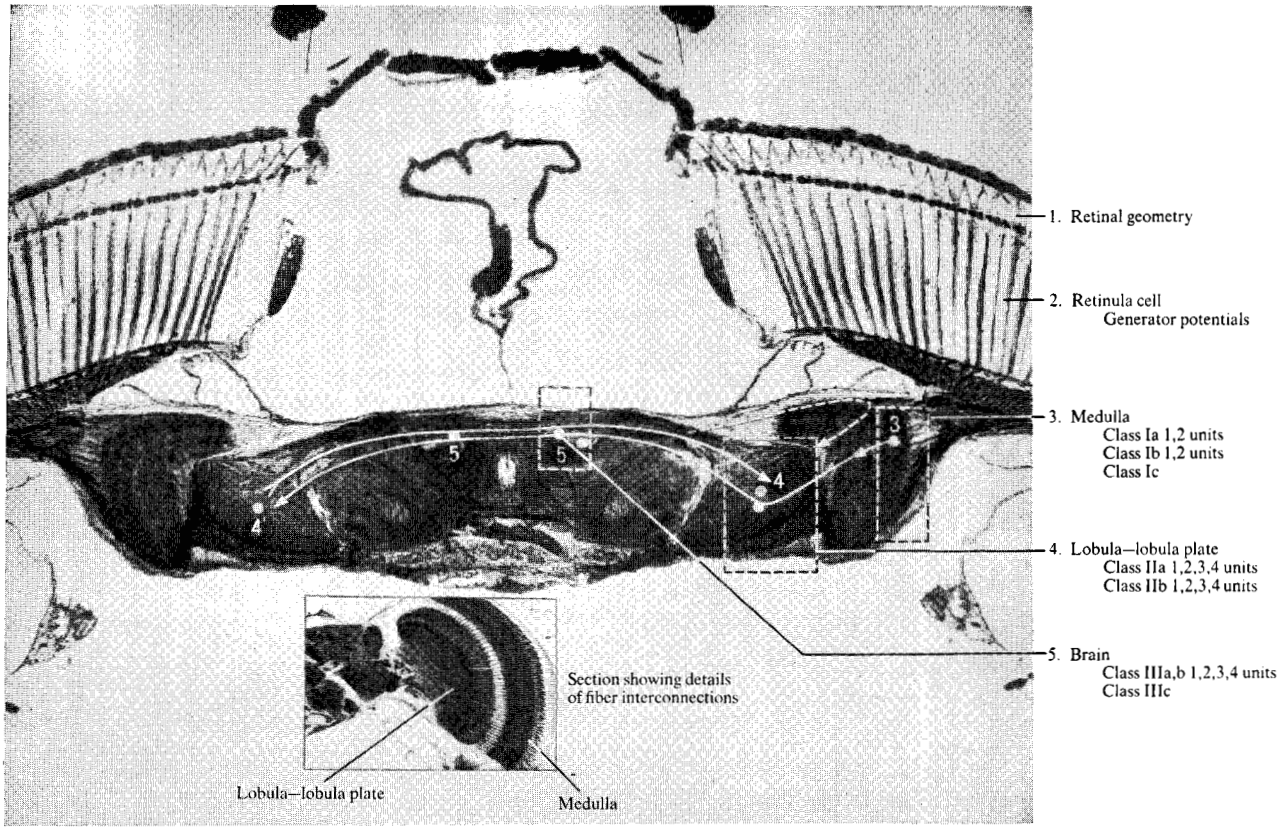


Figure 3 Histological section through center of head of *Calliphora phaenicia* showing the five levels of the visual nervous system under study.

Class I units have been shown to possess the property of abstracting form, the Class II have combined form and motion perception and the Class III combined form and motion relationships from both eyes.[1]

It now is practical to place two or three microelectrodes at select points in the optic lobes and brain carefully to record simultaneously from one to ten separate neuronal units along with pertinent data on the visual stimulus. To study the correlationships accurately requires a maximum A/D sampling rate of 500,000 samples per second per probe and a final maximum timing resolution of spike firing rates to 10 microseconds. The use of this latter figure indicates that 10 interneurons can generate potentially useful information at the maximum rate of 10^6 numbers per second. This points up the first major problem. It is not sufficient to gather data at this rate and store it away in a computer memory for later off-line analysis.

• *Spike separation and optimum probe placement*

On-line real time procedures are required to observe the characteristic wave forms of the individual interneurons (Fig. 4) and to adjust the probes until they form separate distinguishable classes. In addition, probe positioning procedures must locate neuronal units with interesting functional interrelationships relative to the visual stimuli. This requires on-line response analysis procedures to identify response properties and to insure the desired visual field relationship between interneurons. Also, during this process a practical basis must be devised for reducing the response data to a smaller rate per unit time. One form for this is to describe each unit's firing pattern by a TOE (time of crest of each spike). For such data, 10 interneurons typically will generate about 10,000 TOE's per second. However, because these numbers must be known to an accuracy of 10^{-5} seconds over a period of perhaps 20 minutes to an hour, it is necessary to record them to an accuracy of 9 decimal digits.

It is frequently necessary to record the waveform of each individual spike from a probe in addition to its TOE. Anywhere from 10 to 50 points may be required per waveform.

• *On-line analysis for stimulus control*

The adequate description of the relative and overall total functions of such a large number of interneurons requires the ability to search in some optimized manner through

the responses to an extremely large number of possible stimulus conditions. At the stage of investigation being illustrated here, a general concept has been obtained of the visual abstraction process in terms of such visual parameters as light polarization, color, intensity, form and motion. Consider for a moment only the search for abstraction of white light in the categories of intensity, form and motion. Perhaps the following relatively small number of descriptive parameters is adequate to control the search: Pattern average intensity I, pattern boundary shape S, area A and pattern reference location θ_1 and θ_2; form described as a uniform striped pattern of spatial wavelength λ with edge orientation α and finally, pattern motion described as a direction B and velocity V. Assuming only 10 values for each of these 10 variables produces 100 possible variations. Actually, it has been found necessary to scan a much greater range of variation in the on-line analysis to fix upon sets of suitable conditions for recording. The recognition of response characteristics suitable for this process is most complex.

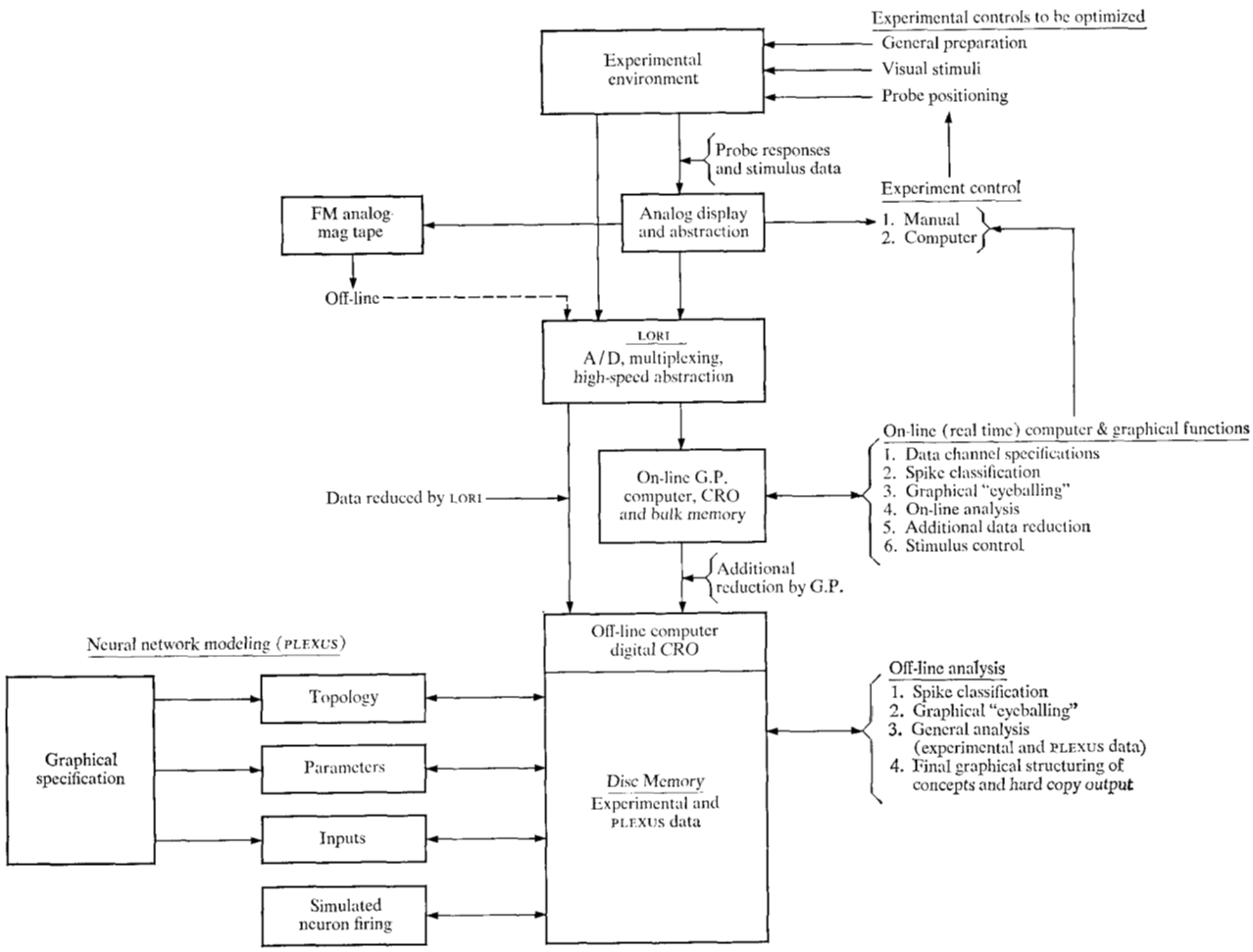
• *Off-line analysis*

The off-line analysis required to structure meaningful detailed concepts of the pattern recognition process is even more complex. For this it has been found necessary to place within the general framework of the combined man-computer interrogation of the experimental data a very rapid, detailed and complex method of structuring theoretical models of the neuronal system that permits the creation of literally thousands of possible models in the course of a few days that can be tested and compared to experimental results with the same speed, detail and generality used for the experimental data. This has been achieved by a program called PLEXUS,[5] which takes full advantage of the graphical communications and function key manipulative control capabilities of the basic computer system designed for this research.

The experimental system

The data acquisition and analysis system developed at the California Institute of Technology for this type of research

Figure 4 Experimental data acquisition and analysis system.



is illustrated schematically in Fig. 4. The general purpose off-line computer illustrated is an IBM 360/44 with three to five 2311 disc units, 128,000, bytes of core memory and two 2250 CRO's. Its multiprogramming operational system (described in reference 6) permits its simultaneous use by several investigators as long as the system is not collecting large amounts of experimental data. The general purpose computer illustrated in Fig. 5 for on-line analysis also has been the 360/44 up to the present time. However, in expanding the usefulness of the system for several experimental setups smaller computers with digital tape memory are suitable and more economical.

• *LORI*

The high A/D sampling rates and rapid abstraction requirements made it necessary to develop a special purpose interface between the experimental and the general purpose computers. This is LORI shown in Fig. 4; it consists of A/D devices, each sampling at a 500 kHz rate into an 8-channel multiplexer. Each channel has adjustable sampling rates. Each of these channels either can be sent directly to the on-line or off-line general purpose computers or stored momentarily in LORI for additional abstraction.

LORI performs three general classes of abstraction.

1. Direct A/D sampling with adjustable sampling intervals.
2. Detection of a spike discharge and the generation of a TOE vector, which is the time of crest magnitude of each spike on a probe channel.
3. The detection of the wave shape of individual spikes as a series of up to 100 samples per spike.

• *Spike classification*

Four methods are available for classification of multiple units on a given electrode. These are presented here in order of increasing power.

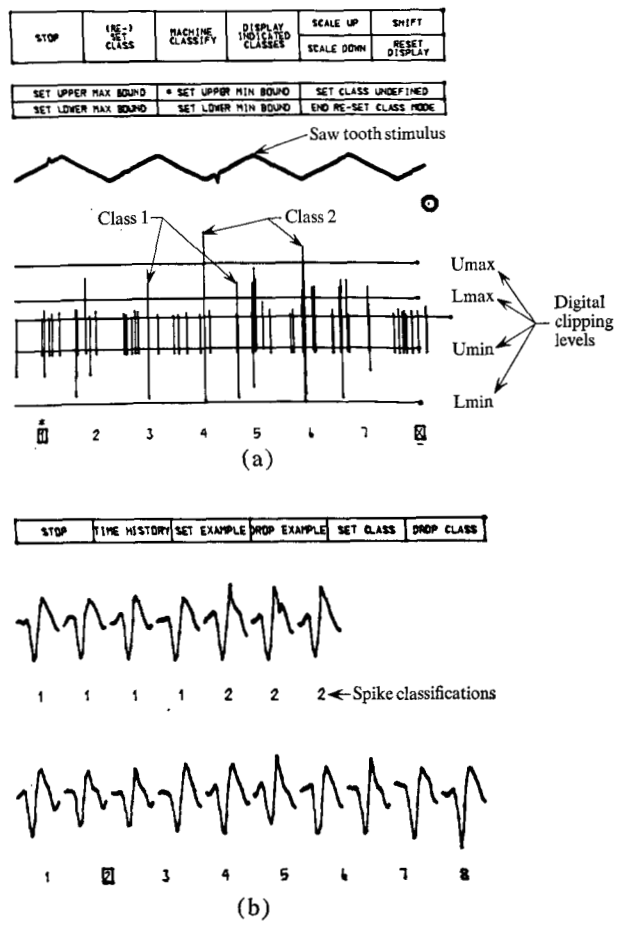
1. If there is clearly only one or two units whose peaks are separated by 20% or more, they can be classified by an analog window discriminator circuit ahead of LORI which outputs pulses on separate channels in the proper time sequences. These are converted to digital times-of-events by LORI.

2. If separation of peaks is clear, but less than 20%, the spikes can be classified by LORI after being converted to digital form (since this has more precise crest voltage detection. Two unit separations at either the analog or LORI level is accomplished by a logical NAND operation (subtraction).

3. For less clear cases, or when more than two units appear at one electrode, computer-aided classification is used. For this method, each waveform (that crosses a threshold) is sent to the G. P. as a time-of-event plus a series of A/D samples of the waveform. A variety of machine and machine-aided techniques have been developed to handle the more difficult cases.[7] Graphical display for this is illustrated by Fig. 5. Waveform classification procedures should be available both on-line and off-line.

4. When separation is required in multiple probe experiments, another method is occasionally useful. In certain cases (especially the brain) two or more units can be picked up, one or more of which also are picked up by another probe in the brain or lobula-lobula plate region which happen to be on the same tract. Spike synchronization, therefore, occurs with a small time lag (Fig. 6).

Figure 5 Graphical display used in spike separation procedures. (a) Here each spike is plotted as a single line terminated by the first and second peak magnitude. A variable section of a record is displayed along with pertinent stimulus data and with the TOE's of each spike properly represented in time. Eyeballing these data frequently provides adequate proof that they can be separated by pulse height only. (b) If pulse height separation is questionable, individual spike waveforms can then be displayed and samples picked for tentative classification by the computer routines. These results are then checked for adequacy.[7]



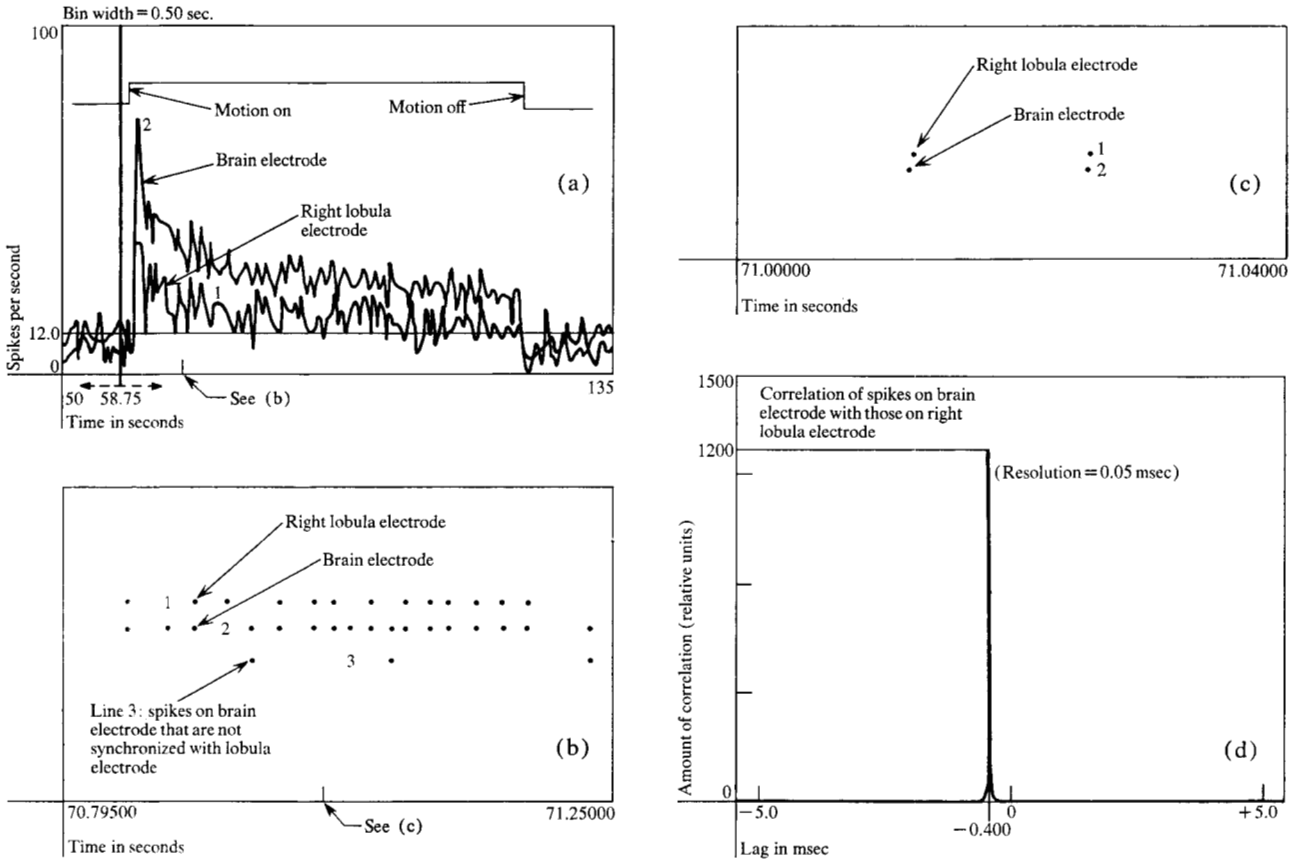


Figure 6 Double probe experiment showing simultaneous recording of Class IIa and IIIa units. Stimulus is vertical stripes ($\lambda = 16°$); horizontal motion started at 61 sec, stopped at 123 sec. Pattern diameter 30°, velocity 30°/sec. (a) Overall response of both units. Firing rate averaged in 0.5 sec bins. (b) Short section of (a) with each spike represented as a dot. Upper line of dots corresponds to right lobula electrode, middle to brain electrode, and lower to unsynchronized brain spikes. (c) Short section of (b) showing relative timing. (d) Cross correlation of the times of events of the two units showing a propagation time between the two electrodes of 0.400 msec, with the brain leading.

An important phase of these procedures is the initial display where each spike is represented by a vertical line between its maximum and minimum as shown in Fig. 5a. Scanning such records and correlating spike frequencies of different line positions and lengths with simultaneously displayed stimulus data greatly facilitates the choice of classes. Typical spike waveforms then can be chosen for display (Fig. 5b) to help determine the next step of classification which might compare only peak magnitudes or might correlate several or many points of the waveform. The importance of this capability for initial scanning is illustrated by the conditions of Fig. 5. A first glance at the spikes in this figure at the analog oscilloscope level might lead to the belief that there is only one class instead of two since the crest magnitudes of both classes are nearly the same. The distinction between the two classes becomes much clearer when examples can be compared as presented in Fig. 5.

Data analysis

Data acquisition and analysis procedures consist of three basic components: (1) simultaneous acquisition of several responses and a single stimulus and correlation of individual responses with stimulus, (2) study of variations in responses for precisely repeated stimulus conditions, and (3) study of effects of changing parameters of *intensity*, *form*, *motion*, etc.

To accomplish the latter objective, two or more stimulus variations are presented cyclically, separated by the same period (t_0). All subcycles corresponding to the same stimulus can be averaged and the resulting averages compared (essentially a comparison of PST histograms). Also, the variation of individual responses from the average can be studied. Available analysis routines range from a rather general display of raw data through procedures such as latency and PST histograms, auto- and cross-correlation and special purpose subtraction routines of

various sorts. The interactive graphical display, in providing a rapid means of looking at data in many different ways, is of great value in these procedures. It is important that many of the above analyses and displays be available for both on-line and off-line analysis. The details are best described in relation to specific results.

• *Combined analysis and graphical "eyeballing"*

The highly flexible graphical display system developed for this research is indispensible for on-line and rapid analysis. At present four vectors of data are displayed simultaneously. TOE responses are displayed either as straight line connected points of averaged TOE's per bin width or, for short time intervals, are plotted as discrete points (Fig. 6). Vectors sampled as A/D are plotted as averaged amplitudes per bin width. Rapid methods are available for changing the boundaries of the sampling window, the bin widths and all other scaling factors. A long vector can be "walked" through the window at a uniform rate or in steps. Subvectors of data can be generated quickly, and so forth.

The use of this flexibility, along with on-line analysis, is illustrated in Figs. 6 and 7. Figure 6a shows the simultaneous display of TOE vectors from two probes and a vector representing the sudden application and removal of a constant velocity visual pattern. This gross graphical picture indicates certain similarities between the two responses, one of which is from a probe in the central brain and the other in the lobula-lobula plate region of one eye. By quickly abstracting a shorter 0.455 second section and displaying it as discrete events (Fig. 6b) it begins to look as if certain spikes occur at the same time. Further expansion to an interval of 0.04 seconds shows that these are not coincident but displaced in time by about 0.4 milliseconds (Fig. 6c). To search the whole record graphically for the evidence of such a relation would be time consuming. It is only a matter of seconds, however, to perform and display the correlation analysis of Fig. 6d.

Figure 7 illustrates the power of PST analysis for the determination of the relative functions of an interneuron in terms of responses to *intensity*, *form* and *motion*. For these data responses were obtained for the sequential presentation of four types of stimuli, each separated by a sufficient time period that the previous pattern was "forgotten." The sequence was then repeated 20 times. The G. P. analysis separated the responses to the four classes of stimuli and graphically presented the four averaged curves of Fig. 7. These curves provide a wealth of significant information never before disclosed.

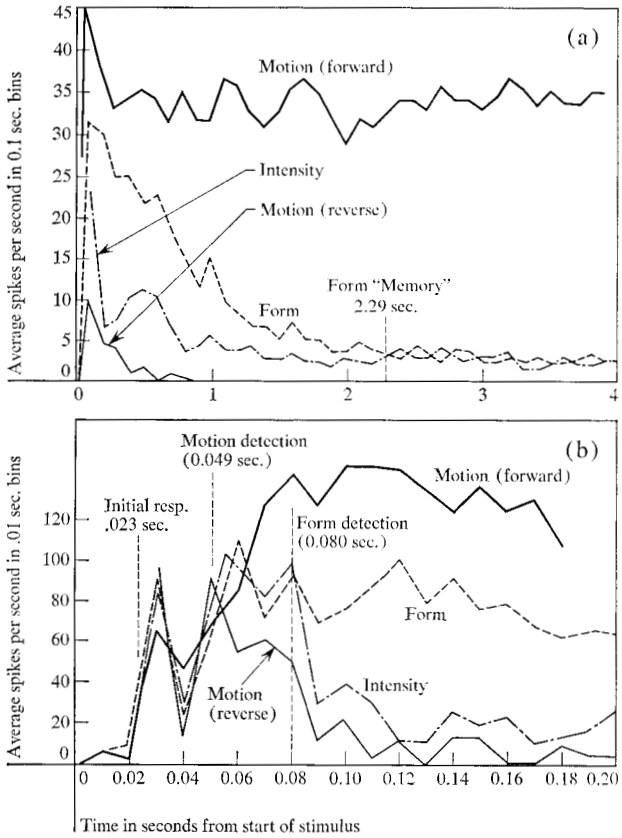


Figure 7 Computer displayed averaged responses of a selective motion detection neuronal unit to four types of visual stimulus representing *intensity, form, forward motion* and *reverse* or *inhibitory motion*. (a) Vectors displayed over total pertinent period of pattern recognition processes. (Not short time memory for static form perception.) (b) Detailed display of first 0.2 seconds of responses showing individual response times to different properties of the stimuli.

• *PLEXUS*

A detailed description of the programming features of PLEXUS is given in reference 5. Its use in relation to data analysis is illustrated by Fig. 4. PLEXUS-generated information and experimental data share the same memory in the IBM 360/44. All new assumptions for a model are generated rapidly by graphical methods (Fig. 4). All elements of such a model (topology, parameters, inputs and network responses) can be stored in memory for rapid retrieval in the development of new models. Actual experimental data can be used for model inputs and model responses can be analyzed by all the methods available to experimental data analysis.

The concepts of topological representation and the methods of model data retrieval developed in this program have general applications to other forms of complex modeling and it is planned to extend the system concept to a wider range of living nervous system analysis.

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