PLASTICITY IN SMALL NEURONAL ASSEMBLIES

George L. GERSTEIN

Departments of Physiology and of Biophysics
School of Medicine, University of Pennsylvania
Philadelphia, Pennsylvania, USA

Abstract. When spike activity of two or more individual neurons is simultaneously recorded, it is possible to detect various types of neuronal connections to and between the observed neurons by making appropriate statistical analysis of the data. Such methods are reviewed. This approach is particularly useful in seeking changes of neuronal connectivity associated with behavioral plasticity. Examples from experiments with *Aplysia* and crayfish are given.

INTRODUCTION

The process of learning and the plasticity of behavior have received careful scientific attention for about a century. Yet the underlying neuronal mechanisms continue to remain almost completely obscure. Theoretical statements of what are likely candidate mechanisms can be found as early as in Descartes, but are far more thoroughly expressed in modern terms by Hebb (7). Basic to such theories is the idea that during the learning process some change occurs in the functional connectivity of the neuronal network. Three recent reviews (1, 9, 14) deal with the efforts that have been made to find changes in excitability and firing patterns of single neurons during the learning process. The only direct tests of the “connectivist” hypothesis have come however in work with *aplysia* (12), and then only for habituation and dishabituation. Thus the concept that a pre-existing neuronal network alters its functional connectivity during behavioral plasticity still needs experimental proof.

This paper discusses a direct approach to the problem based on the simultaneous recording of spike trains from several separate neurons.
Although the examples are drawn from invertebrate preparations (with the attendant possibility of using identified neurons), this approach may also suffice for the study of the vertebrate brain during behavioral plasticity.

**ANALYTIC METHODS FOR TWO OR MORE SPIKE TRAINS**

Whenever such data are available, the basic objective is to seek meaningful temporal relationships between the several spike trains. Such temporal relationships can arise from a number of different physiological (or even artifactual) sources, particularly if the experimental situation includes some form of repeated short stimulation. If temporal relationships are found, they may be dynamic both quantitatively or even qualitatively. They may be phasic with respect to the applied stimuli, or tonic in the context of the general experimental situation.

As shown below, it is possible to interpret such observations on spike trains and their relationships in terms of an equivalent “wiring diagram” which includes not only the two (or more) neurons actually being recorded but also various interneurons whose existence may be inferred. By appropriate experimental design it is possible to seek situations which produce changes in such “wiring diagrams”.

**Cross-correlation**

This analytic approach has been extensively used, and has recently been reviewed by Moore et al. (13). The calculation produces a numerical comparison of one spike train with the other spike train at a shifted (earlier or later) time. A number of statistical difficulties can arise if one or both spike trains are approximately periodic. Perhaps the most serious difficulty with cross correlation is that it represents an average measurement over the entire length of available data, and hence washes out any dynamic aspects of the temporal relationships between the two spike trains. In common with all other analytic methods of this general type, several ambiguities arise in the interpretation of a cross correlogram in terms of a “wiring diagram” between the two neurons. Nevertheless it is a sensitive method that should always be used.

**Joint PST scatter diagrams**

This analytic approach has recently been introduced (4) and additional developments have been described (5). The basic principle of this calculation is the construction of a scatter diagram from the two (or more) spike trains. Nonuniform distributions of dots in this scatter diagram can be
directly interpreted in terms of a “wiring diagram” between neurons. The joint peri-stimulus time (PST) scatter diagram represents a generalization of both PST histograms and of cross correlation, and manages to preserve certain dynamic aspects of the temporal relationships between the spike trains.

The general mode of construction of joint PST scatter diagrams can be seen in Fig. 1. Here the spikes of neuron A appear along the ordinate, and the spikes of neuron B along the abscissa, each in laboratory time.

Parallel to the two axes the instants are marked at which stimuli were delivered to the preparation. Three steps of construction are now needed: (i) At the time of each spike of the B neuron, replicate as a line of points parallel to the ordinate all spikes of the A neuron. When this has been done for all B spikes, the reciprocal construction has also been made, i.e. at the time of each A spike all B spikes are replicated as a line of points parallel to the abscissa. The resulting field of points will have a woven, cloth-like appearance, but is not shown. (ii) Construct the series of squares along the diagonal of the plane, as shown in Fig. 1. Each square is bounded by the time of presentation of stimuli. (iii) On a new coordinate plane superimpose these squares, thus adding together the point distributions found within each original square. This resulting summed square is the fundamental form of the joint PST scatter diagram.
Variations of point density in the scatter diagram can be associated with various possible connections between and to the observed neurons. In Fig. 2 are four scatter diagrams, each derived from computer simulation of the adjoining neural network. The top left shows the case of two neurons, that do not intract and that are not affected by the stimulus.

The resulting scatter diagram is an approximately uniform distribution of points. A cloth-like texture is visible. At the top right is seen the case of two neurons that are driven by the stimulus. The resulting scatter diagram shows two bands of increased point density parallel to the axes, and at a distance from them that corresponds to the latency of each neuron's response. At the bottom left are two neurons that are not affected by the stimulus, but that have a direct excitatory synaptic connection. The scatter diagram for this situation shows a narrow band of increased point density that is parallel to the principal diagonal of the plane, and at a distance from it that corresponds to the propagation and synaptic delay time. This distance is barely visible at the resolution used. Finally, at the bottom right is the case of two neurons that are excited by the stimulus, and that are connected by an interneuron. The interneuron is itself inhibited by the stimulus. The corresponding scatter diagram shows...
bands of increased point density parallel to the axes as well as a diagonal band. Note, however, that the diagonal band is interrupted in the region near the origin.

Thus variations of point density along a diagonal band correspond to stimulus locked variations in the strength of the interaction between the two observed neurons. Such dynamic aspects of the temporal relationships between spike trains are completely lost in cross correlation. On the other hand, neither method detects dynamic changes in interactions that are unrelated to the stimulus structure of the experiment. For additional details and examples see Gerstein and Perkel (5).

The principal purpose of the analytic approaches described is to determine from two or more spike trains what connections exist to and between the observed neurons. The scatter diagram is considerably more

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Fig. 3. Cross correlation histograms for two neurons with direct synaptic connection (top) and two neurons with shared synaptic input (bottom). These were produced by computer simulation of a network in which a neuron (with a Poisson interval distribution) has synaptic connections to two other neurons (each with independent noise input, and a Poisson interval distribution). The locations of the peaks along the time axis (abscissa) are arbitrary.
powerful than cross correlation for this purpose. However, an important ambiguity still exists. Diagonal bands of increased (or decreased) point density can be caused either by direct synaptic connection between the observed neurons (as shown in Fig. 2) or by shared input to the two observed neurons from a usually unobserved common source. There are considerable quantitative differences between the two situations, as shown in the cross correlations of Fig. 3. These are taken from a computer simulation in which neurons B and C share excitatory input from neuron A. The upper cross correlation is for neurons A and B which have direct synaptic connection. The lower cross correlation is for neurons B and C which have no direct connection, but only shared input. Taking into account the background, the peak caused by shared input is approximately one quarter the height and twice the width of the peak caused by direct synaptic connection. Similar quantitative differences between the two situations appear in the diagonal bands of scatter diagrams. The basic qualitative ambiguity of interpretation in an experimental situation remains, however, and additional information must be used.

APPLICATIONS TO EXPERIMENTS

One area in which simultaneous recordings from several neurons together with analysis of the several spike trains can be useful is in studies of sensory processes within the nervous system. The basic problem here is to detect ensembles of neurons, and to see whether such ensembles depend on the nature of the incoming sensory stimulus. I have been able to show that in some cases the interaction between two sensory neurons remains unchanged as stimulus parameters are varied, whereas in other cases the interaction between the two observed neurons changes profoundly as stimulus parameters are varied (3). No clear case can yet be made for stimulus coding in this form, and much work remains to be done.

Another area in which simultaneous recordings from several neurons can be most useful is in investigations of the neurophysiological substrate of plasticity. The general question to be examined here is whether there are any changes in the connections between or to observed neurons that are associated with changes in the behavior of the preparation. This is unfortunately a technically most difficult type of experiment, since it is necessary to maintain observation of the same two (or more) neurons through an entire sequence of behavioral conditioning procedures. Furthermore, at least in vertebrate brains, the likelihood of finding connected neurons is low (1). Therefore, our laboratory has recently spent some time on two invertebrate preparations.
Details of the experimental conditions and results are in Kristan and Gerstein (11), and Kristan (10). Simultaneous recordings were made with intracellular electrodes from three neurons in a dissected, isolated ganglion that was maintained in appropriate conditions. Electric stimuli were applied to two of the axon bundles entering the ganglion. The “Test” (T) stimulus was barely above threshold to affect the observed neurons; the “Priming” (P) stimulus was some three times stronger. Time parameters were chosen to imitate a classical conditioning paradigm. The experiments were designed to evaluate the effects of pairing the two stimuli, each coming over one axon bundle.

The results are characterized in Fig. 4, which shows scatter diagrams for the activity of neurons in two experiments (a and b). Each row shows responses to the T stimulus before stimulus pairing, immediately after stimulus pairing, and finally some time later. In each scatter diagram there are bands of increased density parallel to the coordinate axes.

Fig. 4. Joint PST scatter diagrams for neurons of pleural ganglion of Aplysia. Electrical stimulation sequence is modelled on classical conditioning paradigm. Each row shows complete experimental sequence for a different pair of neurons. See text. From Kristan and Gerstein (11).
which represent the direct responses of the two neurons to the stimulus, and which are little changed by the manipulations of the experiment. In experiment a (top row) before stimulus pairing there is a diagonal band of increased density both near the stimulus instant (the origin) and at later times between stimuli. Thus the two neurons show both a phasic and a tonic temporal coherence. After stimulus pairing, the diagonal density near the stimulus instant is unchanged, while the diagonal density at later times essentially has disappeared. Thus the two neurons still show a phasic temporal coherence, but have lost their tonic temporal coherence. Finally the third scatter diagram (taken some time later) shows a diagonal band of density very similar to that before pairing.

The series of scatter diagrams in experiment b (lower row) show complementary changes. Here stimulus pairing increases the tonic temporal coherence between the two neurons, while it reduces the phasic temporal coherence. In all these experiments intracellular recordings were available, so that the source of temporal coherence could be identified as a strong, shared synaptic input to the observed neurons. Most important, however, is that such temporal coherence can be a dynamic, time varying property of a group of neurons with both phasic and tonic changes.

Crayfish claw

Reflex opening and closing of the claw in an intact animal can be obtained by mechanical stimulation of sensory hairs on the appropriate part of the claw. Repeated reflex closing upon an obstacle will under some conditions cause a complex form of adaptation in the animal, so that even if the obstacle is removed, the claw will close no further than its former position. Although this is a somewhat exasperating preparation, it is in some respects convenient for a study of the neural changes underlying behavioral plasticity (6, 8).

Studies of the anatomy, innervation and mechanical properties of the crayfish claw abound in the literature (2, 15, 16). The opener and the closer muscles are each innervated (for purposes of the slow movements used here) by a single excitatory and a single inhibitory motor axon. A special stretch receptor near the joint and hundreds of sensory hairs provide input to the system. All cell bodies and a complex neuropil are found in the first thoracic ganglion.

With suction electrodes it is possible to record from individual identified axons at the periphery near the muscles. All four motor axons and some small number of sensory axons may simultaneously be examined in a particular experiment while the animal undergoes behavioral training.

If a single sensory hair pit is mechanically stimulated, spikes are evoked on the appropriate motor axons. This is shown in Fig. 5, with the
PST histograms for the activity of a sensory axon coming from the hair pit, as well as for the activity of the closer exciter axon. The long latency before the motor discharge is largely propagation time to and from the ganglion. However, upon examination of the scatter diagram for the activity of these two neurons (Fig. 5), no sign of any diagonal density distribution can be seen. This suggests that there is at least one inter-neuron between the sensory and motor neurons. An alternate interpretation would be that the connection is monosynaptic but that the motor neuron has a long time constant for temporal summation of presynaptic activity.

Fig. 5. Left: PST histograms for a sensory axon and for the closer exciter axon of the crayfish claw. Right: Joint PST scatter diagram for same data. Courtesy of T. J. Wiens.

Under some conditions there is evidence for temporal coherence of the closer exciter and the opener inhibitor motor neurons. This is shown in Fig. 6. The scatter diagram for data taken during the behavioral training paradigm shows a faint diagonal band at the times corresponding to reflex closing of the claw. This is absent in the scatter diagram for data taken during the test situation, although this might be a matter of insufficient statistics. The third scatter diagram in Fig. 6 also shows the training situation, but was made with higher time resolution from the instant at which closing began; the diagonal band is clearly visible.

Again, it can be seen in this experiment that the temporal coherence of some neurons is a dynamic, time varying quantity, although it has
not yet been possible to show that the coherence changes at the same time as the behavior. Additional work is required, particularly on the interneurons in order to locate more clearly the neural changes associated with this behavioral plasticity.

DISCUSSION

The two examples above demonstrated how temporal coherence of certain neurons varies both with respect to stimulus instants and during experimental manipulations. It is probably reasonable to consider that temporal coherence among neurons is a cardinal sign of functional grouping. (There may be many other indicators concerned, for example, with the properties of membrane potentials.) If this is so, then the approach sketched above can be used to study the dynamic variations of functional groupings, particularly during the changes associated with behavioral plasticity. The two examples given, however, involved relatively primitive invertebrate ganglia with at best only primitive behavioral indices of plasticity.

To what extent then, can such methods help in observing the neural changes that accompany plasticity of behavior in more complex vertebrate preparations? The methods discussed can easily be extended to any number of simultaneously observed neurons, although as the number exceeds three, there are difficulties in direct representation (5, 11) and the amount of required computer power rapidly increases. Some number of neurons in the range of 10 to 100 surely represents the practical limit from this viewpoint as well as because of the geometric relations of the surface and volume of the tissue being studied.

There seem to be two extreme possibilities: If the changes involved in the establishment of even some simple conditioned reflex in a verte-
rate brain are represented in very slight changes in activity of a diffuse network of many thousands or tens of thousands of neurons, then the scatter diagram and similar methods are not sufficiently sensitive as detectors, nor can a significant fraction of the relevant neuronal population be sampled.

If, on the other hand, the changes of plasticity in a vertebrate brain are represented simultaneously by relatively strong changes of connectivity in many small groupings of neurons that are working more or less in parallel, then there is some hope of unravelling the mechanisms by these methods. Which of these extreme possibilities for the organization of the nervous system is more correct remains to be seen. In the meantime it would seem to be a prudent research strategy to seek variations of neuronal connectivity in relatively limited functional areas of the vertebrate brain that have been identified by ablation techniques as being essential for some particular (unfortunately rather complex) behavioral task.

REFERENCES


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George L. GERSTEIN, Departments of Physiology and of Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174, USA.