THE ANALYSIS OF MULTIPLE NEURAL SPIKE TRAINS

{Running head: MULTISPIKE ANALYSIS}

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Abstract: A common experimental method in neuroscience involves the recording of the activity of a single neuron. However, studies of the functional connectivity of collections of neurons and their behavior require the simultaneous recording of their activity. Current technology permits such recordings of over a hundred neurons. These recordings yield large data sets that present challenging problems in their analysis and their interpretation in biological terms. In this paper, we describe various techniques for detecting functional connections between neurons and describing the nature of those connections.

Keywords and phrases: Coherency, ross-intensity, diffusion process, gravitational clustering, Markovian interval process, multivariate point process, snowflake plot

1 INTRODUCTION

The brain consists of many cells called neurons which are the fundamental units that process information. One way that information is transmitted between neurons is through changes in their electrical activity. Most notable is the nerve impulse or action potential, which is a large fluctuation in voltage that is propagated towards other cells. For many purposes, the action potential is usually short enough to be considered a spike and modeled as a point event; a typical recording will yield a sequence of spikes, or a spike train. A common experimental method in neuroscience that dates back to its earliest days is based on the recording of the activity of a single neuron. It has been especially useful for studies of the effects of sensory inputs.

However, a substantial part of recent research in neuroscience involves the study of the cognitive or attentional state of subjects. Such studies require the simultaneous recording of many neurons. In addition, studies of the functional connectivity of collections of neurons require similar data. Current technology permits such recordings of over a hundred neurons. The resulting large data sets present challenging problems in their analysis and their interpretation in biological terms. In this paper, we describe various techniques for detecting functional connections between neurons and describing the nature of those connections.

In Section 2 we describe the requisite neurophysiology. In Section 3 we describe the various methods that have been proposed and used for the analysis of simultaneously recorded spike trains. In Section 4, we conclude with a discussion of avenues for further research.

2 PHYSIOLOGICAL BACKGROUND

We begin with a brief account of the physiology of the neuron. This is drawn from more extensive treatments intended for mathematics, signal processing, and statistics audiences are in the books by MacGregor (1987) and Tuckwell (1988, 1989); from neurophysiology texts by Levitan and Kaczmarek (1991) and Shepherd (1994); and the research literature, some of which is cited below.

Neurons differ widely according to their location and function, but they do share certain basic features. In short, a neuron gathers electrochemical signals at its soma or cell body and at its dendrites, which is a treelike projection emanating from the soma. The neuron integrates these signals. When the result of that integration exceeds a certain threshold, the neuron emits an impulse or action potential down another projection called the axon. The axon branches repeatedly, ending at swollen terminals called knobs. These knobs are adjacent to another neuron's cell body or its dendritic tree, to which they send a signal across a small gap known as the synapse. The cell that sends the signal is called presynaptic and the cell that receives it postsynaptic.

The soma contains a nucleus with its genetic materials, and organelles for the synthesis of proteins and their turnover and to meet its energy needs. The entire cell is encased by a plasma membrane, a lipid double layer which prevents the mixing of the cell contents with that of the extracellular space. The membrane also has important electrical properties. Typically, its interior is negatively charged and its exterior is positively charged because of different ionic concentrations inside and outside the neuron. The movement of ions across the membrane is largely determined by the voltage and ion concentration gradients, and the permeability of the membrane to the various ion species, such as calcium, chloride, potassium, and sodium. When there are no external signals, the membrane potential is at or near its resting state, which is in the range of -40 to -90 millivolts. Much of the cell's energy is expended in maintaining this potential difference by activating certain proteins at the membrane that transfer ions through channels across the membrane.

Signals received by the postsynaptic neuron move the membrane potential away from rest. The synaptic connections are either excitatory or inhibitory. The response of the postsynaptic cell to an excitatory input is called an excitatory postsynaptic potential or EPSP. This response is due to the release of a chemical neurotransmitter by the presynaptic cell's knob during the spike, which in turn increases the permeability of the postsynaptic membrane to certain ions, including sodium and potassium. The inhibitory postsynaptic potential or IPSP is similar, but with the permeability of potassium and chloride ions increasing while that of sodium ions remaining low. As their names indicate, EPSPs (IPSPs) move the membrane potential toward (away from) the firing threshold.

The dendritic tree and postsynaptic sites on the soma are characterized, at least to a first approximation, by passive electrical properties. That is, the inputs to them are summed linearly with the resting potential, both temporally and spatially. In contrast, a special location known as the trigger zone (usually at the base of the axon, or axon hillock) is characterized by active electrical properties, meaning that there are voltage-dependent ion channels. When the membrane potential at the trigger zone exceeds the firing threshold, a fast depolarization occurs. The axon's membrane is also active; hence the depolarization propagates down the axon and its branches without change. That signal is known as a nerve impulse, action potential, or spike. The spike for a neuron has a characteristic shape; however, the duration of the spike is sufficiently short to be regarded as a point event. Finally, a sequence of spikes from a neuron is called a spike train.

Multiple spike train recordings can arise from a single microelectrode that is implanted in the extracellular space near several axons. Current technology also allows for extended recordings from multiple extracellular microelectrodes; hence, simultaneous spike trains from over a hundred units are now possible. Regardless of how the multiple spike trains are obtained, they must first be sorted before any meaningful analysis of the data are done. That is, each spike must be identified with a particular neuron that generated it. This step requires detailed measurements on the shape of the spike itself. The extracellular action potential waveforms differ in detail according to the neuron and the relative position of the measuring microelectrode. These differences provide a basis for attributing the spikes to the neurons that generate them. There are two general classes of algorithms to do this: feature clustering and template matching. Feature clustering takes several properties of a waveform such as its height and duration and clusters them. Template matching classifies a spike on the basis of the overlap of its waveform with a set of previously determined template waveforms. For a more detailed description of these methods and a recent automatic sorting procedure, see Fee, Mitra, and Kleinfeld (1996).

3 Methods for detecting functional connections

In this section, we describe the following approaches that have been used to study multiple spike trains: moment methods, intensity function based methods, frequency domain methods, graphical methods, and parametric methods. The categories are somewhat arbitrary, for there is some overlap among them and most of these methods have their roots in the theory of point processes: see, for example, Daley and Vere-Jones (1988), Karr (1991), and Snyder and Miller (1991). One exception is the gravitational clustering method below which draws upon the cluster analysis and pattern recognition literature.

3.1 Moment methods

We start with the case of two neurons A and B which have firing times $A_1 < A_2 < \cdots < A_{n_A}$ and $B_1 < B_2 < \cdots < B_{n_B}$ over some recording period $[0, T]$. Also, let $N_i(s,t)$ be the number of spikes from neuron i occurring in the interval $(s,t]$ for $i = A, B$.

Assuming regularity, the joint spike intensity function is

$$\lambda(t,u) = \lim_{h,k\to 0} \frac{1}{hk} P\left\{N_A(t,t+h) > 0 \text{ and } N_B(u,u+k) > 0\right\}.$$

When the two neurons are independent, $\lambda(t,u) = \lambda_A(t)\lambda_B(u)$, where $\lambda_i(t)$ is the marginal intensity function for neuron i; and there is further simplification when the two processes are independent stationary Poisson processes. To estimate $\lambda(t,u)$, we generally need M replicates of the process over a time interval, say $[0,T]$. Subdivide the square $[0,T]^2$ into smaller squares of length h, let S_{ij} be the subsquare with center $(ih/2, jh/2)$, and estimate λ there by

$$\hat{\lambda}\left(\frac{ih}{2}, \frac{ij}{2}\right) = \frac{1}{Mh^2} \sum_{m=1}^{M} I\left(\text{data from trial } m \text{ in } S_{ij}\right).$$

Hypotheses about the joint intensity can then be assessed using χ^2 goodness-of-fit tests.

Assuming (at least second order) stationarity, one measure of the dependence between A and B is the cross-intensity function

$$\lambda_{AB}(u) = \lim_{h,k\to 0} \frac{1}{hk} P\left\{N_A(u+t, u+t+h) > 0, N_B(t,t+k) > 0\right\}.$$

When the two neurons are independent, $\lambda_{AB}(u)$ is the product of the individual intensities $\lambda_A\lambda_B$, so that large values of $\mid \lambda_{AB}(u) - \lambda_A\lambda_B \mid$ indicates dependence. In the neuroscience literature $\lambda_{AB}(u)$ is also called the cross-correlation function even though it is not bounded. Brillinger (1976) proposed the following estimate of $\lambda_{AB}(u)$:

$$\hat{\lambda}_{AB}(u) = \sum_{i=1}^{n_B} \sum_{j=1}^{n_A} I\left\{A_j - B_i \in (u-h, u+h)\right\},$$

where I is an indicator function. Under suitable regularity, the estimates $\hat{\lambda}_{AB}(u)$ at a finite number of points $\{u_l : 1 \le l \le L\}$ are approximately independent Poisson random variables. Hence, the cross-intensity can be used to estimate $\lambda_{AB}(u)$ at those points. This estimate is also called the cross-correlation histogram or cross-correlogram: for examples of its use and interpretation, see Aersten and Gerstein (1985), Knox (1974), and Moore, Segundo, Perkel, and Levitan (1970).

Taking a different viewpoint, Doss (1989) studied the function $K(t_1,t_2)$, which was proposed by Ripley (1976, 1977) in the context of spatial statistics:

$$K(t_1,t_2) = \frac{1}{\lambda_A} E\left[N_A(t_1,t_2) \mid B \text{ fires at } t=0\right] = \frac{1}{\lambda_B} E\left[N_B(-t_2,-t_1) \mid A \text{ fires at } t=0\right].$$

When the two neurons are independent, $K(t_1, t_2) = (t_2 - t_1)$ regardless of the marginal intensities λ_A and λ_B. Given data over a time interval $[0, T]$, Ripley proposed the estimate

$$\hat{K}(t_1, t_2) = \frac{T}{n_A n_B} \sum_{i=1}^{n_B} \sum_{j=1}^{n_A} I\left[A_j - B_i \in (t_1, t_2)\right].$$

Doss showed that under regularity conditions, as $n_B \to \infty$, $\hat{K}(t_1, t_2)$, is consistent and asymptotically normal with a variance that can also be consistently estimated from the data.

Doss also noted that K and λ_{AB} are related thus:

$$K(t_1, t_2) = \frac{1}{\lambda_A \lambda_B} \int_{t_1}^{t_2} \lambda_{AB}(u)\, du.$$

Even though these two measures of dependence are mathematically equivalent, in practice they are quite different. First, the cross-correlogram is the more common of the two. Next, the regularity conditions imposed by Doss and Brillinger do not imply each other. And while $\hat{\lambda}_{AB}$ is similar to a density estimate with variance on the order of $(hT)^{-1}$, the estimate $\hat{K}(t_1, t_2)$ is similar to that of a distribution function with variance on the order $1/n_B$. Hence, the two methods are complementary.

In practice, the cross-correlogram is the more common of the two. Typically, the analysis for more than two neurons proceeds in a pairwise fashion, although it is widely recognized that such an analysis can miss important three-way or higher order interactions. For one early attempt at dealing with this problem see Gerstein, Perkel, and Subramanian (1978). The next three sections describe several methods that attempt to deal directly with them.

3.2 Intensity function based methods

A more elaborate but related approach starts with a models the intensity functions of the counting processes of several neurons as functions of each other. The approach is due to Cox and Lewis (1972), who used it in a reliability context. Applications to neuroscience are due to Borisyuk, Borisyuk, Kirillov, Kovalenko, and Kryukov (1985), Chornoboy, Schramm, and Karr (1988), and Utikal (1997a,b). In this section, we largely follow Utikal's treatment.

For $(p+1)$ neurons consider the $(p+1)$-variate counting process $\mathbf{N} = (N, N^{(1)}, \ldots, N^{(p)})$ that monitors the spikes which occur at the random times $\{T_1, T_2, \ldots\}$, $\{T_1^{(1)}, T_2^{(1)}, \ldots\}$, ..., $\{T_1^{(p)}, T_2^{(p)}, \ldots\}$, respectively. As the notation indicates, this discussion assumes a target neuron with counting process N and trigger neurons with counting processes $N^{(i)}$ for $i = 1, \ldots, p$; however, in principle the neurons could be treated symmetrically. Under certain regularity conditions, the counting processes can be decomposed into the sum of an intensity process $\lambda(t)$ and a martingale $M(t)$:

$$\begin{aligned} dN(t) &= \lambda(t)dt + dM(t) \\ dN^{(i)}(t) &= \lambda^{(i)}(t)dt + dM^{(i)}(t), \text{ for } i = 1, \ldots, p. \end{aligned}$$

The models of neural interaction say that the intensity functions depend upon functions of the time passed since the most recent firings. Specifically, define the backward recurrence times of $N^{(i)}$ at time t to be $(t - T^{(i)}_{N^{(i)}(t)})$. Then the models for $\mathbf{N}$ have the form above with

$$\begin{aligned} \lambda(t) &= \alpha(t - T_{N(t)}, t - T^{(1)}_{N^{(1)}(t)}, \ldots, t - T^{(p)}_{N^{(p)}(t)}) \\ \lambda^{(i)}(t) &= \alpha^{(i)}(t - T_{N(t)}, t - T^{(1)}_{N^{(1)}(t)}, \ldots, t - T^{(p)}_{N^{(p)}(t)}), \end{aligned}$$

where the functions α and α^i are assumed to be unknown; further information about could be incorporated into them.

Cox and Lewis (1972) called such a process a Markov interval process for the case $p = 1$. Chornoboy, et al. (1988) used the following additive model for $\lambda(t)$:

$$\lambda(t) = \alpha\left(t - T_{N(t)}\right) + \sum_{i=1}^{p} \alpha_i \left(t - T^{(1)}_{N^{(1)}(t)}\right).$$

Utikal (1997) argues that the additive model is not appropriate for the rather sudden jumps in the intensity function that are due to inhibitory or excitatory inputs. He therefore proposes the multiplicative or proportional hazards model

$$\begin{aligned} \lambda(t) &= \lambda_0(t - T_{N(t)})\alpha(t - T^{(1)}_{N^{(1)}(t)}, \ldots, t - T^{(p)}_{N^{(p)}(t)}) \\ \lambda^{(i)}(t) &= \alpha^{(i)}(t - T_{N(t)}, t - T^{(1)}_{N^{(1)}(t)}, \ldots, t - T^{(p)}_{N^{(p)}(t)}), \end{aligned}$$

which allows the use of standard statistical packages to compute certain test statistics. At this point, specific parametric models may be imposed to test the different kinds of interactions. Utikal gives an explicit example involving inhibition and excitation, including a delay term for the transmission of a signal from a trigger neuron to the target neuron. Utikal proposes several test statistics to assess the parameters of this semiparametric model.

3.3 Frequency domain methods

Measures of dependence in the frequency domain are based on the Fourier transform of the processes. As in the spectral theory of stationary Gaussian processes, they provide an alternative to time domain methods, which have certain disadvantages. For instance, the cross-intensity function is analogous to a covariance function; thus, it is unbounded and is a dimensional quantity; Kirkwood (1979) discusses further limitations and other disadvantages of cross-correlation functions. On the other hand, regression techniques involve correlational measures which are bounded; in the Fourier domain, they involve the coherence and phase as measures of association and partial association: see Rosenberg, Amjad, Breeze, Brillinger, and Halliday (1989). They also provide system identification techniques for studying synaptic interactions: see Brillinger (1975) and Brillinger, Bryant, and Segundo (1976). In this section, we describe some of these techniques.

Assume once again that we have stationary processes with counting measures $N_i(t)$ for neuron $i = A, B$. In that case, the joint spike density $\lambda_{AB}(t, u)$ is a function of the time difference $(u - t)$; we call that $\lambda_{AB}(u - t)$ also. In most cases of interest, the processes become independent as the lag increases:

$$\lim_{|u|\to\infty} \lambda_{AB}(u) = \lambda_A \lambda_B,$$

so that the processes are mixing. Bartlett (1963) defined the cross-spectrum between two point processes at frequency ω as the Fourier transform of the cross-covariance density $q_{AB}(u) = \lambda_{AB}(u) - \lambda_A \lambda_B$,

$$f_{AB}(\omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} q_{AB}(u) e^{-iu\omega}\, du.$$

The auto-spectrum for the single process N_A is

$$f_{AA}(\omega) = \frac{\lambda_A}{2\pi} + \frac{1}{2\pi} \int_{-\infty}^{\infty} q_{AA}(u) e^{-iu\omega}\, du,$$

where the first term is due to the singularity of the autocovariance at zero. Bartlett's cross-spectrum is related to the empirical Fourier transform of the process itself,

$$\hat{N}_i(\omega;T) = \int_0^T e^{-it\omega}\, dN_i(t)$$

thus:

$$f_{AB}(\omega) = \lim_{T\to\infty} \frac{1}{2\pi T} E\left[\hat{N}_A(\omega;T)\overline{\hat{N}_B(\omega;T)}\right] \text{ for } \omega \neq 0,$$

where the bar indicates the complex conjugate. This expression leads immediately to an estimate of the cross-spectrum.

The cross-spectrum leads to a measure of dependence called the coherence, which comes from the problem of predicting a linear functional of one process from that of the other. Specifically, consider the linear combinations $\int a(t)dN_A(t)$ and $\int b(t)dN_B(t)$, where $a(t)$ and $b(t)$ have Fourier transforms $\hat{a}(\omega)$ and $\hat{b}(\omega)$, respectively. Then the mean-squared error of prediction

$$E \mid \int a(t)dN_A(t) - \mu - \int b(t)dN_B(t) \mid^2$$

is minimized by

$$\hat{b}(\omega) = \hat{a}(\omega)\frac{f_{AB}(\omega)}{f_{AA}(\omega)}.$$

The minimum value achieved is

$$\int \mid \hat{a}(\omega) \mid^2 \left(1- \mid R_{AB}(\omega) \mid^2\right) f_{AA}(\omega)d\omega,$$

where

$$\mid R_{AB}(\omega) \mid^2 = \frac{\mid f_{AB}(\omega) \mid^2}{f_{AA}(\omega) f_{BB}(\omega)}$$

is the coherence of the process at frequency ω. In addition,

$$\mid R_{AB}(\omega) \mid^2 = \lim_{T\to\infty} \mid \text{corr}\left(\hat{N}_A(\omega;T), \hat{N}_B(\omega;T)\right) \mid^2 .$$

Thus, the coherence is between zero and one; when it is zero, one process is of no use in linearly predicting the other, and when it is one, one process gives a perfect linear prediction of the other. Next, the phase spectrum is

$$\theta_{AB}(\omega) = \arg R_{AB}(\omega) = \arg f_{AB}(\omega)$$

is useful in assessing timing relations between the processes. In the simple case that B is a lagged version of A with lag h ($A_j = B_j + h$), then $\theta_{AB}(\omega) = -h\omega$.

For estimating the coherence and phase from observations over a time period $[0, LT]$, Brillinger proposed breaking up the time interval into L disjoint sections of length T, computing the empirical Fourier transform of the counting process, $\hat{N}i(\omega; T, l)$ in each interval $l = 1, \ldots, L$, and estimating the cross-spectrum thus

$$\hat{f}_{AB}(\omega) = \frac{1}{2\pi LT} \sum_{l=1}^{L} \hat{N}_A(\omega; T, l) \overline{\hat{N}_B(\omega; T, l)},$$

to get the following estimate of the coherence:

$$\mid \hat{R}_{AB}(\omega) \mid^2 = \frac{\mid \hat{f}_{AB}(\omega) \mid^2}{\hat{f}_{AA}(\omega) \hat{f}_{BB}(\omega)}$$

The estimate of the phase spectrum follows similarly. Rosenberg, et al. (1989) contains the details of inference using these measures (for example, using the transform $\tanh^{-1} \mid \hat{R}_{AB}(\omega) \mid^2$ instead of the coherence to improve the normal approximation, and setting critical values). They also show how to use the partial coherence $\mid \hat{R}_{AB|C}(\omega) \mid^2$, which measures the improvement by neuron B when neuron C is already included in the prediction of neuron A. See also Brillinger and Villa (1994) for applications of these methods to the study of neurons from *Aplysia Californica*, a sea hare that has been the subject of many neurophysiological investigations.

3.4 Graphical methods

All of the approaches discussed in this paper use graphical displays. However, we have isolated the two methods in this section because they are graphical procedures that have not been studied systematically from a theoretical standpoint.

The first is called a snowflake plot. Perkel, Gerstein, Smith, and Tatton (1975) developed it to study networks of three neurons. They postulated that the analysis of relationships between the neurons depends upon the differences between their firing times. If T_{ij} is the j^{th} firing time of neuron i, then the cyclic differences $T_{1j} - T_{2k}$, $T_{2k} - T_{3l}$, and $T_{3l} - T_{1j}$ sum to zero. Hence, they can be displayed in two dimensions. Perkel used a triangular coordinate system which treats the three neuron pairs symmetrically. The axes make a 120° angle with each other. The vertical axis corresponds to an interval the firings of the first two neurons; the signed distance along that axis is given by $T_{1j} - T_{2k}$ (upwards if the sign of this difference is positive). The other two axes correspond to the other two pairs of neurons, and are treated similarly. Thus, the point on the snowflake plot corresponding to T_{1j}, T_{2k}, T_{3l} is given by drawing the three perpendiculars to the axes at the given distances and plotting the point where they meet. The Cartesian coordinates of that intersection is

$$\left(\frac{1}{\sqrt{3}}[(T_{3l} - T_{1j}) - (T_{2k} - T_{3l})], T_{2k} - T_{1j}\right).$$

Next, let the span L be the time between the first firing and the last firing among the three neurons. The original proposal for snowflake plot suggested that all such points with span less than L (therefore all points) be plotted. While this is feasible for three neurons, it becomes less so with larger numbers of neurons, so it becomes necessary to define smaller spans and only plot points that fall within them. Of course, the choice of appropriate span depends on an assessment of the length of time during which neural interactions can be sustained: for example, can the firing of the first neuron now affect another neuron ten seconds hence ? Thus, any implementation should experiment with several choices of time window.

Perkel, et al. bounded the plot in a hexagon, hence the name snowflake plot. They used extensive computer simulations to show how different networks' firing patterns appeared on the snowflake plot. For instance, they showed that if the first neuron caused the second one to fire at a lag h with high probability (and the third neuron was firing independently of the other two), then there is a dense set of points along a line perpendicular to the first axis at a distance h from the origin. They showed the results of other networks that included inhibition, feedback loops, and coincidence detectors. They also noted that with just these synaptic types, there were over seven hundred distinct networks of three neurons. One limitation of this method is that there is not a one-to-one correspondence between a particular network and its resulting snowflake plot: several distinct networks can give similar plots. Therefore, they recommended its use for screening purposes; that is, to identify a class of possible networks from a plot rather than to expect to identify a particular network.

The extension of this graphical procedure to p neurons yielding correspondingly high dimensional data is straigtforward in some respects but presents considerable challenges. The relation among the cyclic differences above reduces the dimension of the data by only one to $(p-1)$. Thus, the problem here is that of detecting structure in high-dimensional data that is noisy. Projection pursuit is one methodology with this specific aim: see, for instance, Huber (1985) for further discussion and references.

One aim of projection pursuit is to identify interesting low dimensional projections of data. Here, the word "interesting" is often construed to mean "far from Gaussian" (Huber, p. 443), using some measure of entropy. An important issue is the computational feasibility of this procedure; that is, one major concern is the number of projections needed to be assured that a thorough search has been done. But in the problem of multiple spike trains, several directions are distinguished from our prior substantive knowledge. That is, candidates for interesting one-dimensional projections would look for evidence of pairwise excitation or inhibition. Similarly, candidates for interesting two-dimensional projections could be based on our knowledge of the various kinds of higher order interactions. Thus, the candidates for interesting projections is at least partly well defined; in short, this type of data set is well suited for exploratory data analysis using an interactive projection pursuit algorithm. The implementation of this more elaborate graphical aid, along with a careful study of its theoretical properties remains to be done.

The other graphical approach that we describe here is related to gravitational clustering, which is used in the pattern recognition literature, and was applied to this problem by Gerstein, Perkel, and Dayhoff (1985). The idea behind this approach is the following. First, the activity of neurons is mapped into motions of particles in Euclidean space of appropriate dimension. The forces exerted on particles by others are due to "charges" that represent interactions between the corresponding neurons. (Even though a charge is used here, indicating electrical attraction and repulsion, we use the term gravitational clustering because of earlier work on pattern recognition, that used the term: see Wright, 1977.) The particles are allowed to move about until they begin to cluster as a result of the charges, and the resulting aggregation of the particles into smaller subgroups then presumably represent the functionally related, or cooperative, subgroups that are sought. Of course, detailed knowledge about the nature of the connection is not available from this technique.

Gravitational clustering is an attractive approach to such problems, but a number of cautionary remarks are in order. First, there is no evidence yet that the gravitational clustering approach models a biophysical mechanism. It is a formal mathematical tool to try to detect functional connections among neurons. Gerstein, et al. recognize this limitation, saying that they are not interested in "dynamic realism but are using these dynamics only as a means to allow appropriate particles to aggregate" (p. 884). It is important, therefore, to consider variations of this model to see if the substantive conclusions about the functional connections change much when the details of the gravitational algorithms are changed. Next, this clustering procedure has the same difficulty that any clustering algorithm has: namely, without some external validation, it is not possible to say with any confidence that the derived clusters are indeed correct, rather than being artifacts of the clustering process. A related point here is that there are no guidelines on deciding when to stop the aggregation process; when a certain connection between two neurons is strong, the algorithm should quickly join them into one cluster; more extensive simulation studies are needed to understand the quantitative relationship between the time to joining and the strength of connection. This problem is similar to the difficulty in hierarchical clustering algorithms, where the choice of the appropriate time to stop splitting the sub-clusters is not clear, especially when there is no external validation.

3.5 Parametric methods

When there is extensive information about a particular network, many detailed questions are bound to arise. In that case, it is often fruitful to propose parametric models to try to answer such questions.

One example comes from Brillinger's (1988) analysis of data from a network of neurons from *Aplysia*. Let the spike trains of two neurons, say A and B be modeled as the point processes $A(t)$ (the number of spikes by A up to time t) and $B(t)$, respectively. Suppose that the effect of A on B is of interest. If A fires at time τ, let the postsynaptic effect on

B be $a(t-\tau)$; a is called the summation function. If $\gamma(t)$ is the time since B last fired, then the potential at the trigger zone for B is

$$V(t) = \int_0^{\gamma(t)} a(u)dA(t-u) = \sum a(t-\tau_j).$$

Brillinger allows the firing threshold V_f to be the random function of t, $V_f(t) = V_f + \epsilon(t)$, where $\epsilon(t)$ is zero mean noise used to represent the contributions of unmeasured neurons influencing B. In order to include B's own effect on its internal potential, spontaneous firings, and its refractoriness, Brillinger suggested adding a recovery term $\theta_1\gamma(t)+\theta_2\gamma(t)^2+\theta_3\gamma(t)^3$ to the membrane potential. Then the probability that B fires at time t given its past is given by the probit model

$$p_t = \Phi\left(\sum_{u=0}^{\gamma(t)-1} a(t)A(t-u) + \theta_1\gamma(t) + \theta_2\gamma(t)^2 + \theta_3\gamma(t)^3\right);$$

hence, the likelihood given the firing times of A and B is

$$\prod_t p_t^{B(t)}(1-p_t)^{1-B(t)}.$$

Brillinger suggests that the parameters of this model be estimated by maximum likelihood, which because of the probit model can be done using standard GLIM routines.

For the *Aplysia* data, Brillinger showed that the estimates of θ_i were all significant, and also provided an estimate of the summation function. Thus, a detailed study of the neuron's recovery is possible from this analysis. This method in principle extends to large numbers of neurons; it can also be modified to include other effects such as adaptation. See also Brillinger and Villa (1994) for a recent example of the use of semiparametric modeling. The use of a linear summation function can be extended to include higher order nonlinear terms. Such terms better model presynaptic signals that are bursty, regular, or very fast. Such methods are the point process versions of the Volterra expansions for Gaussian processes. For an account of the theory, see Brillinger (1975, 1992); and for examples of its use, see Brillinger, Bryant, and Segundo (1976).

Another example of a parametric model is motivated by Gerstein and Mandelbrot (1964), who proposed the first random walk model for the firing of a single neuron. Their model can be derived using the following argument. Let $\{N_i^E(t) : i = 1, \ldots, n_E\}$ and

$\{N_j^I(t) : j = 1, \ldots, n_I\}$ represent the excitatory and inhibitory input Poisson processes, respectively. Suppose that N_i^E and N_j^I have rates λ_i^E and λ_j^I, and magnitudes α_i^E and α_j^I, respectively. Then the stochastic differential equation for $V(t)$ is

$$dV = \sum_{i=1}^{n_E} \alpha_i^E dN_i^E - \sum_{j=1}^{n_I} \alpha_j^I dN_j^I.$$

In the limit that the intensities tend to infinity and the magnitudes of the inputs tend to zero appropriately, the Poisson inputs are well approximated by white noise dW. Thus, $dV = \mu dt + \sigma dW$, where μ is the mean input per unit time, and σ is the standard deviation of that noisy input. Thus, $V(t) = V_0 + \mu t + \sigma W(t)$ is a Brownian motion starting at V_0 with drift μ and diffusion or variance σ^2. For a constant firing threshold V_f, the random time to firing is $T = \inf\{t > 0 : V(t) = V_f\}$. And when the net input is excitatory, $\mu > 0$ and T is a proper random variable with the inverse Gaussian density

$$f(t; d, \nu) = \frac{d}{\sqrt{2\pi t^3}} \exp\left[-\frac{(d - \nu t)^2}{2t}\right],$$

where $\nu = \mu/\sigma$ is the standardized drift and $d = (V_f - V_0)/\sigma$ is the standardized distance from the initial and firing potentials. For an account of other random walk or diffusion models of single neuron activity, see Ricciardi (1994).

Iyengar (1985) extended this work to a "Zeitgeber" model, in which an external stimulus (the "time-giver") drives two other neurons. This model assumes that the noise affecting each neuron is the sum of two components: a shared component and an independent specific component. The other assumptions for the model are similar to those of Gerstein and Mandelbrot above, so that the parameters of this model have similar physical interpretations. These assumptions lead to a correlated two-dimensional Brownian motion, $(X_1(t), X_2(t))$, where the correlation is related to the noise variance ratios thus. Let the noise variances be σ^2 for the shared noise and σ_i^2 for the noise particular to the i^{th} neuron. Then,

$$Corr\,(X_1(t), X_2(t)) = \left[\left(1 + \frac{\sigma_1^2}{\sigma^2}\right)\left(1 + \frac{\sigma_2^2}{\sigma^2}\right)\right]^{-1/2}.$$

Here, the firing time distributions are given by the joint distribution of (τ_1, τ_2), where $\tau_i = \inf\{t : X_i(t) = a_i\}$ and a_i is the distance between the resting potential and the firing threshold for the i$^{\text{th}}$ neuron. The derivation of the joint distribution of (τ_1, τ_2) involves the solution of the heat equation inside a wedge in $\Re^2$, with specified initial and boundary conditions, and the use of the strong Markov property. As an aside, recall that the inverse Gaussian distribution has statistical properties that mirror those of the Gaussian distribution, and admit easy estimation and testing procedures. The statistical properties of this model for a bivariate inverse Gaussian have not been studied or studied and compared with other proposals that are in the literature. Furthermore, extensions of this model to more neurons leading to higher dimensional analogs of this model also have not been done.

4 DISCUSSION

Some of the methods above, such as the use of the cross-intensity function, are rather generic in that they are used for point processes arising in many areas, not just neuroscience. Other methods, such as Brillinger's use of a recovery function, are tailored to the biophysical details of the neuron. Still other methods, such as Utikal's interacting counting process approach are flexible enough to model both neurophysiological phenomena and other phenomena (in this case, reliability of components). All of these methods have a role in neurophysiology. The generic methods play an important role during the initial phase of an investigation when little is known about the network. As more information is gathered and initial hypotheses are refined, parametric approaches come to the fore to assess them.

The routine gathering of such data is still relatively recent; therefore, there are many outstanding problems. For example, the graphical methods that have been proposed have both proved themselves to be useful screening devices, but they do not have adequate theoretical support. That is, a careful study of the properties of these methods to find out when they work well and what their limitations are, along with computational procedures to store and process the huge data sets have yet to be done.

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