

# EXTRACELLULAR RECORDING FROM MULTIPLE NEIGHBORING CELLS: A MAXIMUM-LIKELIHOOD SOLUTION TO THE SPIKE-SEPARATION PROBLEM

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## INTRODUCTION

In recent years considerable attention among extracellular electrophysiologists has focused on the problem of simultaneously recording the activity of multiple neurons in behaving animals. Such recordings, it is hoped, will provide much-needed insight into the dynamics of neural ensemble computation and coding. Of particular interest are recordings from neighboring neurons, for example cells that lie within a single column of neocortex. Such cells are likely to share functional roles and to possess the anatomical interconnectivity needed for ensemble coding, making them plausible participants in local computational and signaling circuits.

Single cortical columns can be as little as  $30\mu\text{m}$  in cross-section, and so it is difficult to introduce multiple independent electrodes into the same column *in vivo*. It is important, therefore, to distinguish the extracellular traces of action potentials from different cells gathered by a single electrode. The problem is made significantly easier by the use of a multi-tip electrode, for example the four-wire bundle commonly called a *tetrode*, that provides several slightly different electrical view-points on the same group of cells. We have recently adapted the tetrode technology, introduced by Recce and O'Keefe<sup>1</sup> for chronic recording in rat hippocampus, for use in behaving monkey experiments<sup>2,3</sup>.

In the current paper we discuss a solution to the problem of separating waveforms from multiple cells in a tetrode recording. We adopt an explicitly probabilistic approach, constructing a parametric latent-variable model from which the data are presumed to be generated. We find estimates of the parameters of the model using maximum-likelihood techniques, and then, using these parameter values, infer the values of the latent variables, in particular the times of firing of the various cells. This two-stage maximum-likelihood process reflects a commonly made approximation to the full Bayesian posterior over the latent-variables<sup>4,5</sup>. The correct estimates of the firing

times should be made by integrating over the possible parameter values; for strongly peaked posteriors, however, we can approximate this integral by evaluating the posterior at the most probable value of the parameters. Given a weak prior, this point is the same as the maximum-likelihood estimate.

## DATA COLLECTION

Information about the construction of the electrode is available in an earlier paper.<sup>3</sup> Here we mention only those parameters of the construction and data collection that are relevant to the spike recognition problem.

The tetrode is a bundle of four individually insulated 13 $\mu$ m-diameter wires twisted together and cut so that the exposed ends lie close together. The potential at the tip of each electrode is amplified (custom electronics), low-pass filtered (9-pole Bessel filter,  $f_c = 6.4$  kHz) to prevent aliasing and digitized ( $f_s = 12.8$  to 20 kHz) (filters and A/D converter from Tucker Davis Technologies). This data stream is recorded to digital media; subsequent operations are currently performed off-line.

In preparation for inference, candidate events (where at least one cell fired) are identified in the data stream. The signal is digitally high-pass filtered ( $f_c = 0.05f_s$ ) and the root-mean-square (RMS) amplitude on each channel is calculated. This value is an upper bound on the noise power, and approaches the actual value when the firing rates of resolvable cells are low. Epochs where the signal rises above three times the RMS amplitude for two consecutive samples are taken to be spike events. The signal is upsampled in the region of each such threshold crossing, and the time of the maximal subsequent peak across all channels is determined to within one-tenth of a sample. A 1 ms section is then extracted at the original  $f_s$  such that this *peak time* falls at a fixed position in the extracted segment. One such waveform is extracted for each threshold crossing.

## GENERATIVE MODEL

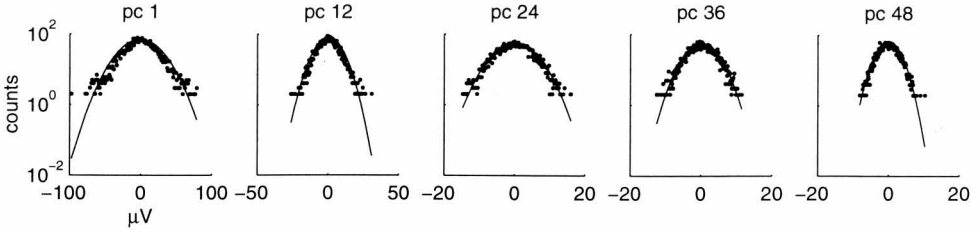
Our basic model is as follows. The recorded potential trace  $V(t)$  is the sum of influences that are due to resolvable *foreground* cells (which have a relatively large effect) and a *background* noise process. We write

$$V(t) = \sum_{\tau} (c_1^{\tau} S_1(t - \tau) + c_2^{\tau} S_2(t - \tau) + \dots) + \eta(t) \quad (1)$$

Here,  $c_m^{\tau}$  is an indicator variable that takes the value 1 if the  $m$ th cell fires at time  $\tau$  and 0 otherwise. If cell  $m$  fires at  $\tau$  it adds a deflection of shape  $S_m(t - \tau)$  to the recorded potential. The effect of all background neural sources, and any electrical noise, is gathered into a single term  $\eta(t)$ . For a multichannel probe, such as a tetrode, all of  $V(t)$ ,  $\eta(t)$  and  $S_m(t)$  are vector-valued.

In this paper we take the waveform shape  $S_m(t)$  to be constant. In other work<sup>6</sup> we discuss the possibility of variation in the underlying waveforms independent of the common noise source  $\eta(t)$ . The  $c_m^{\tau}$  are assumed to be independently Bernoulli distributed for each  $\tau$  and  $m$ , with constant firing probabilities, except that we will enforce a refractory period between spikes from the same cell.

The distribution of the noise,  $\eta(t)$ , may intuitively be expected to be Gaussian; if the waveform of a spike on the cell membrane is constant (and the preparation well shielded), the noise in the recording is composed of thermal noise at the tip, noise in the



**Figure 1.** Distribution of background noise. We extract 1ms segments from a bandpassed recording sampled at 16 kHz from a four-channel electrode, avoiding the foreground spikes identified as described in the text. Each segment is thus a 64-dimensional object. We find the principal components of the ensemble of such vectors, and construct histograms of the projections of the vectors in these directions. A few of these histograms are shown on a log-scale (points), as well as a zero-mean Gaussian fit to the distribution projected along the same axes (lines).

signal amplification and conditioning electronics, and superimposed waveforms due to the electrical activity of many “background” cells too far away to be distinguished in the sorting process. The thermal and electronic noise are known to be Gaussian. Provided that the number of cells contributing to the background noise is large, and their firing relatively independent, the central limit theorem suggests that their influence too may be taken to be well approximated by a Gaussian, although not necessarily an isotropic one. The empirical distribution of the noise is shown in figure 1. It is clear that the Gaussian is a reasonable description, although a slight excess in kurtosis is visible in the higher principal components.

The distribution of  $\eta(t)$ , then, is described by a single correlation function. In practice the noise will be correlated over only a short time-lag and such a function will be equivalent to a Toeplitz covariance matrix,  $\Sigma_\eta$ . The parameters of the model to be discovered, then, are  $\Sigma_\eta$ ,  $S_m$  and  $p_m$ . Once these are estimated we can proceed to find estimates for the spike times  $c_m^\tau$ , which are the quantities we ultimately seek.

## ESTIMATING NOISE PARAMETERS

The covariance of the background noise is measured directly from segments without foreground spikes. We can use the covariance to whiten the noise so that further inference proceeds in the context of decorrelated noise. To do this we fit an autoregressive (AR) process of order greater than the measured correlation length to the background, using the Yule-Walker equations. We then subtract the forward predictor given by this model. It is important to note that the resultant signal is not white: only the background is decorrelated. The complete signal is quite non-Gaussian and so cannot be decorrelated by second-order methods.

## ESTIMATING WAVEFORMS

We now extract candidate spike waveforms from the whitened signal around the threshold crossings, as was described before. We will write  $V^i$  for the  $i$ th extracted event and assume that it represents a spike from a single cell (that is, only one of the  $c_m$  is non-zero). This is an unreasonable assumption; we can shore it up partially by eliminating from our collection of  $V^i$  segments that appear heuristically to contain overlaps (for example, double-peaked waveforms). Ultimately, however, we will need to

make our inference procedure robust enough that the parameters describing the model are well estimated despite the errors in the data.

The benefit of this assumption is that it allows the waveforms to be modeled as arising from a mixture distribution, each waveform coming from a single multivariate Gaussian cluster with mean  $S_m$  (which is now viewed as a vector). We can achieve robustness by introducing additional mixture components, one centered at zero to account for false triggers of the event extraction heuristic and others with broad covariances to “mop up” any remaining overlaps. In what follows we will not write these clusters explicitly.

We consider the case of  $M$  cells, each of which generates events drawn from a multivariate Gaussian distribution with mean  $S_m$  and identity covariance (assuming that the noise has been whitened). For conciseness, we write the probability of a given observation  $V^i$  under the  $m$ th Gaussian as  $G_m(V^i) = (2\pi)^{-2/d} \exp\left(- (V^i - S_m)^T (V^i - S_m) / 2\right)$ . The prior probability of a spike arising from the  $m$ th cell is written  $p_m$ , and the parameters are collected into a single vector  $\theta$ . Neglecting, for the moment, the refractory constraint, the log-likelihood of the mixture model, given a sequence of observations  $\{V^i\}$ , is simply

$$l(\theta; \{V^i\}) = \sum_i \log \left( \sum_m p_m G_m(V^i) \right). \quad (2)$$

The effect of the refractory period on this likelihood will be discussed below.

The EM approach to the fitting of such a Gaussian mixture model is well known<sup>7, 8</sup>. We introduce indicator variables  $c_m^i$ , similar to the  $c_m^r$ . For each  $i$  exactly one of the corresponding  $c_m^i$  assumes the value 1 and the rest 0, thus indicating the cluster from which the given observation was drawn. If the  $c_m^i$  could be observed and thus used to augment the data  $V^i$  the (complete data) log-likelihood would be

$$l(\theta; \{V^i, c_m^i\}) = \sum_i \sum_m c_m^i \left( \log p_m + \log G_m(V^i) \right). \quad (3)$$

The indicator variables allow us to bring the logarithm inside the summation, considerably simplifying the task of model fitting.

The EM procedure now proceeds in two steps, iterated to convergence. In the first (E) step we find the expected value of the log-likelihood (3) under the distribution  $P(c_m^i | \{V^i\}, \theta)$ . Since the log-likelihood is linear in the variables  $c_m^i$ , this simply involves replacing the  $c_m^i$  with the corresponding expected value. We write  $r_m^i$  for  $E[c_m^i]$  (these are often called the *responsibilities*) and obtain

$$E[l(\theta; \{V^i, c_m^i\})] = \sum_i \sum_m r_m^i \left( \log p_m + \log G_m(V^i) \right), \quad (4)$$

with, by Bayes’ rule,

$$r_m^i = P(c_m^i | \{V^i\}, \theta) = \frac{p_m G_m(V^i)}{\sum_{\tilde{m}} p_{\tilde{m}} G_{\tilde{m}}(V^i)}. \quad (5)$$

The second (M) step involves maximizing this expected log-likelihood over the model parameters  $\theta$ . This is easily seen to reduce to independently fitting each of the Gaussians to the data, weighted by the corresponding responsibilities.

We now consider the alterations to this standard approach that are necessary to accommodate the refractory constraint. The likelihood  $l(\theta; \{V^i, c_m^i\})$  is identical to (3) in most cases, but for sequences of  $c_m^i$  that violate the constraint it diverges to  $-\infty$ . In taking the expected value of the log-likelihood, however, the probability of such

divergence is 0 and so the E step results in a form similar to (4), except that the values of  $E[c_m^i]$  are different. We will use  $s_m^i$  to denote these new responsibilities, reserving  $r_m^i$  for the values in (5).

To obtain the new responsibilities, consider first the simple case where only two spikes have been observed and the second appears within a refractory period of the first. We have a joint distribution over  $c_m^1$  and  $c_n^2$  with

$$P(c_m^1, c_n^2) = \begin{cases} 0 & \text{if } m = n \\ r_m^1 r_n^2 / Z & \text{otherwise} \end{cases} \quad (6)$$

where  $Z = \sum_m \sum_{n \neq m} r_m^1 r_n^2$  is an appropriate normalizing constant. The expected values we seek are then just the marginals of this joint distribution, e.g.

$$s_m^1 = \sum_{n \neq m} r_m^1 r_n^2 / Z = r_m^1 (1 - r_m^2) / Z \quad (7)$$

where we have used the fact that  $\sum_n r_n^i = 1$ .

This result easily generalizes to the case of many spikes

$$s_m^i = \frac{r_m^i}{Z^i} \prod_{i,j \text{ refractory}} (1 - r_m^j) \quad (8)$$

where  $Z^i$  is the appropriate normalizer.

The M step is still a weighted Gaussian estimation, the weights now being the new responsibilities  $s_m^i$ .

## SPIKE TIME INFERENCE

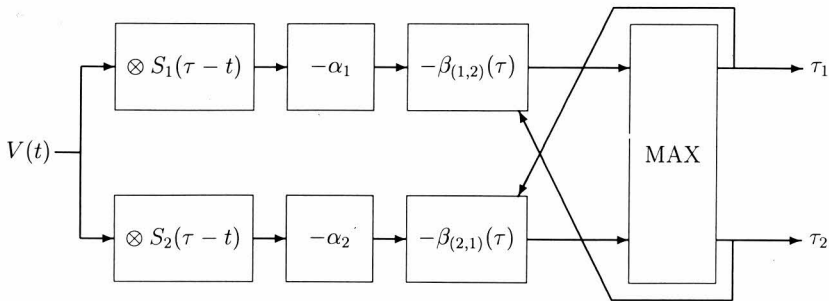
Three issues are left unresolved by this clustering process. First, the event identification heuristic could be improved upon once the true spike shapes have been determined. Second, if all events are to be clustered the sorting process must occur off-line, ruling out experiments in which rapid feedback about the cells' responses is needed. Third, superposed events have been discarded, rather than resolved into their constituent spike forms.

These issues are addressed by building matched filters for the identified spike waveforms. The filters are applied to the recorded signal to identify spike occurrences, thus improving event detection. The same filtering process can also be applied to further data from the same site as they are collected, facilitating real-time experimental monitoring of subsequent data. The algorithm is easily implemented on DSP hardware. Finally, as we shall see, the filtering process resolves spike superpositions.

Our filtering process arises as a direct solution to the maximum likelihood statement of the problem. For clarity, we will restrict ourselves here to the case of a single-channel continuous signal with spikes from two cells corrupted by white Gaussian noise. This simple formulation demonstrates the essence of the approach. The treatment is easily extended to four discrete-sampled channels with multiple cells.

We consider a recorded signal  $V(t)$  which is composed of spikes of two shapes  $S_1(t)$  and  $S_2(t)$  occurring at times  $\tau_1^i$  ( $i = 1 \dots N_1$ ) and  $\tau_2^i$  ( $i = 1 \dots N_2$ ) respectively, and which is corrupted by stationary Gaussian noise with zero mean and standard deviation  $\sigma$ . The log-likelihood of the model given by the times  $\{\tau_1^i\}$  and  $\{\tau_2^i\}$  is proportional to

$$l(\{\tau_m^i, S_m\}; V(t)) \propto -\frac{1}{2\sigma^2} \int \left( V(t) - \sum_{i=1}^{N_1} S_1(t - \tau_1^i) - \sum_{i=1}^{N_2} S_2(t - \tau_2^i) \right)^2 dt. \quad (9)$$



**Figure 2.** Block diagram illustrating the adaptive-threshold filter-based detection scheme for two spike shapes. The symbol  $\otimes$  represents convolution. Consult text for an explanation of the other symbols.

We wish to maximize this likelihood with respect to the model.

We expand the square (bearing in mind that spikes from a single source cannot overlap), and, with some rearrangement and removal of terms independent of the times  $\tau_m^i$ , we obtain the following expression:

$$\begin{aligned}
 l(\{\tau_m^i, S_m\}; V(t)) \propto & \sum_{i=1}^{N_1} \left( 2 \int V(t) S_1(t - \tau_1^i) dt - \int S_1(t)^2 dt \right) \\
 & + \sum_{i=1}^{N_2} \left( 2 \int V(t) S_2(t - \tau_2^i) dt - \int S_2(t)^2 dt \right) \\
 & - \sum_{\tau_1^i, \tau_2^j \text{ close}} \left( 2 \int S_1(t - \tau_1^i) S_2(t - \tau_2^j) dt \right).
 \end{aligned} \quad (10)$$

The notation “ $\tau_1^i, \tau_2^j$  close” means that the sum is taken over those pairs of times where the two associated spike shapes overlap.

The integrals within the first two summations dictate the matched filter form. The output of the  $m$ th matched filter (which has impulse response  $S_m(-t)$ ) is compared to the threshold set by the squared power of the filter,  $\alpha_m$ , to compute a time-dependent quantity  $\mathcal{F}_m$ .

$$\alpha_m = \frac{1}{2} \int (S_m(t))^2 dt \quad (11)$$

$$\mathcal{F}_m(\tau) = \int V(t) S_m(t - \tau) dt - \alpha_m \quad (12)$$

Disregarding, for the moment, the final term in (10), this implies that we increase the likelihood of the model by choosing times  $\tau_m^i$  which fall at the peaks of  $\mathcal{F}_m$ , provided that those peaks are positive and do not fall closer together than one spike-width.

The final term in (10) describes the interaction between the spikes. We can view it as a time-dependent modification of the threshold  $\alpha_m$  caused by a spike in cell  $n$ . If a spike in the  $n$ th cell has been detected at  $\tau_n$ , then the adjustment at time  $\delta$  after the detection is  $\beta_{(m,n)}(\tau_n + \delta)$ , leading to output  $\hat{\mathcal{F}}_m$ .

$$\beta_{(m,n)}(\tau_n + \delta) = \int S_m(t - \delta) S_n(t) dt \quad (13)$$

$$\hat{\mathcal{F}}_m(\tau) = \int V(t) S_m(t - \tau) dt - \alpha_m - \sum_{j \neq i} \beta_{(m,n)}(\tau) \quad (14)$$

Thus, whenever a spike is detected according to (12), the thresholds for other spike shapes are transiently altered by the term  $\beta$ . This allows the correct resolution of superpositions. The process is illustrated in figure 2.

This adaptive-threshold process is thus seen to find the maximum likelihood estimate of the spike times, given the signal, the noise characteristics and the spike shapes.

## DISCUSSION

The algorithms presented here are optimal, in the maximum-likelihood sense, given the simple Gaussian generative model described. They resolve overlaps correctly and can be run in real time. In this respect, they represent a significant advance over commonly used approaches to this problem.

The model examined may well prove to be too simple to apply to all experimental preparations<sup>9,10</sup>. It is, however, possible to extend this same framework to describe non-Gaussian variability as well as dynamic variation of spike shape during bursts<sup>6</sup>.

## ACKNOWLEDGMENTS

This work has benefited considerably from important discussions with both W. Bialek and S. Roweis. J. Hopfield has provided invaluable advice and mentoring to MS. Funding for various components of the work has been provided by the Keck Foundation, the Sloan Center for Theoretical Neuroscience at Caltech, the Center for Neuromorphic Systems Engineering at Caltech, the Office of Naval Research and the National Institutes of Health.

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