Simultaneous Paired Intracellular and Tetrode Recordings for Evaluating the Performance of Spike Sorting Algorithms

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Abstract

Objective evaluation of spike sorting algorithms such as those used to decompose tetrode recordings into distinct spike trains requires *a priori* knowledge of the correct classification for a given recording. Intracellular recording can unambiguously assign spikes to a single neuron, and thus provide correct classification if signals from that neuron concurrently appear in a tetrode recording. Simultaneous single or paired intracellular and tetrode recordings are used here to evaluate a contemporary spike sorting algorithm for isolated as well as overlapped events. These data are also used to demonstrate that overlapping extracellular spikes combine additively, and to introduce a means for quantifying variability in action potential shape.

Key words: tetrode, spike sorting, spike linearity, overlap resolution, NSCA

1 Introduction

Tetrodes (1) offer significant advantages in addressing the problem of sorting spikes from multiunit recordings into distinct trains (2; 4; 5; 6). These bundles of fine wires have four close-packed recording sites to generate multiple views of the electrical landscape at the electrode tip. A cell which is closer to one site will produce a larger waveform there than at the other, slightly more distant recording sites. As each cell has a unique spatial position, and thus a unique pattern of distances to each group of recording sites, each neuron should, in principle, present a characteristic pattern of waveforms across the multiple channels amenable to algorithmic extraction.

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[2] Supported by NIH, NIMH, the Keck Foundation, the Alfred P. Sloan Foundation, NSF, and ONR.

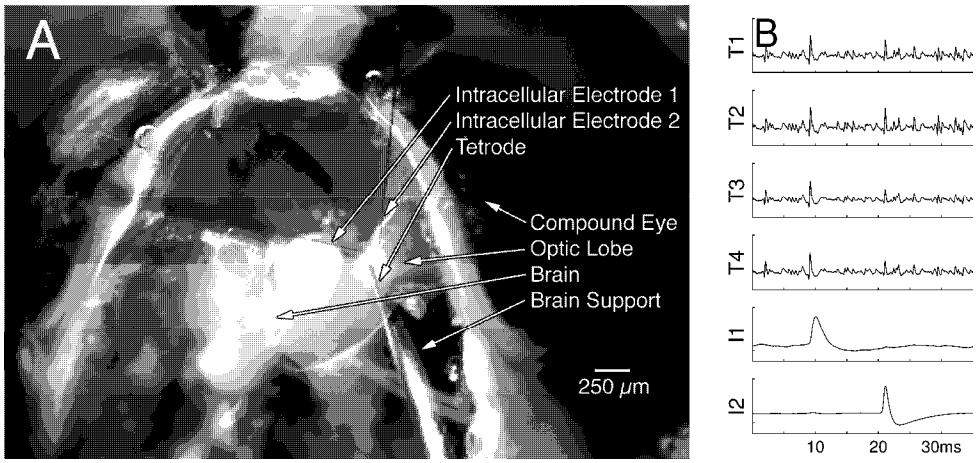

Fig. 1. **A**: Photomicrograph of locust head capsule taken directly following a recording. Spontaneous activity was recorded for up to 15 minutes at a time. The retinotopic organization of the insect optic lobes, combined with the existence of large integrative neurons in the lobula, allowed simultaneous intracellular impalement of tetrode-recorded neurons at distances of up to 100 μm from the tetrode tip. **B**: Example of simultaneously recorded data. T_1–T_4 are the four tetrode extracellular voltages; I_1 and I_2 the two intracellular voltages. Based on spike amplitudes, rising phase shapes, after-hyperpolarization amplitude, and synaptic background, the I_1 electrode, here, was likely impaled in a distal dendrite far from the spike initiation zone, the I_2 electrode in a primary neurite relatively near the spike initiation zone.

Evaluating such spike sorting algorithms requires an independent verification of firing times for the collection of neurons being recorded. While synthetic data can be used for this purpose, doing so requires assumptions about signal characteristics which may interfere with objective evaluation. An *in situ* approach, such as intracellular recording, is therefore preferable. We elected to combine dual intracellular recording with tetrode recording, allowing simultaneous verification of signals from two neurons. This provides not only the desired spike assignment confirmation, but also allows quantification of variability in extracellular action potential shape and explicit testing of the assumption of linearity in the extracellular medium during overlapped events.

2 Methods

Experiments were carried out *in vivo* on adult female locusts (*Schistocerca americana*). Animals were restrained dorsal side up, the head was immobilized with beeswax, and a watertight beeswax cup was built around the head for saline superfusion. A window was opened in the cuticle of the head capsule between the eyes, and air sacs on the anterior surface of the brain carefully removed. For stability, the esophagus was sectioned anterior to the brain, and the gut removed through a subsequently ligatured distal abdominal section.

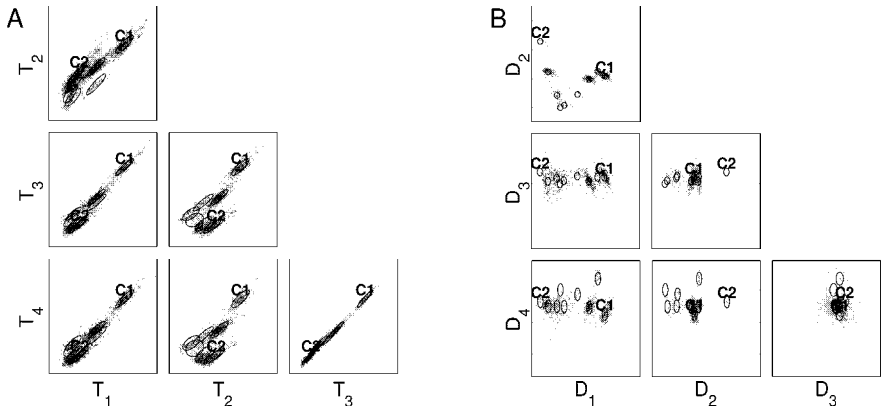

Fig. 2. Clustergrams of sorting output. Ellipses indicate fits generated from cluster analysis of the first four noise-whitened principal components. Clusters C_1 and C_2 correspond to the impaled neurons from I_1 and I_2, respectively. **A**: Action potential peak heights for tetrode channels T_1–T_4 plotted against each other (axes in scaled volts). **B**: The same data transformed to the optimal 4D linear discriminant subspace D_1–D_4 (axes in arbitrary units), markedly improving the discriminability of clusters. Cluster C_2, difficult to isolate in **A**, is clearly separated in **B**.

The brain was treated with protease (Sigma type, XIV), gently desheathed, and supported with a small metal platform. The head capsule was continuously superfused with oxygenated room-temperature physiological saline (in mM: 140 NaCl, 5 KCl, 5 $CaCl_2$, 4 $NaHCO_3$, 1 $MgCl_2$, 6.3 HEPES, pH 7.0).

Intracellular recordings were made using conventional sharp glass microelectrodes pulled with a horizontal puller (Sutter P-87), filled with 0.5 M KAc, for resistances of 100–300 MΩ. Intracellular recordings were done in bridge mode using an Axoclamp 2A amplifier (Axon Instruments) from the third optic lobe (lobula). Data were collected from 28 single neuron and 6 paired intracellular recordings, all with simultaneous tetrode recordings, from 7 animals.

Tetrode recordings were made using electrodes composed of 4 strands of 15 μm insulated tungsten wire (California Fine Wire) twisted at approximately 1 turn/mm and glued with common cyanoacrylate (3). The tip was freshly cut at an acute angle using fine surgical scissors before each penetration for impedances of 0.4–0.7 MΩ at 1 kHz. Tetrodes were placed in the lobula, 50–100 μm medial to the site of intracellular penetrations (see Figure 1A).

All signals were amplified, low-pass filtered at 10 kHz (8-pole analog Bessel with gain, BrownLee Precision), digitized at 50 kHz with 16-bit resolution (Tucker Davis Technologies), and written to compact disc. The continuous voltage recordings were digitally high-pass filtered at 250 Hz and events were obtained by threshold detection on either the tetrode or intracellular signals only. Each set of extracted events was clustered using Expectation Maximization (EM) techniques (4; 5) implemented with in-house MATLAB code.

3 Results and Discussion

Figure 1B shows a typical recording from this preparation that will be used as a running example. Each of the large spikes appears across the four tetrode channels with a characteristic amplitude signature, some with simultaneous action potentials in the intracellular channels. The tetrode recording contains signals from (at least) four identifiable cells, two of which were impaled with the intracellular electrodes. Figures 3A–B show the spike-triggered average waveforms of the six channels, as triggered on I_1 and I_2, revealing the tetrode waveform associated with action potentials from the impaled cells.

3.1 Spike sorting performance

The performance of a spike-sorting algorithm based on maximum likelihood techniques (4; 5) on these recordings was evaluated. The algorithm consists of two stages: (a) automated robust fitting techniques are first used to discover clusters of events, and then (b) optimal filters based on these clusters are used to decompose the signal into distinct spike trains.

During the first, clustering, stage, the purity of each cluster is of highest interest (see Figure 2). Cluster C_1 contained 862 spikes of which 831 (96.3%) came from a single cell (as verified by I_1). Only 3 additional spikes from that cell (0.4%) were misassigned to other clusters. Cluster C_2 contained 190 events with 183 (96.3%) of these from the other impaled cell (I_2). Ten spikes from this cell (5.5%) were misassigned.

At present a strong test of the second, filtering, stage has not been performed due to difficulties with suitable decorrelation (whitening) of the signal. However, a preliminary run on unwhitened data was performed to detect C_1 spikes. There were 1052 such I_1-identified events, including overlaps. Of these, 999 were correctly detected by C_1 filter (95.0%), however 124 additional spikes were incorrectly grouped in with these (11.8%). Such false-positives are likely to be reduced once the filtering procedure is correctly applied.

3.2 Linearity of overlaps

To evaluate the electrical linearity of the extracellular medium, overlapping events were modeled using spike-triggered averages of non-overlapped events. Instances when the two impaled cells fired at nearly the same time were selected, and the recordings of these events were compared to the sum of M_1 and M_2 appropriately time shifted as determined from the intracellular signals

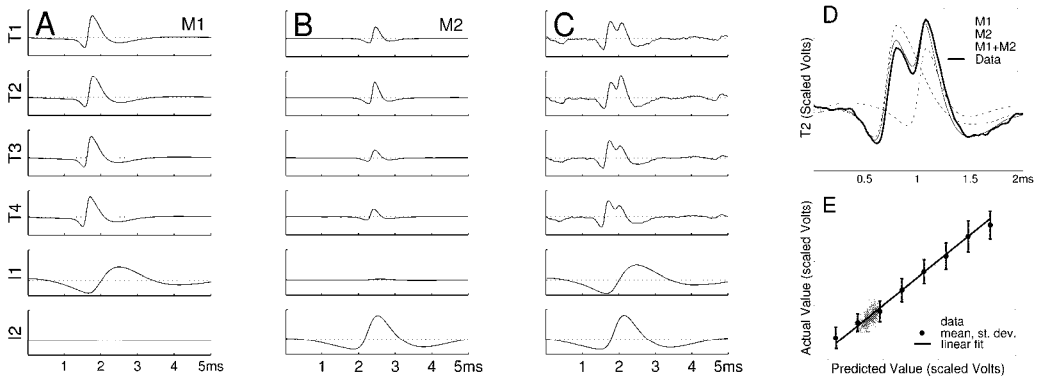

Fig. 3. **A**, **B**: Spike-triggered average waveforms of the six channels, triggered on each of the two intracellular channels. M_1 and M_2 are the means of I_1 and I_2 events, respectively. I_1 and I_2 have been high-pass filtered at 250 Hz for accurate event detection, resulting in waveforms uncharacteristic of standard intracellular records. **C**: Example overlap event. **D**: Expanded view of overlap T_2, with the recorded waveform (heavy line), time-shifted M_1 and M_2 (dotted lines), and predicted additive combination $M_1 + M_2$ (fine line). **E**: Sample-by-sample predicted versus actual values for overlaps (dots, $n_{event} = 88$), with linear fit (solid line, $y = 1.016x - 0.009$), and standard deviations (filled symbols).

(see Figure 3). For additively combined spikes, the simple sum of the models was expected to accurately predict the shape of overlap events. Actual voltage is shown as a function of predicted voltage in Figure 3E, along with a linear fit that closely matches the unity slope line, demonstrating that the additive assumption is valid across the observed voltage range.

3.3 Spike Waveform Variability

The variability of extracellular spikes was examined by computing the point-by-point covariance of all I_1-triggered tetrode events. The covariance matrix was decomposed into stationary and non-stationary parts by an EM algorithm equivalent to factor analysis in the Fourier-transformed space (see Figure 4) which, by analogy with PCA, we call Non-Stationary Components Analysis. The stationary component corresponds to additive background noise, whereas the non-stationary component corresponds to intrinsic waveform variability.

4 Conclusions

In conclusion, preliminary analysis of simultaneous paired intracellular and tetrode recordings demonstrates that one spike sorting algorithm (4; 5) performed 96% correct isolation and classification of signals from a tetrode. Fur-

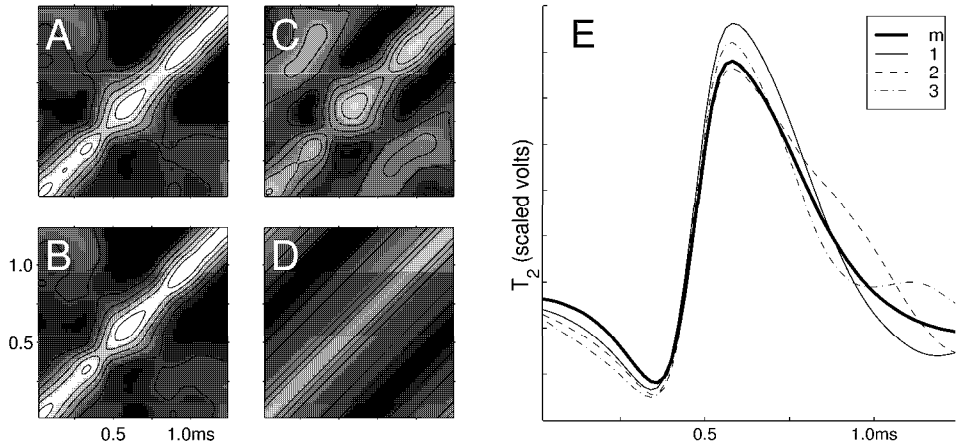

Fig. 4. Covariance decomposition of T_2 from I_1 events (**A**) into non-stationary (**C**) and stationary (**D**) components. Reconstruction (**B**) from the two components closely matches the original (compare **B** to **A**). **E**: Modes of variability. The mean (heavy line) and first three modes of non-stationary variability are shown (scaled by 2). Intrinsic spike waveform variability consisted of increased amplitude (thin line), increased breadth (dashed line), or secondary peak inclusion (dot-dashed line).

ther, overlapping extracellular waveforms were demonstrated to combine additively. Lastly, spike waveform variability was shown to be decomposable into stationary non-spike noise, and several modes of non-stationary variability. To obtain copies of these data sets, please contact the corresponding author.

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