Spike sorting

Rodrigo Quian Quiroga

What is spike sorting? A gold standard in neuroscience is to record extracellularly the activity of single neurons with thin electrodes implanted in the brain (Figure 1A). Extracellular recordings pick up the spikes of neurons nearby the electrode tip and the job of the experimenter is to determine which spike corresponds to which neuron. This identification is done based on the shape of the spikes, given that, in principle, each neuron fires spikes of a particular shape, depending on the morphology of its dendritic tree and the distance and orientation relative to the recording site, among other factors. Spike sorting is the grouping of the detected spikes into clusters based on the similarity of their shapes. The resulting clusters of spikes correspond to the activity of different putative neurons.

Why is spike sorting important? As the algorithms for spike sorting can be quite complicated and given this can be a difficult and time consuming process, it is worth asking whether it is really necessary to do spike sorting rather than taking all the spikes together, as the lump activity of an unknown number of neurons. The problem is that close-by neurons — picked up by the same electrode — can fire in response to different things. This is the case, for example, in the human or rat hippocampus, where nearby neurons fire to unrelated people in the first case and to distant place fields in the latter. But even when nearby neurons have similar responses, it is important to distinguish them and observe their individual tuning properties, firing characteristics, relationship with other neurons and local field potentials, and so on.

One strategy to avoid having to use complex spike-sorting algorithms is to use acute electrodes lowered into the animals' brain during each experiment. Then, the electrode can be placed sufficiently close to a given neuron, decreasing interference of the spikes from others. There are, however, several caveats with this approach: first, it introduces a bias towards recording from high firing (and typically less selective) neurons; and second, it is possible to observe only one or very few neurons at a time.

How is spike sorting done? The first spike sorting algorithms just used amplitude discriminators, which are very fast and easy to implement on-line. But in most cases the amplitude alone is not sufficient to separate the spikes from different neurons. A straightforward improvement (still implemented in many commercial systems) is to use window discriminators, defining one or several boxes along observed spike shapes that the spikes of a given neuron should cross. The drawback of this approach is that the windows should be set (and readjusted) manually, which is not practical for more than a few simultaneously recorded channels. Moreover, spike shapes may overlap, thus making it difficult to select optimal windows to separate them. Another simple spike-sorting approach is to select a characteristic spike shape for each neuron and assign the rest of the spikes via template matching. But again, this requires the intervention of a user for selecting the templates.

Most spike-sorting algorithms have four main steps (Figure 1B). First, a high pass filter (with a cutoff frequency of 300 Hz or higher) is used to filter the high power, low frequency activity in order to visualize the spikes. Second, the spikes are detected, typically by amplitude thresholding (Figure 2A). Third, features are extracted from the detected spike shapes. The goal is to keep only those features that help the classification and get rid of the ones that just reflect random variations. This is one of the key steps in spike sorting, for which several approaches have been suggested: from taking the amplitude, width and energy of the spikes, to extracting the first principal components or taking a selection of wavelet coefficients (Figure 2B). The final step is to cluster the activity of the different neurons in the feature space. Manual clustering algorithms allow the user to cluster-cut ellipsoids or polygons, delimiting the spikes of different neurons in two-dimensional projections of the feature space (for example, plotting the amplitude against the width of the spikes). However, this approach is very subjective and time consuming. Automatic spike sorters typically use methods from machine learning or statistical mechanics, like variations of expectation maximization algorithms or superparamagnetic clustering. The

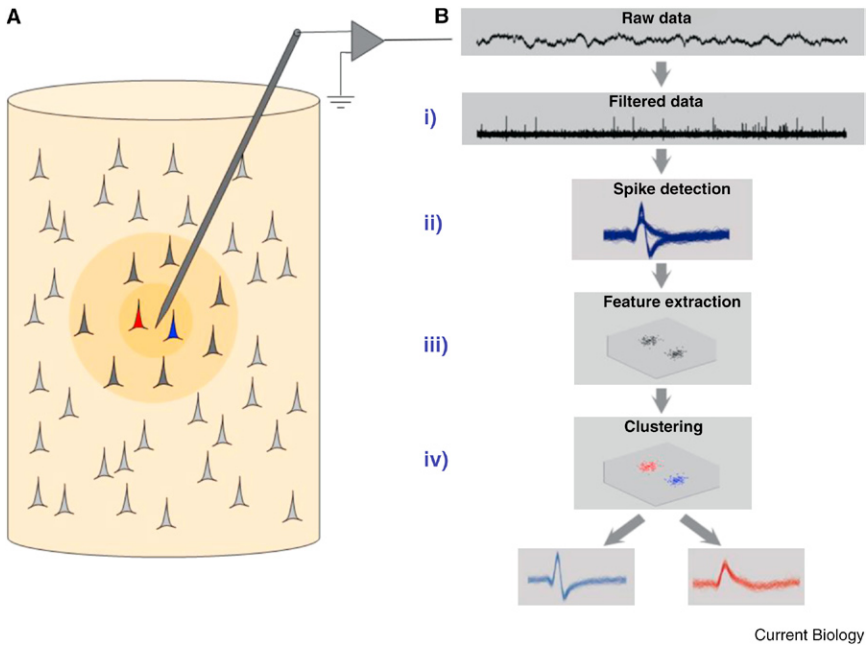

Figure 1. Extracellular recordings and spike sorting.
(A) The extracellular activity of neurons is recorded with microwires inserted in the brain. The spikes of neurons close-by the electrode tip (in red and blue) can be separated via spike sorting; neurons further away (in dark grey) can be detected but not sorted, thus generating the multiunit activity; more distant neurons (in light gray) contribute to the background noise. (B) Main step of spike-sorting algorithms (adapted from Quian Quiroga *et al.* (2004)).

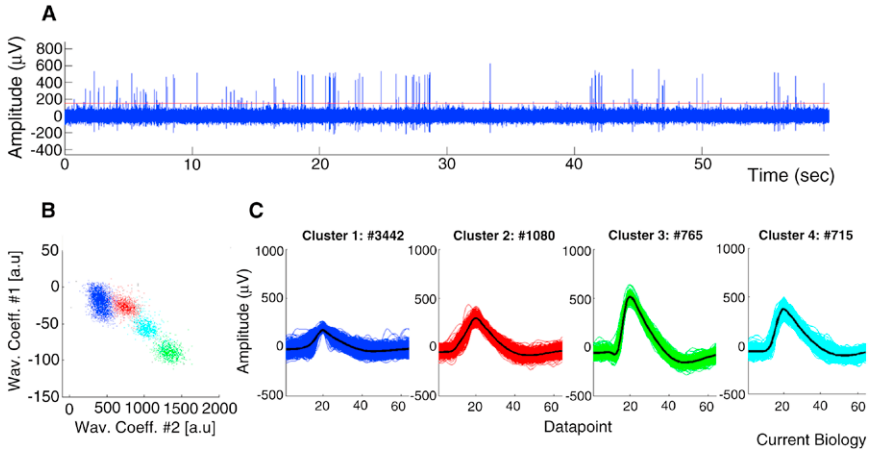

Figure 2. Spike sorting of a recording from the human medial temporal lobe.
(A) One minute of the continuous (high-pass filtered) data, where spikes are observed on top of the background activity. The red horizontal line marks the threshold used for spike detection. (B) Projection of the spike shapes onto the feature space (the first two wavelet coefficients). Note that it is possible to observe four different units. (C) The spike shapes corresponding to three putative neurons (in red, green and cyan) and one multiunit (in blue). The numbers on top show the total number of spikes.

end result of a spike-sorting algorithm is the sequence of spike times, the cluster membership and the spike shapes (Figure 2C).

What about multiunit clusters? These are formed by spikes from many neurons, with shapes that cannot be separated because of a low signal-to-noise ratio. The neurons contributing to the multiunit activity are relatively close to the electrode (for their spikes to be detected), but not close enough to enable the clustering of their shapes (Figure 2C). An important issue is to distinguish between single- and multiunit activity: is a response pattern coming from a single neuron or from a group of them? This distinction is subjective and it is based on the fact that multiunit clusters do not show a stereotypical spike shape (they are formed by the superposition of different spikes), have a relatively low amplitude and violate the refractory period of single neurons.

What are tetrodes? Tetrodes are four close-by micro-electrodes that allow recording the activity of single neurons from different sites. It has been shown that the possibility of observing the same neurons from different sites increases dramatically the single cell yield and also gives more reliable results, because an ambiguous separation from one channel can be disentangled using the information from another. The challenge is to decide how to combine the information from the different channels. Solutions vary from the use of the peak amplitude for each of the channels, the first two principal components, or doing the spike sorting over longer waveforms constructed by the concatenation of the spikes from the different channels.

How many neurons can we see from an electrode? The number is variable and it depends on the electrodes used, the cortical area, noise levels, etc. In general, it is possible to see up to six or seven units per electrode (typically from two to four). Tetrodes increase the accuracy and yield of neurons and, at least in hippocampal recordings, it is possible to see up to 15–20 neurons from a single tetrode.

Why don't we see more neurons? Based on anatomical considerations, it has been argued that the number of neurons identified from single electrodes or tetrodes should be at least one order of magnitude larger than what we currently see. A possible reason for this disagreement is that the majority of neurons remain silent and are therefore not detected. It is also possible that the electrode penetration produces tissue damage. A third alternative is that our state-of-the-art spike sorters are not yet able to discriminate the activity of large numbers of neurons. It is actually likely that the reason for observing less neurons than expected is a combination of these three factors.

How do we test spike-sorting algorithms? A major problem for the development and optimization of spike-sorting algorithms is that there is typically no ground truth with real data. Notable exceptions are simultaneous intra- and extracellular recordings, where the spike sorting outcome with an extracellular recording could be validated with the intracellular data. But in general, it is a common situation that the decision of whether two similar clusters should be kept separated or merged into a single cluster is far from obvious. This problem is exacerbated by the fact that we don't know beforehand how many neurons are present in each recording. There is therefore a need to develop simulations that reproduce (at least) the most important features of real extracellular recordings, to quantify and compare the performance of different algorithms.

What's next? The possibility of recording from hundreds or thousands of neurons simultaneously is the dream of any neurophysiologist and a goal that is within reach, as it is now possible to record from hundreds of channels simultaneously. There is clearly a need to develop fully automatic, fast spike-sorting algorithms to deal with such large number of channels and the massive volumes of recorded data. The advantage of using tetrodes is also clear, but current spike-sorting algorithms still use relatively naïve methods to combine the information from different sites. Further developments of spike-sorting algorithms should go together with the optimization of electrode designs with the general goal of maximizing the number of simultaneously recorded and identified neurons.

Where can I find more?

Lewicki, M. (1998). A review of methods for spike sorting: the detection and classification of neural action potentials. Network: Comp. Neural Syst. 9, R53–R78.

Quian Quiroga, R., Nadasdy, Z., and Ben-Shaul, Y. (2004). Unsupervised spike sorting with wavelets and superparamagnetic clustering. Neural Comput. 16, 1661–1687.

Department of Engineering, University of Leicester, Leicester LE1 7RH, UK and Leibniz Institute for Neurobiology, Brenneckestraße 6, 39118 Magdeburg, Germany.
E-mail: rqqg1@le.ac.uk