Spike Sorting: The Prehistory

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Outline

Ensemble recordings

What is spike sorting?

What do we measure? The origin of the signal.

A short history of spike sorting

Where are we ?

Ensemble recordings

What is spike sorting?

What do we measure? The origin of the signal.

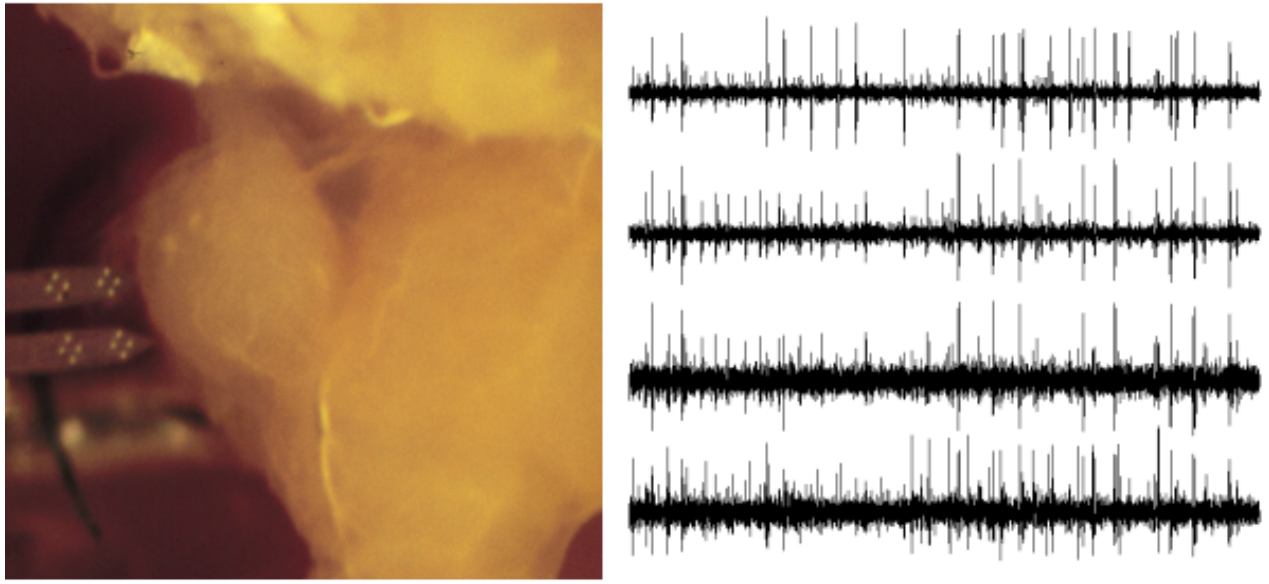
A short history of spike sorting

Why do we want to record from many neurons?

Neurophysiologists are trying to record many neurons at once with single neuron resolution because:

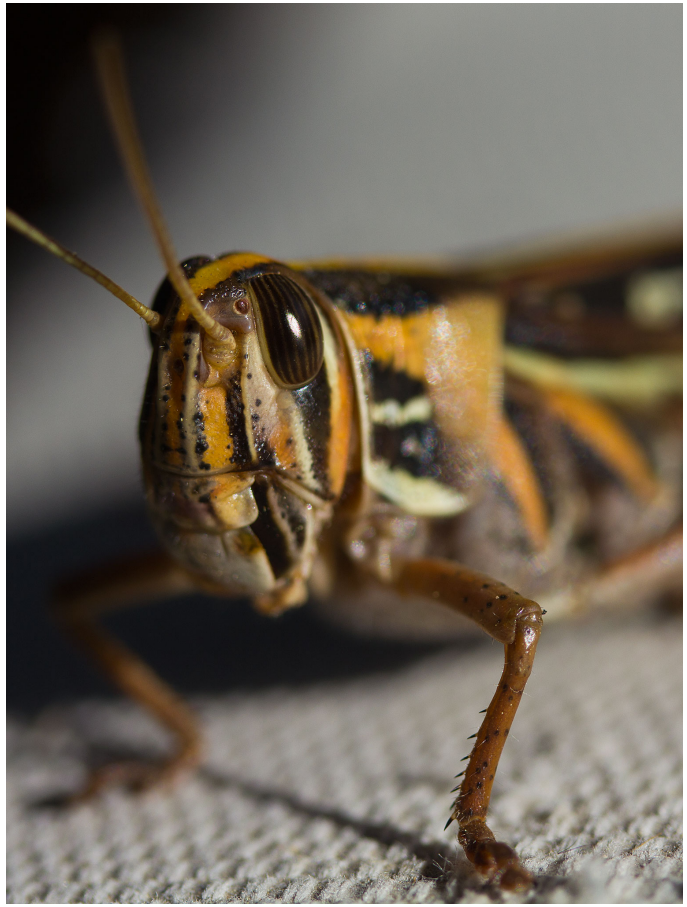
- They can collect more data per experiment.
- They have reasons to think that neuronal information processing might involve synchronization among neurons, an hypothesis dubbed binding by synchronization in the field.

Ensemble recordings: multi-electrode array (MEA)



Left, the brain and the recording probe with 16 electrodes (bright spots). Width of one probe shank: 80 μm. Right, 1 sec of raw data from 4 electrodes. The local extrema are the action potentials. The insect shown on the figure is a locust, *Schistocerca americana*. Source: Pouzat, Mazor and Laurent.

What a *Schistocerca americana* looks like?



"Schistocerca americana headshot" by Rob Ireton - `http://www.flickr.com/photos/24483890@N00/7182552776/`. Licensed under Creative Commons.

Voltage sensitive dyes

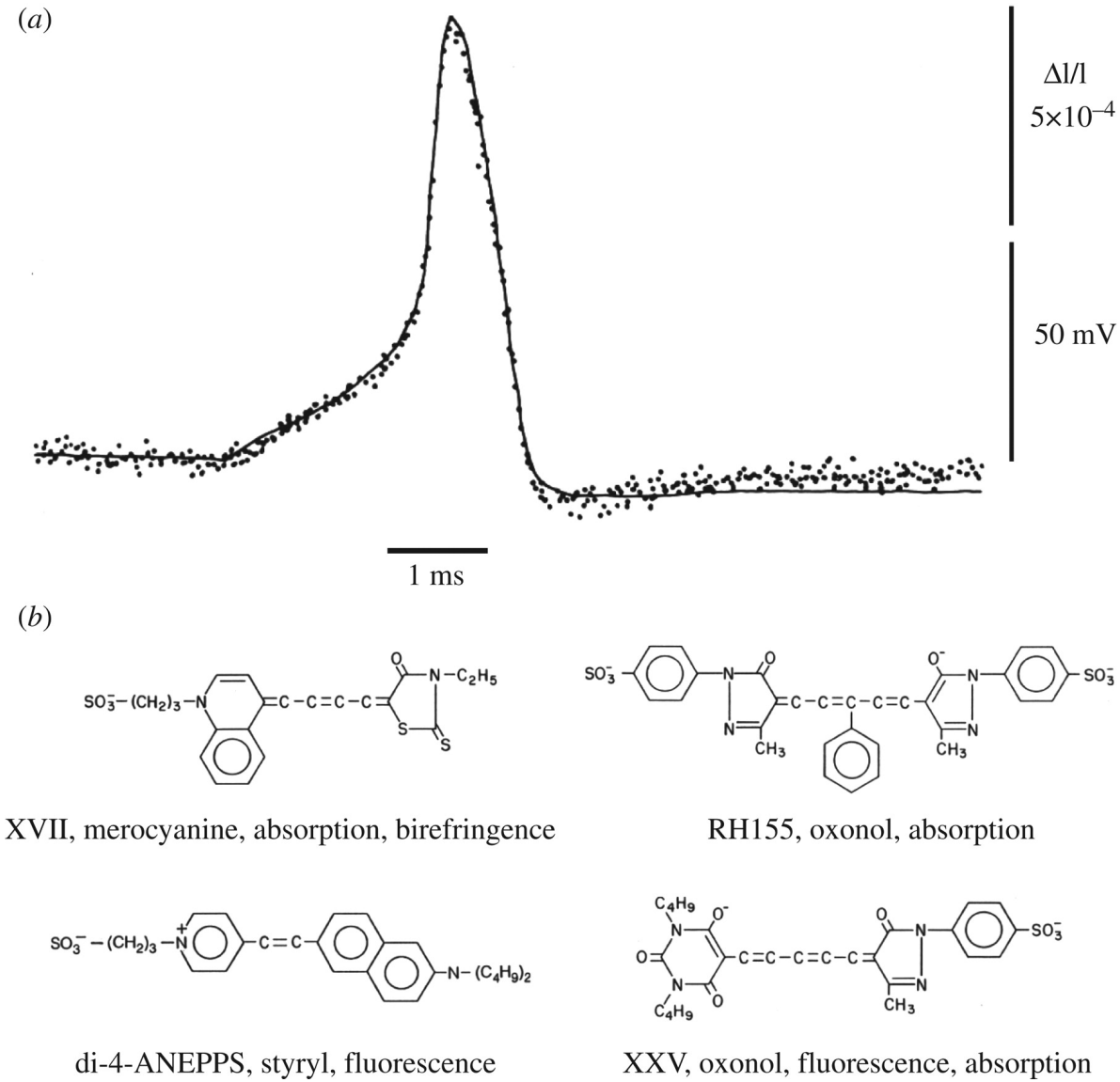


Fig. 2 of Homma et al (2009) *Phil Trans R Soc B* **364**:2453.

Ensemble recordings: voltage-sensitive dyes (VSD)

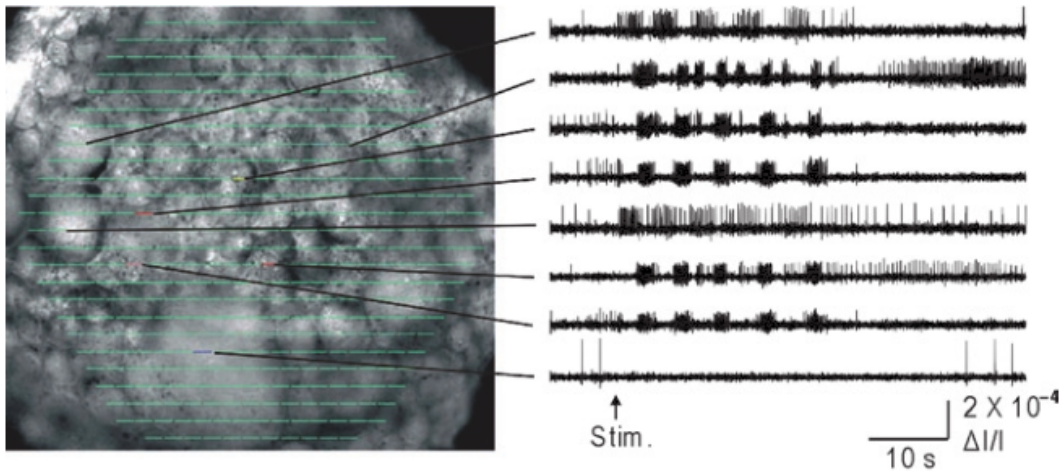


FIGURE 5.3. Superimposing an image of the preparation with the diode array makes it easy to inspect the firing of different neurons. The *left panel* shows an image of the dorsal surface of the pedal ganglion, superimposed in Neuroplex with a display of the 464 acquired optical traces in their corresponding diode positions (*green lines*). The *right panel* shows how clicking on diodes of interest displays their optical data. These are filtered, unaveraged recordings from single diodes, each showing the firing recorded from the indicated ganglion location. A five cycle swim motor program was elicited by a 10 Hz, 1 s, 10 V stimulus to Pedal Nerve 3, delivered at the arrow.

Imaging of the pedal ganglion of *Tritonia* by Frost et al (2010), Chap. 5 of *Membrane Potential Imaging in the Nervous System*, edited by M. Canepari and D. Zevecic.

What a *Tritonia* looks like?

"Tritonia festiva" by Daniel Hershman from Federal Way, US - edmonds4. Licensed under Creative Commons Attribution 2.0 via Wikimedia Commons.

Where are we ?

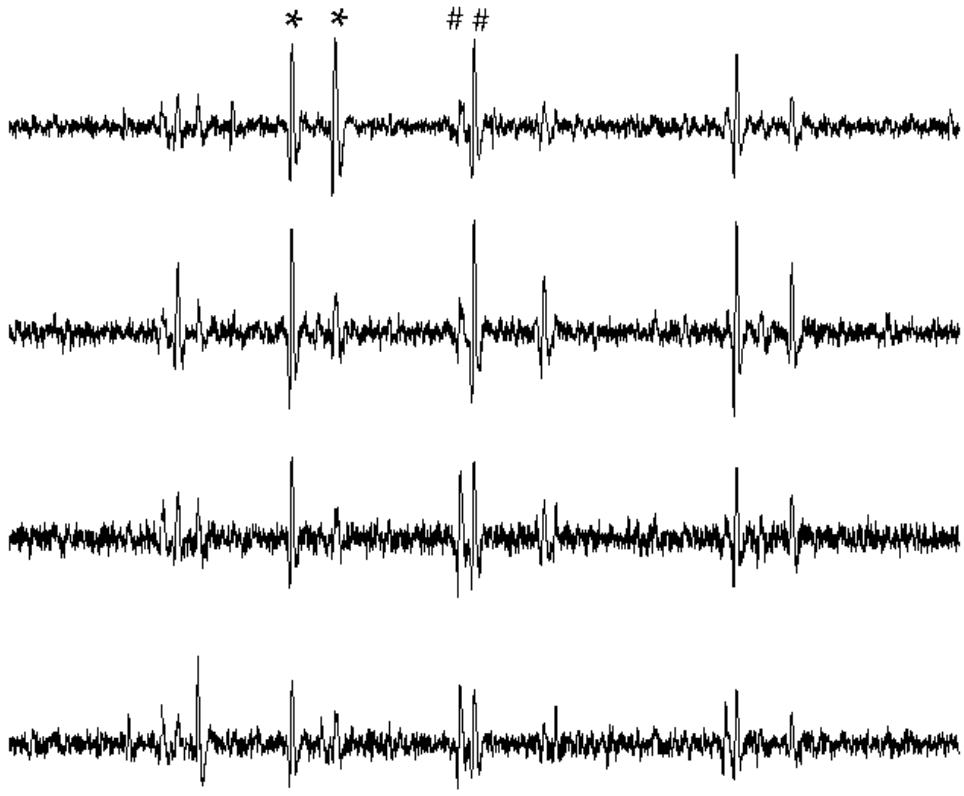
Ensemble recordings

What is spike sorting?

What do we measure? The origin of the signal.

A short history of spike sorting

Why are tetrodes used?



The last 200 ms of the locust figure. With the upper recording site only it would be difficult to properly classify the two first large spikes (**). With the lower site only it would be difficult to properly classify the two spikes labeled by ##.

What do we want?

- Find the number of neurons contributing to the data.
- Find the value of a set of parameters characterizing the signal generated by each neuron (*e.g.*, the spike waveform of each neuron on each recording site).
- Acknowledging the classification ambiguity which can arise from waveform similarity and/or signal corruption due to noise, the probability for each neuron to have generated each event (spike) in the data set.
- A method as automatic as possible.

A similar problem

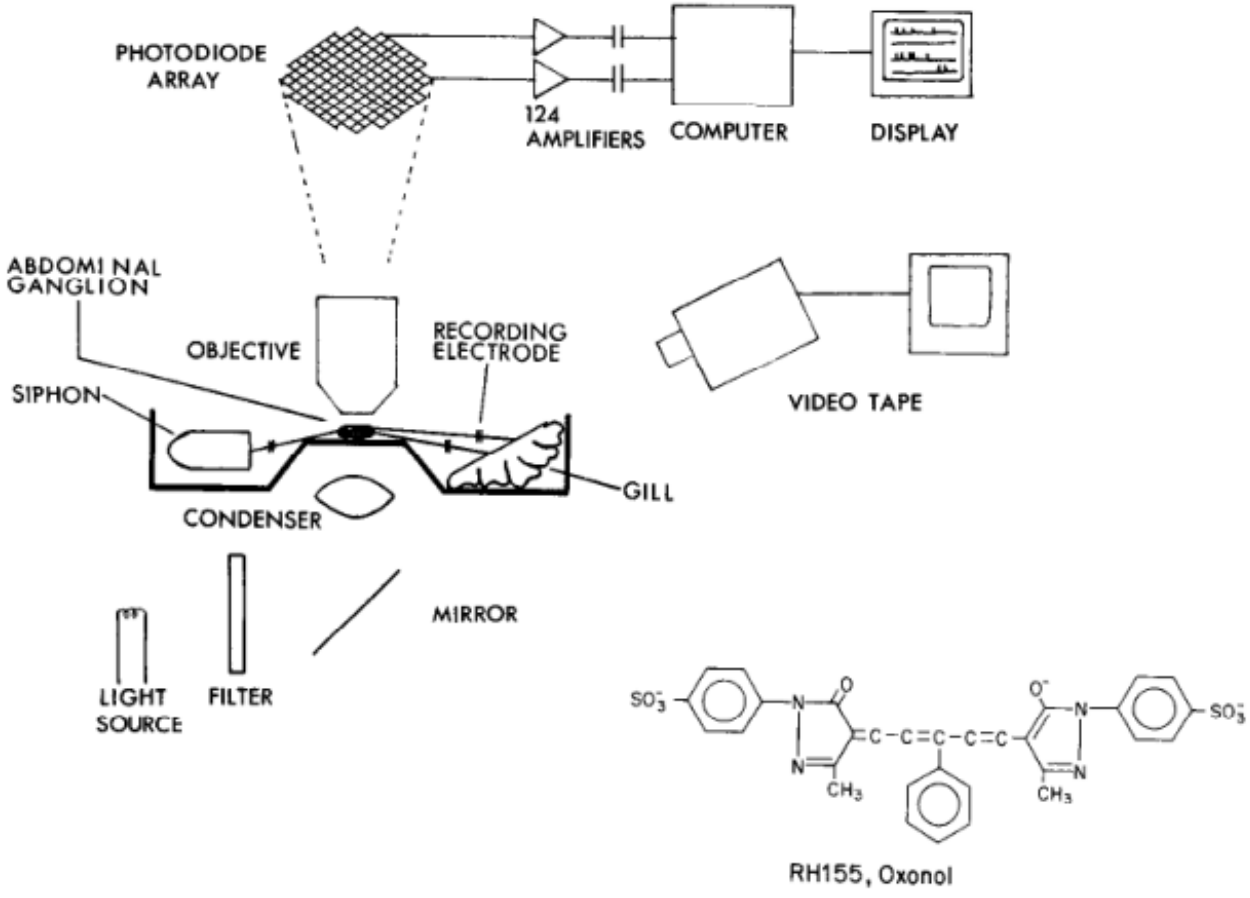
- Think of a room with many people seating and talking to each other using a language we do not know.
- Assume that microphones were placed in the room and that their recordings are given to us.
- Our task is to isolate the discourse of each person.

With apologies to Brueghel (original, without microphones, in Vienna, Kunsthistorisches Museum).

To fulfill our task we could make use of the following features:

- Some people have a low pitch voice while other have a high pitch one.
- Some people speak loudly while other do not.
- One person can be close to one microphone and far from another such that its talk is simultaneously recorded by the two with different amplitudes.
- Some people speak all the time while other just utter a comment here and there, that is, the discourse statistics changes from person to person.

VSD does not eliminate the sorting problem (1)



VSD recording from Aplysia's abdominal ganglion, Fig. 1 of Zecevic et al (1989) *J Neurosci* **9**:3681.

Aplysia

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VSD does not eliminate the sorting problem (2)

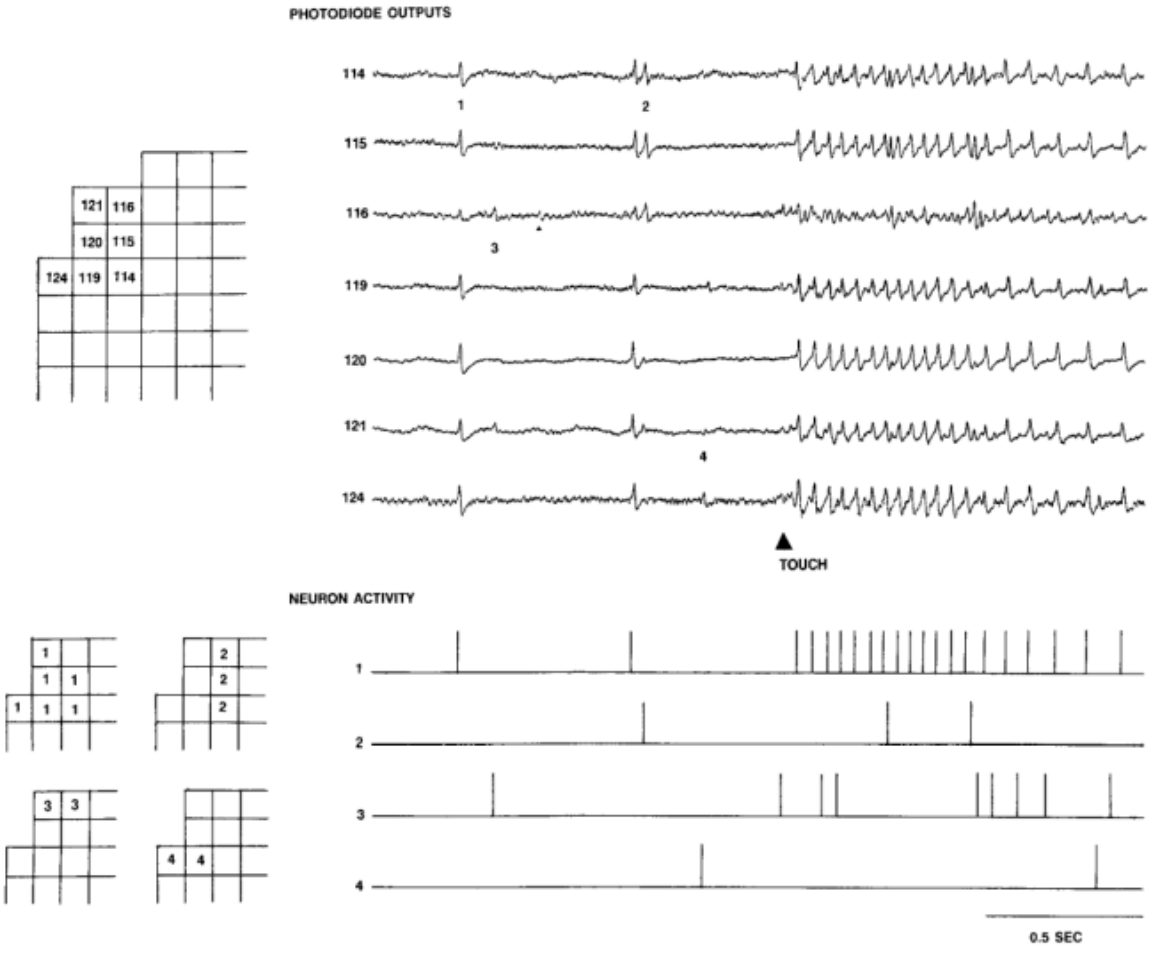


Fig. 2 of Zecevic et al (1989).

Calcium signals have slow kinetics!

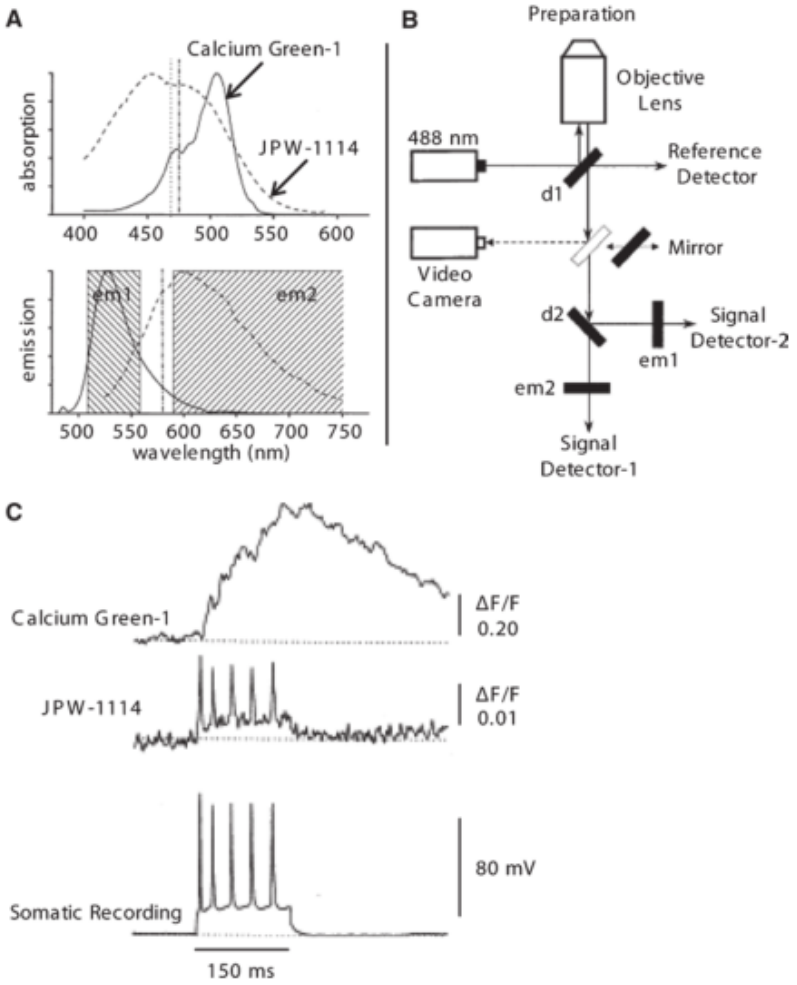


FIGURE 4.3. Combining voltage and calcium imaging using JPW-1114 and Calcium Green-1. (A) Combined excitation and emission spectra of JPW-1114 (*dotted line*) and Calcium Green-1 (*straight line*) with the relative placement the emission filters em1 and em2. (B) Epifluorescence configuration for high-speed random-access, laser-scanning microscopy (similar to the configuration reported in Fig. 4.1b). (C) Representative voltage and calcium signals simultaneously recorded from the proximal dendrite of a cultured hippocampal pyramidal neuron; action potentials were elicited by current injection through a somatic patch pipette also used for somatic recording. Reproduced from Bullen and Saggau (1998) with the permission of Springer.

Fig. 4 of Canepari et al (2010), Chap. 4 of of *Membrane Potential Imaging in the Nervous System*, edited by M. Canepari and D. Zevecic.

Where are we ?

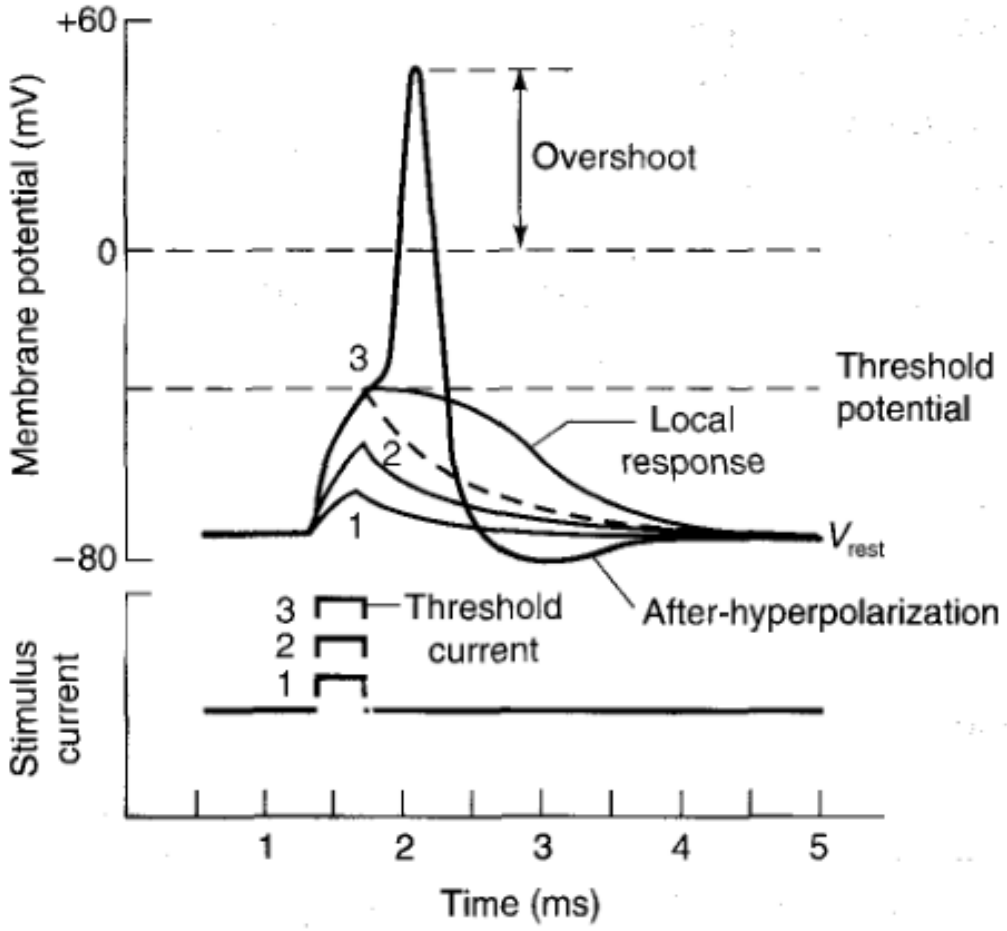
Ensemble recordings

What is spike sorting?

What do we measure? The origin of the signal.

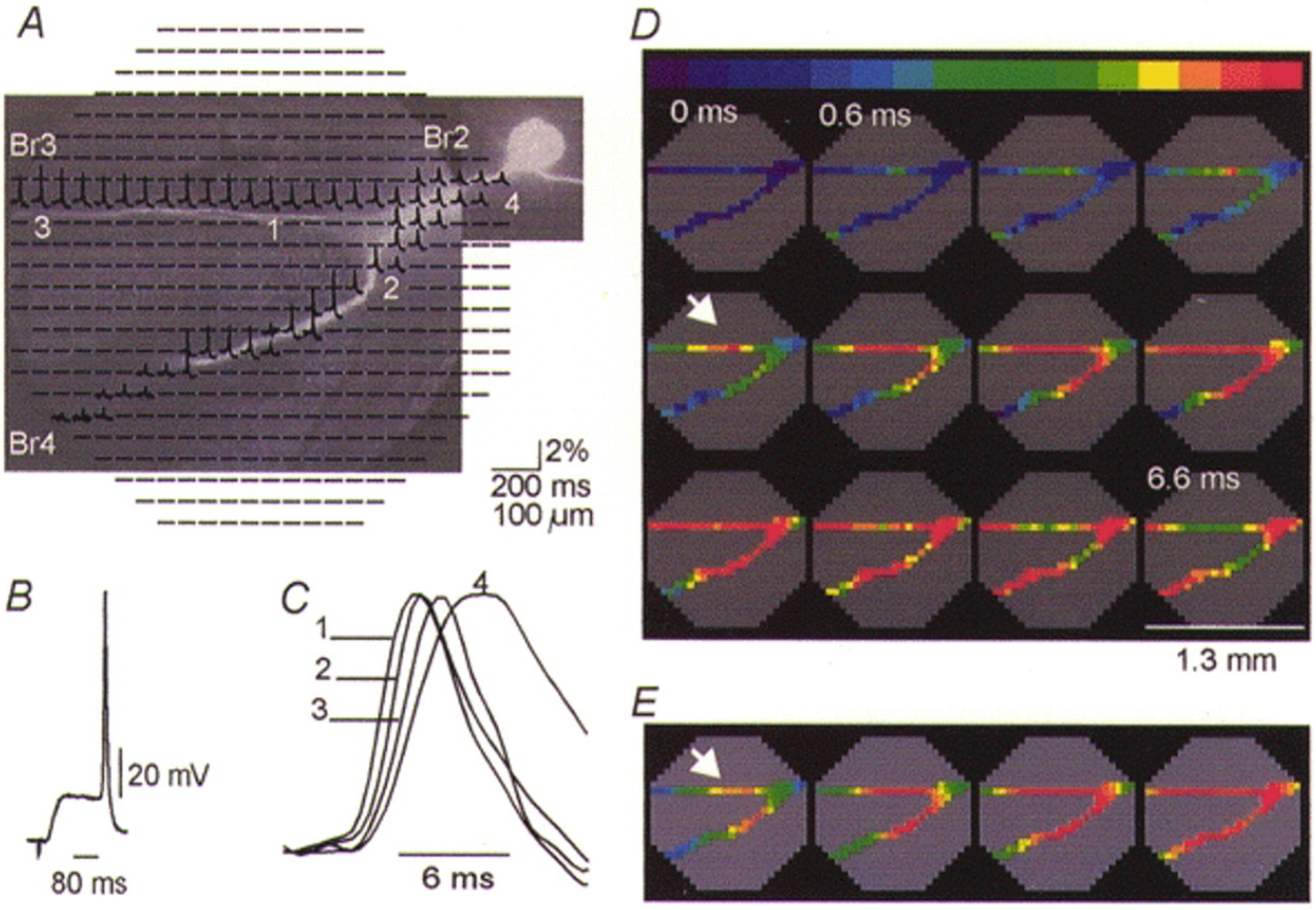
A short history of spike sorting

What makes (most) neurons "special"?



If we *depolarize* neurons "enough", an "active response" of *action potential* is generated. Fig. 5-16 of Randall et al (1997).

The action potential propagates!



VSD recording from the giant metacerebral neuron of *Helix aspersa*. Fig. 2 of Antic et al (2000) *J Physiol* **527**:55.

The giant metacerebral neuron of *Helix aspersa*

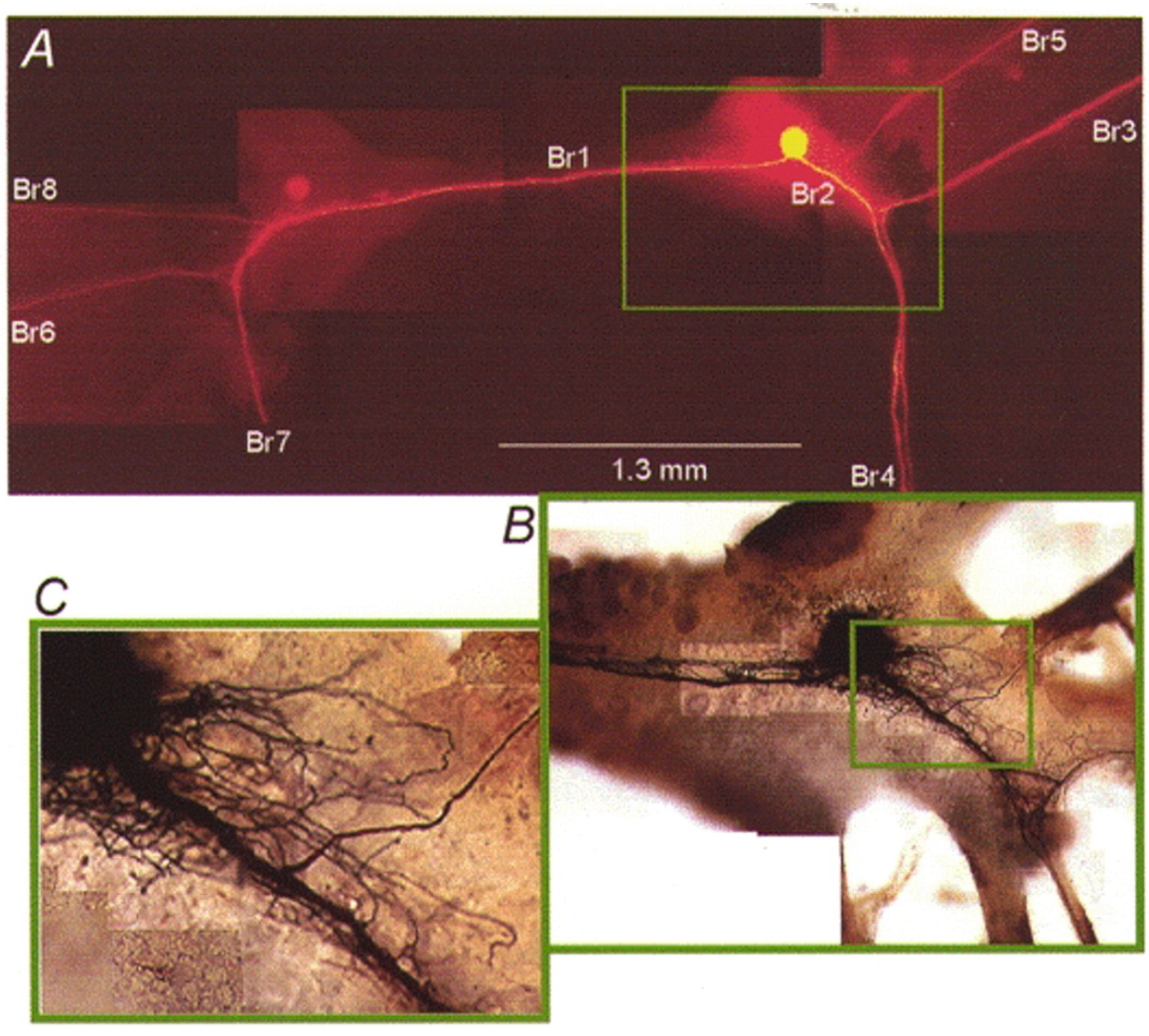
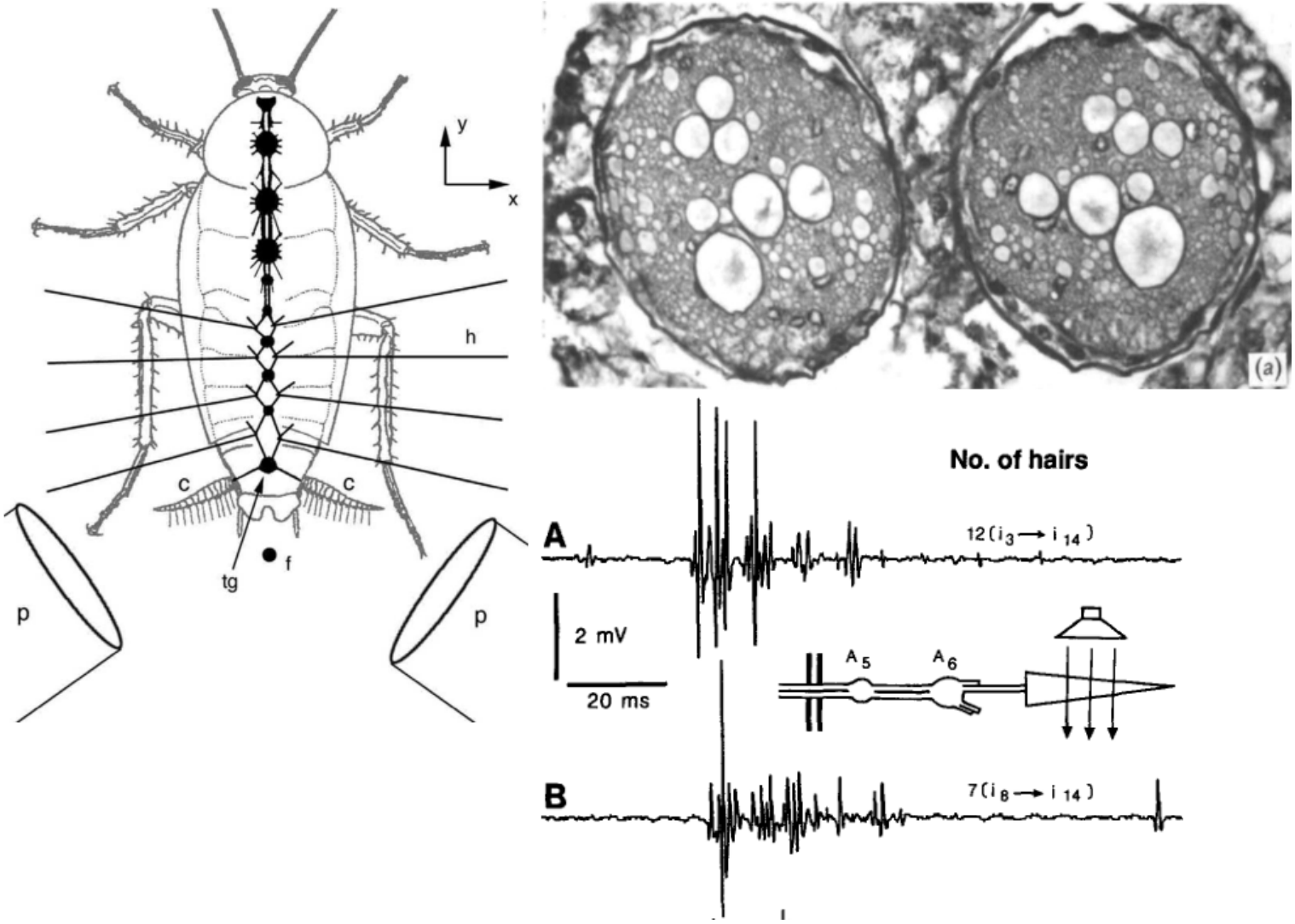


Fig. 1 of Antic et al (2000).

Helix aspersa

"Snail1web". Licensed under Public domain via Wikimedia Commons.

Something you already know



Left, Rinberg and Davidowitz (2002); top, Spira et al (1969); bottom, Hamon et al (1990).

Observed extracellular potential

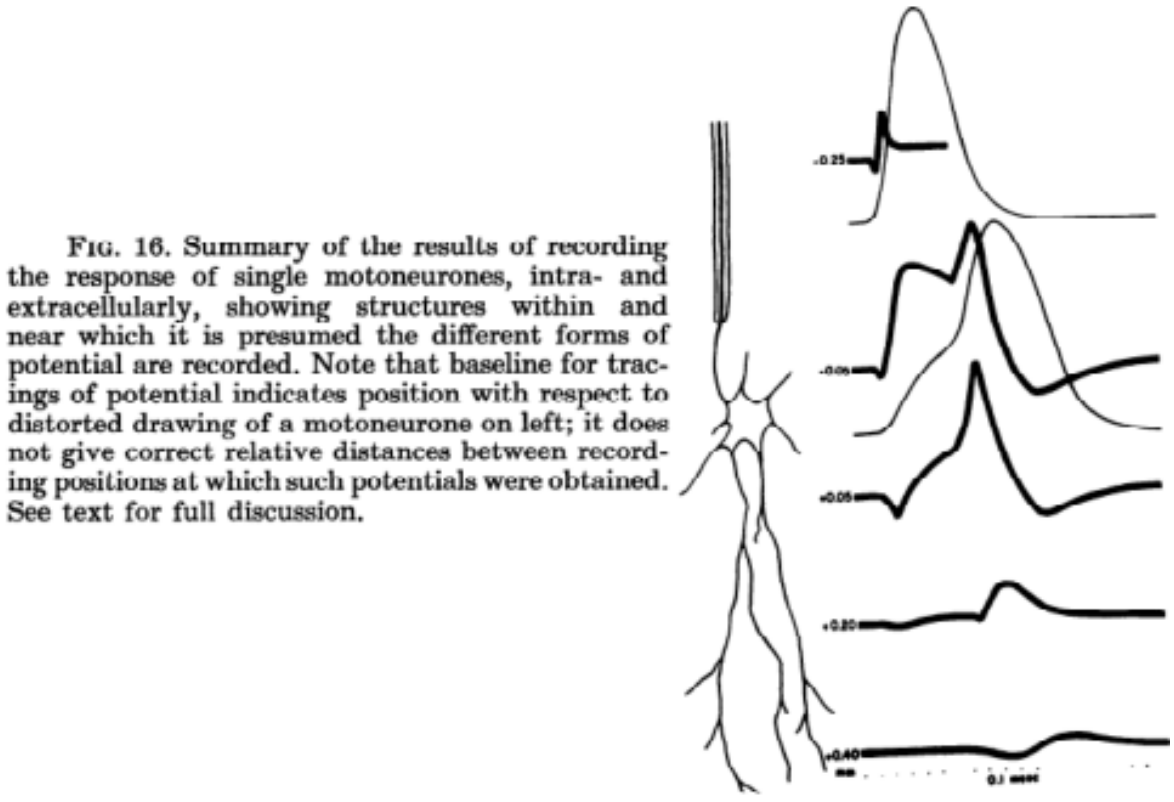
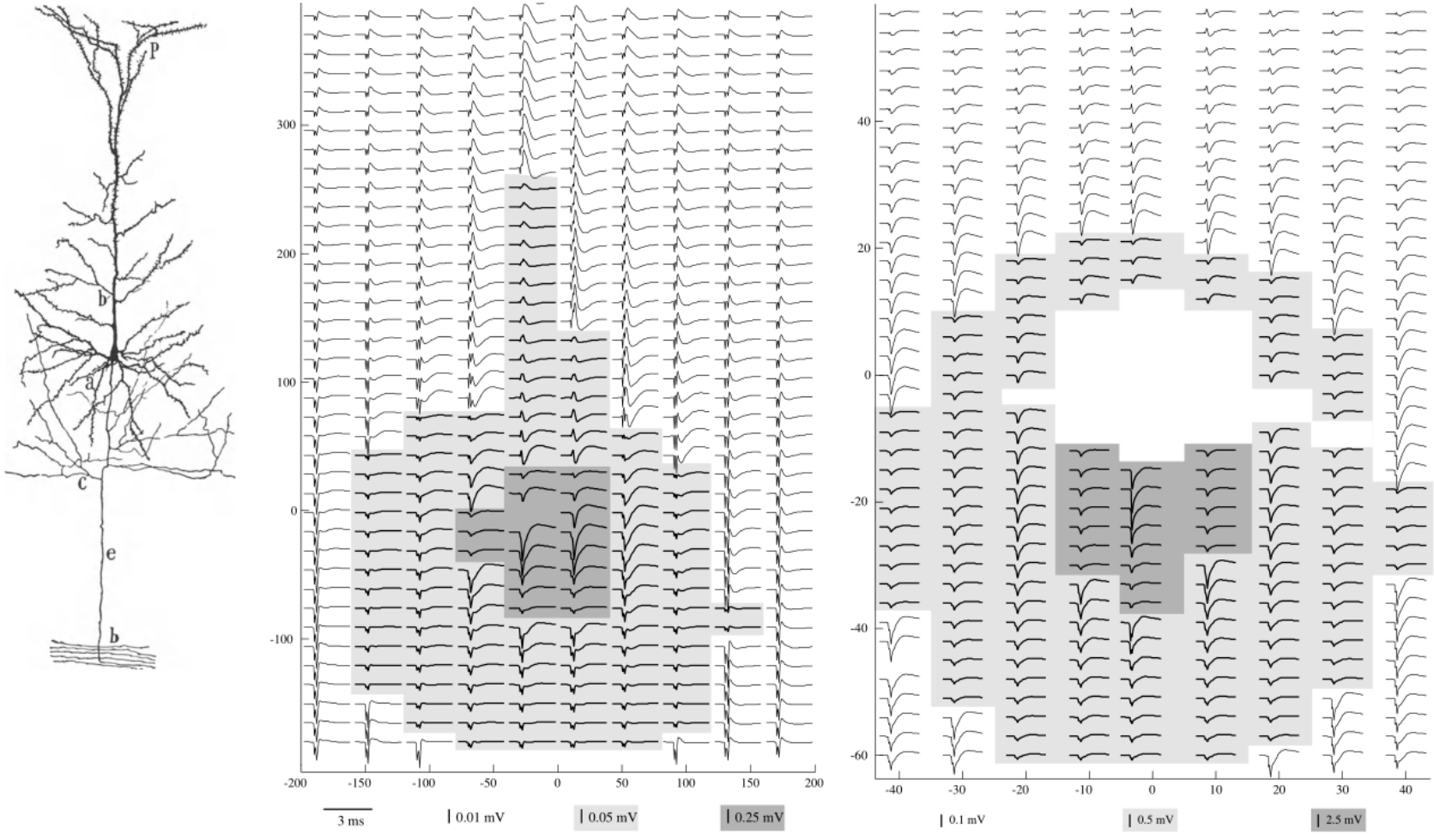


FIG. 16. Summary of the results of recording the response of single motoneurones, intra- and extracellularly, showing structures within and near which it is presumed the different forms of potential are recorded. Note that baseline for tracings of potential indicates position with respect to distorted drawing of a motoneurone on left; it does not give correct relative distances between recording positions at which such potentials were obtained. See text for full discussion.

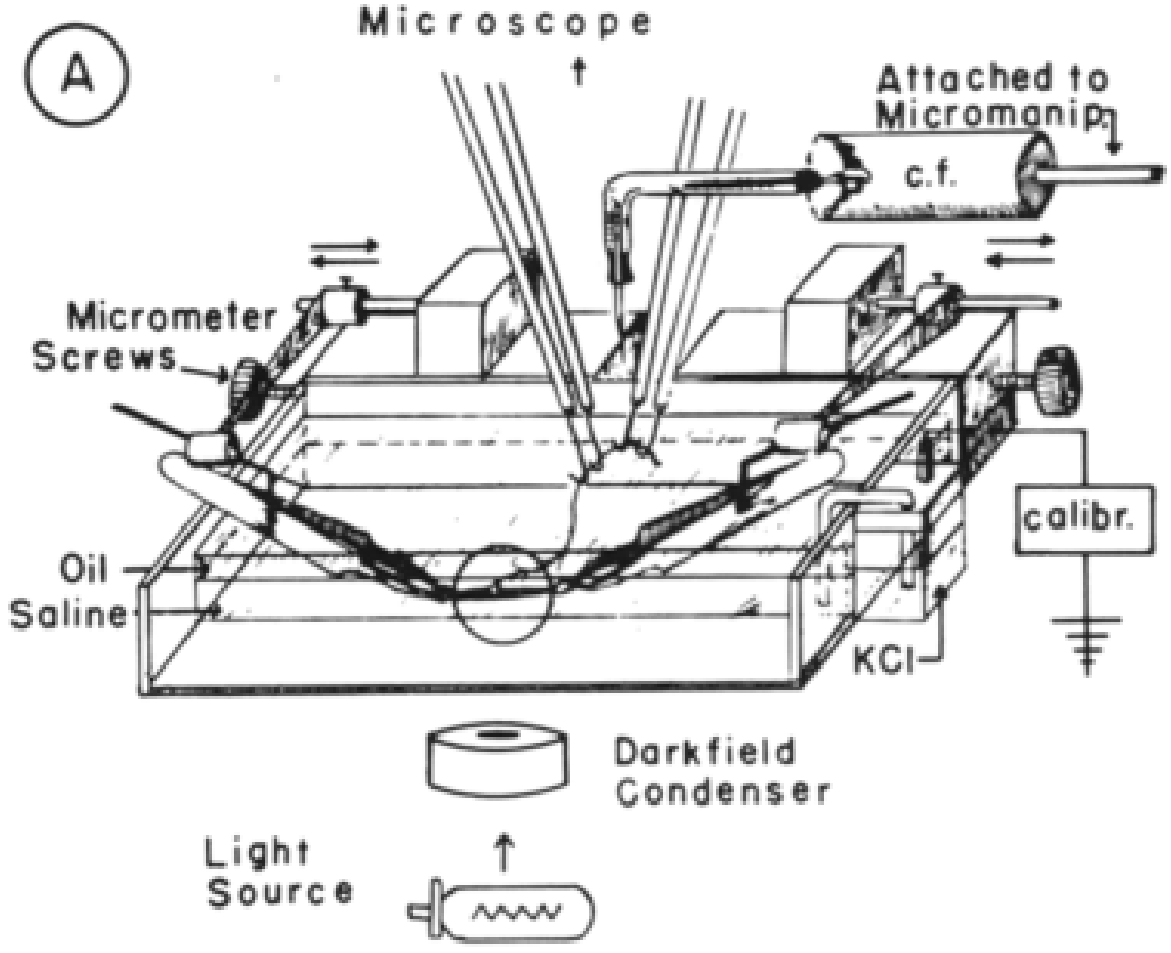
Fig. 16 of Fatt (1957) *J Neurophys* **20**:27. Recordings from Cats motoneurons.

Computed extracellular potential



Left, Fig. 8 of Santiago Ramón y Cajal (1899), middle and right Fig. 2.4 and 2.5 of Holt PhD thesis (Caltech, 1999).

The stretch receptor of *Homarus americanus*



The preparation, Fig. 1 A of Eyzaguirre and Kuffler (1955a) *J Gen Phys* **39**:87.

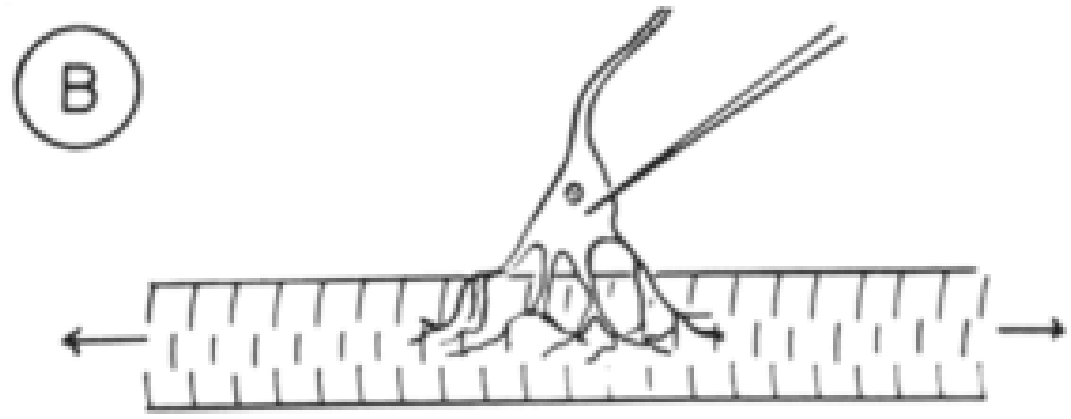


Fig. 1 B of Eyzaguirre and Kuffler (1955a).

Homarus americanus

"Yellow-lobster". Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons.

Mismatch between somatic and axonal AP?

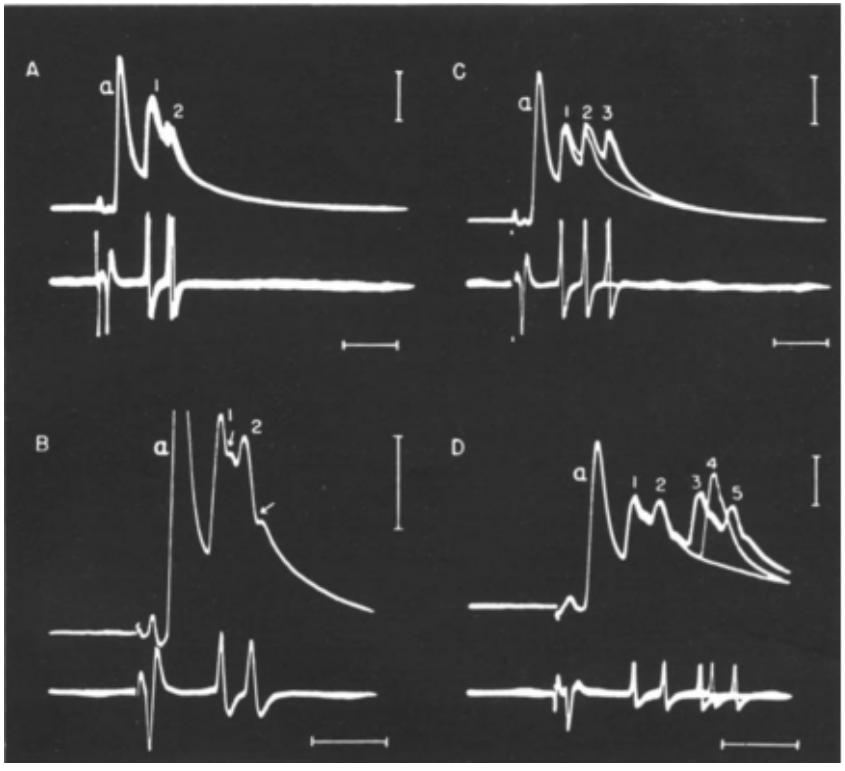


FIG. 13. Simultaneous intra- and extracellular records of grouped discharges in a slow cell. A, intracellular soma record (upper trace) shows superimposed at a 10/sec. stimulation rate components 1 and 2 following the large antidromic spike (*a*). The lower trace shows consistently two afferent axon impulses. B, higher amplification, single sweep, shows that in addition to components 1 and 2 there occurs abortive soma activity (arrows), not associated with axon impulses which are only 2 msec. apart. C, superimposed repeated exposures. Component 3 and its associated axon impulse drop out in several sweeps. D, components 1 and 2 are always present while 3 and 5 do not appear on some traces. Component 4 (fainter trace) is seen once or twice in the absence of 3 and 5. Note that small soma potential components (not numbered) may be only 0.5 to 2 msec. apart. Potential calibration, 25 mv. Time, 5 msec.

Fig. 13 of Eyzaguirre and Kuffler (1955b) *J Gen Phys* **39**:121.

This is not an invertebrate peculiarity

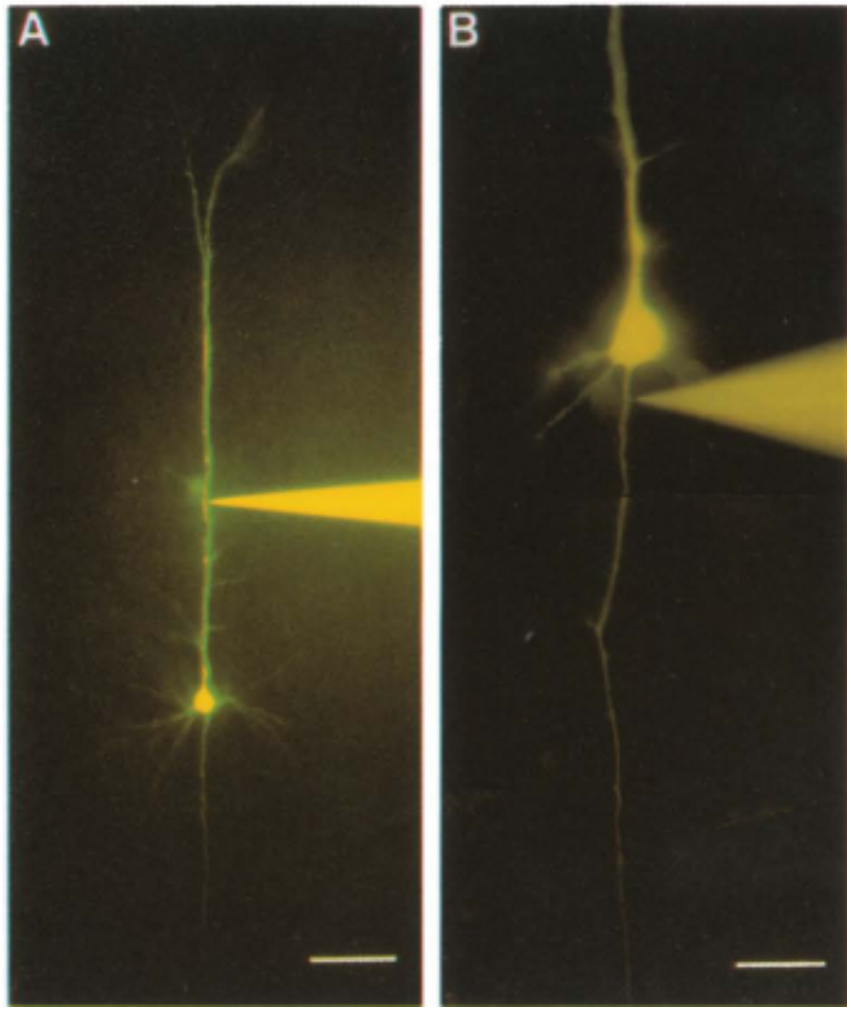


Fig. 7 of Sakmann and Stuart (2009) in *Single-Channel Recording*, edited by B Sakmann and E Neher, Springer

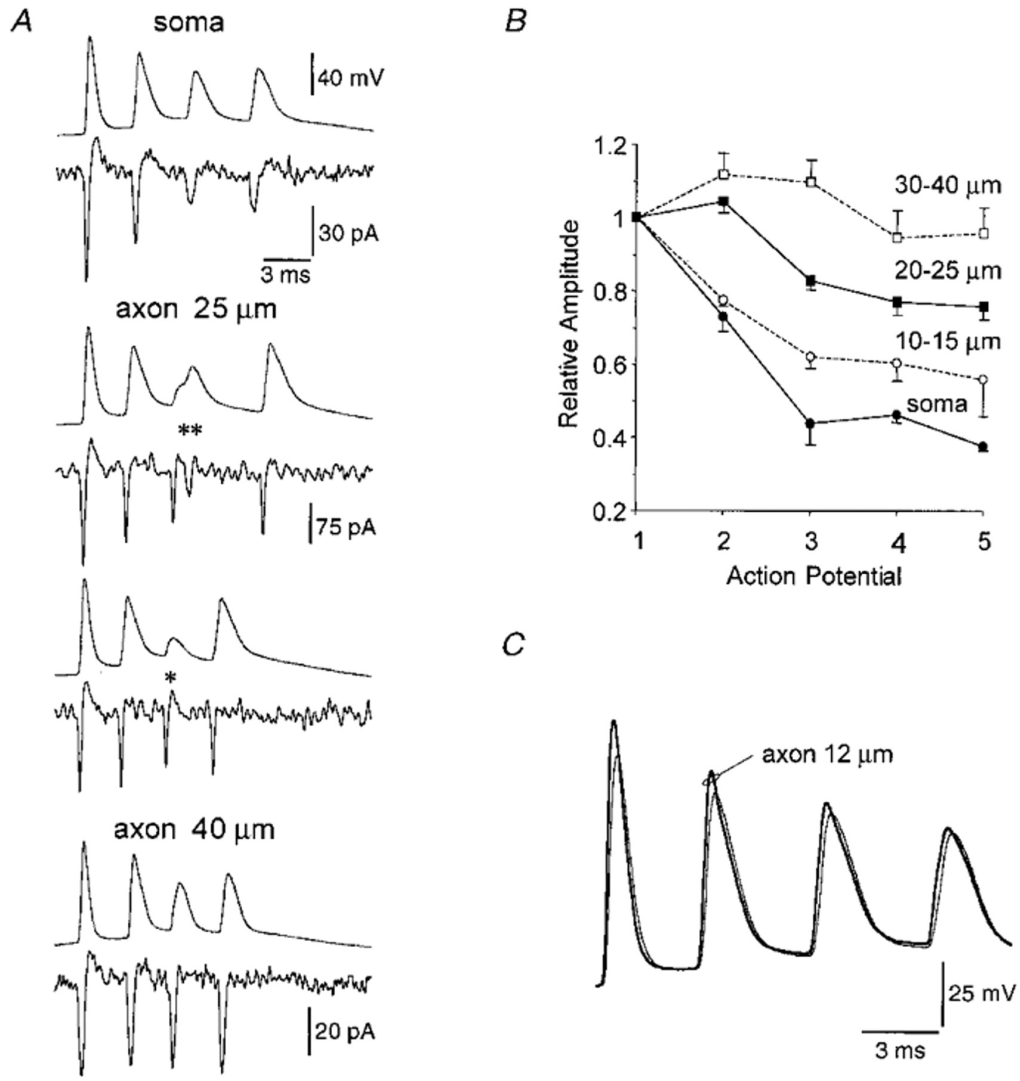


Fig. 5 of Williams and Stuart (1999) *J Physiol* **521**:467.

No more than a HH model is required

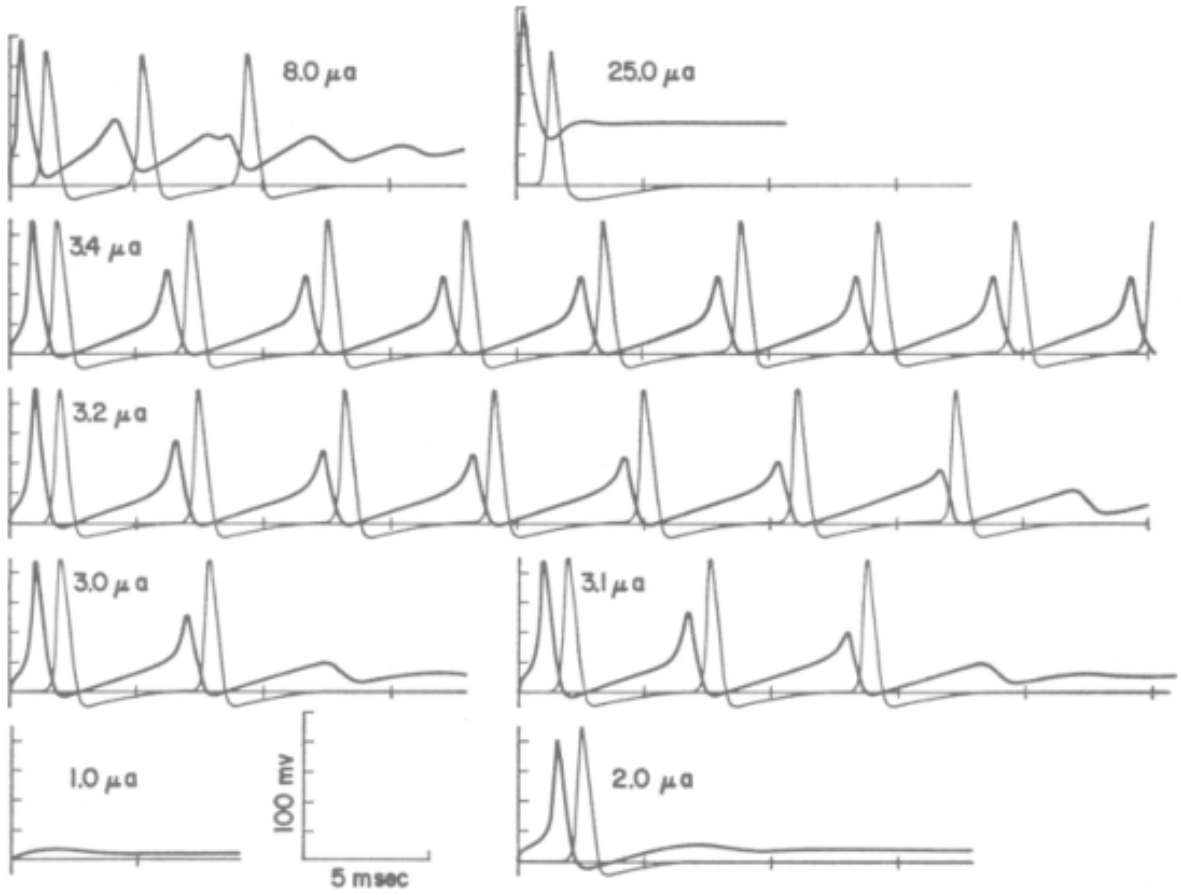
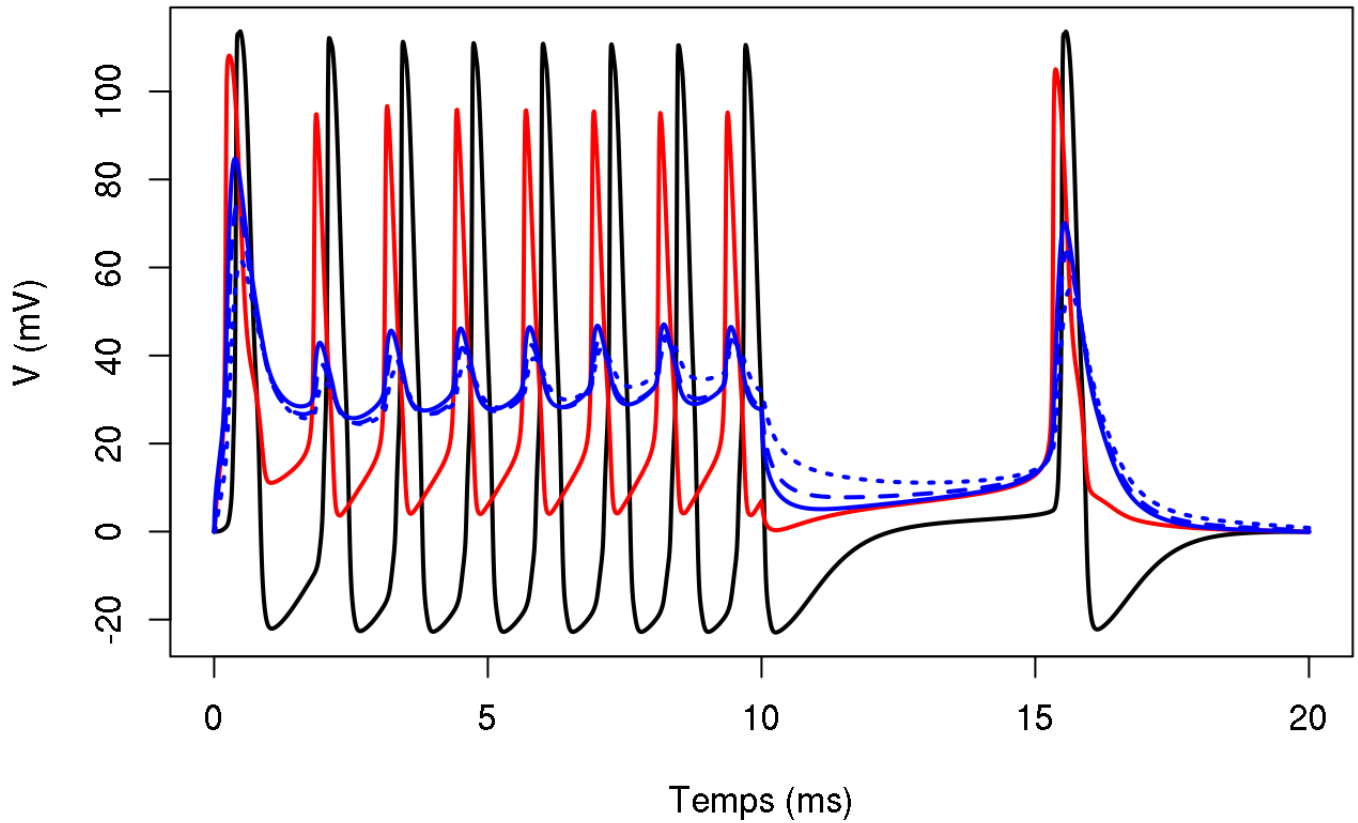


FIGURE 5 Response of the continuous axon to a steady stimulus of various intensities showing time course of the membrane potential V at $x = 0$ (heavy lines) and at $x = 2$ cm (lighter lines).

Fig. 5 of Cooley and Dodge (1966) *Biophys J* **6**:583.



CA3 pyramidal cell simulation. After Traub et al (1994). Current injected in the soma. Voltage response in the soma and dendrite (blue), axon initial segment (red), axon (black).

My conclusion

- Extracellular recordings from regions with neurons having different "sizes", like the neo-cortex, result in a biased sampling of the neuronal populations.
- Since what goes on in the soma is not necessarily what goes on in the axon and since the extracellular potential is dominated by what goes on in the former, we might get wrong output estimations from extracellular recordings.

Where are we ?

Ensemble recordings

What is spike sorting?

What do we measure? The origin of the signal.

A short history of spike sorting

Sorting by eye

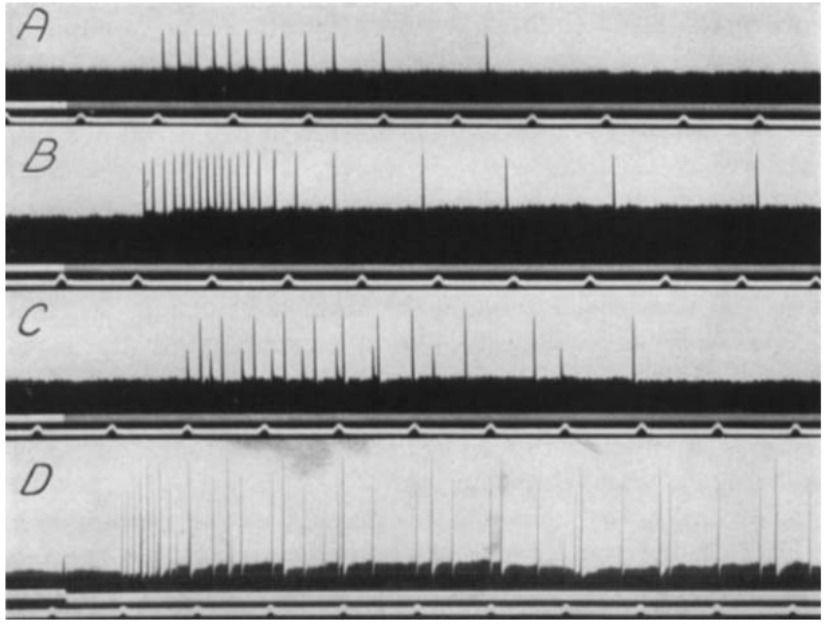


Fig. 4 Action potentials from nerve strands containing several active fibers. A to C) From bundle containing two active fibers. A and B) Stimulation of respective end organs separately. Intensity, 0.1. C) Stimulation of both end organs simultaneously. Intensity, 0.03. D) Record showing discharge in three active fibers. Recording as in figure 2.

As soon as the "the all-or-nothing response of sensory nerve fibres" was established by Adrian and Forbes (1922), neurophysiologists started doing sorting by eye using the spike amplitude as a feature. Fig. 4 of Hartline and Graham (1932) *J Cell Comp Physiol* **1**:277. *Limulus polyphemus* recording.

What a *Limulus polyphemus* looks like?

"Limulus polyphemus (aq.)" by Hans Hillewaert - Own work. Licensed under Creative Commons Attribution-Share Alike 4.0 via Wikimedia Commons.

Automatic window discriminator (1963)

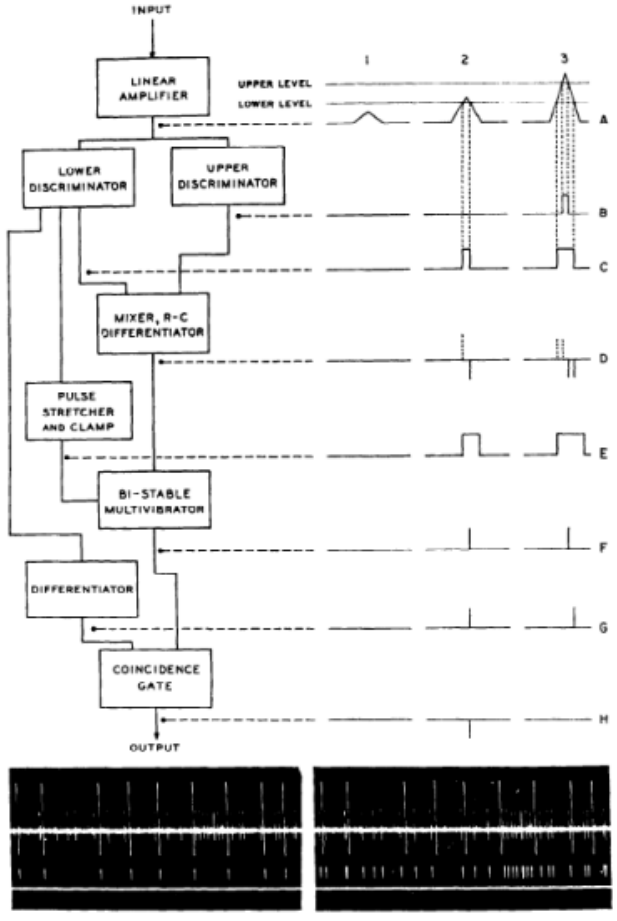


FIG. 4. Above, block diagram indicates the mode of operation of the differential amplitude discriminator which is described in full in the text. To the left, below: upper trace shows multi-unit record of thalamic cellular activity. The action potentials of two single units are clearly discernible. Lower trace shows output of DAD when only the larger spike enters and reverses within the potential window. To the right, below: upper trace is same record, but now the potential window has been moved so that only the small spike reverses within it, while the larger one traverses both lower and upper potential levels. Lower trace is DAD output, showing selection of smaller spike.

Fig. 4 of Poggio and Mountcastle (1963) *J Neurophys* **26**:775. *In vivo* recordings from monkeys thalamic neurons.

Template matching (1964)

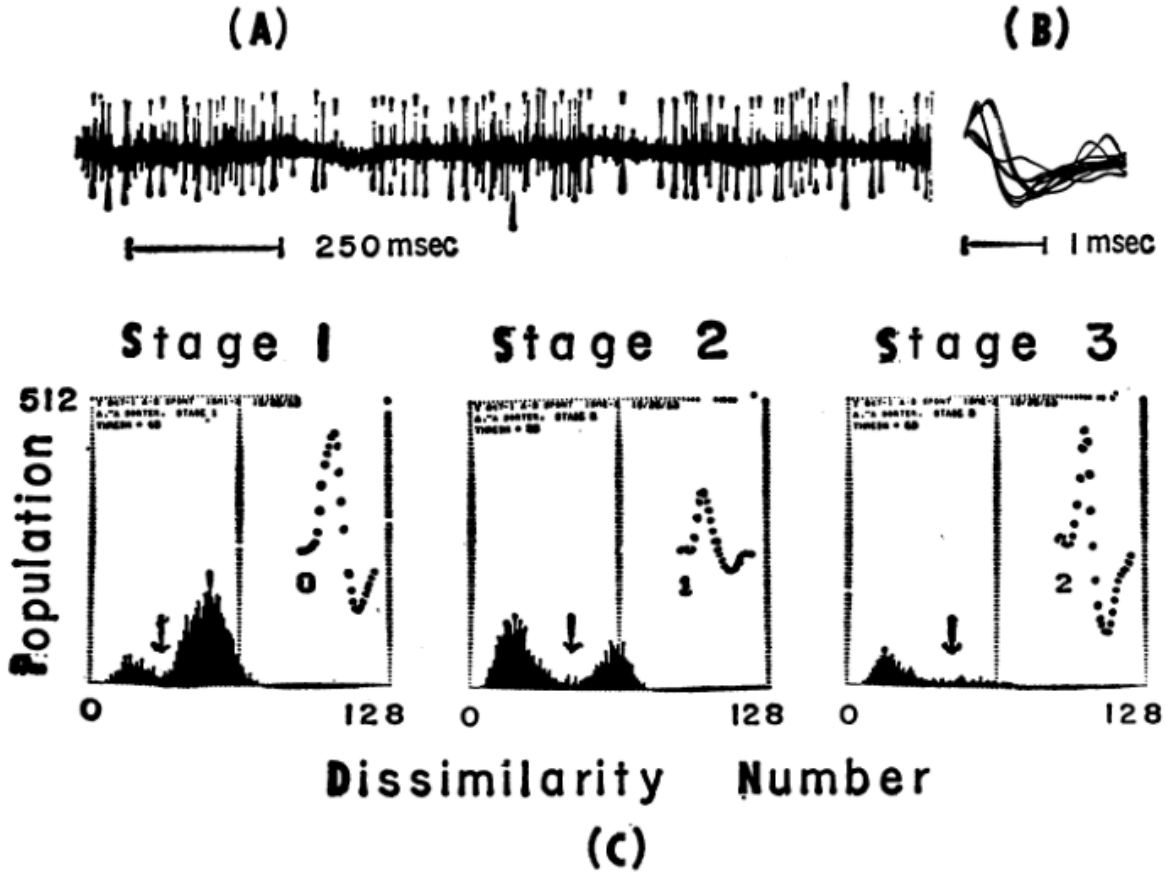
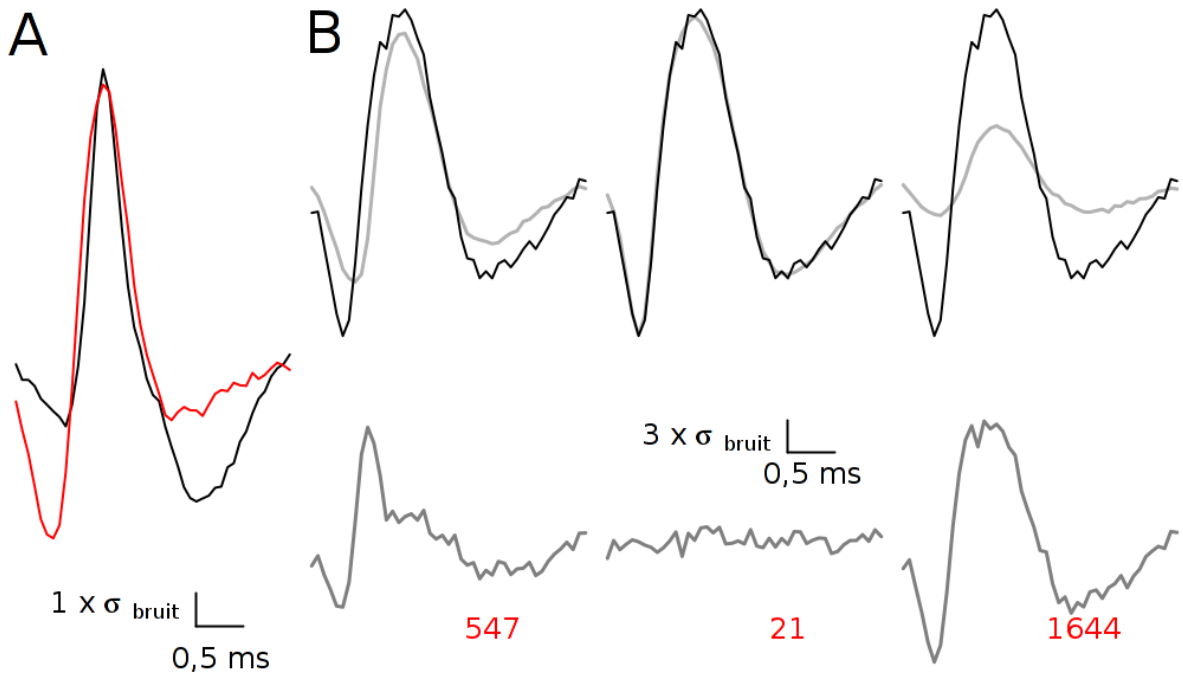


Fig. 1. Action potentials from several adjacent neurons. *A*, Approximately 1.5 seconds of action potential data from several units. *B*, Multiple triggered sweeps of an oscilloscope at high speed to show the three waveforms of action potentials in the data. *C*, Population as a function of dissimilarity number during three stages of the separation (see text).

Fig. 1 of Gerstein and Clark (1964) *Science* **143**:1325. Dorsal cochlear nucleus recordings from anesthetized cats.



Concentrate on part B. 3 templates are subtracted from the same event. Top: event (black) and template (gray); bottom: event-template (in red the residual sum of squares).

Dimension reduction and cluster membership (1965)

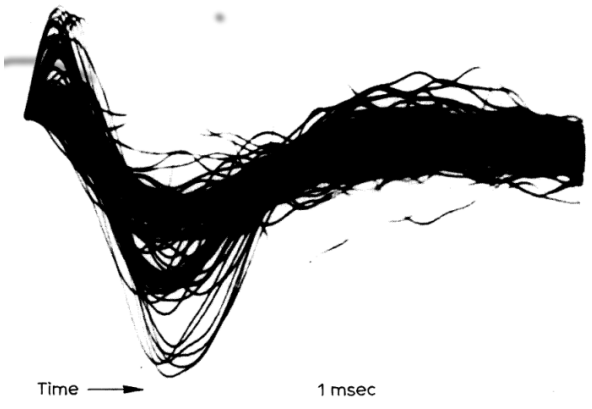


Fig. 1
At least three clearly defined groups of action potentials can be seen in the above photograph. A fourth group is discernible just under the first peak on the left.

Electroenceph. clin. Neurophysiol., **1965**, *18*: 192–195

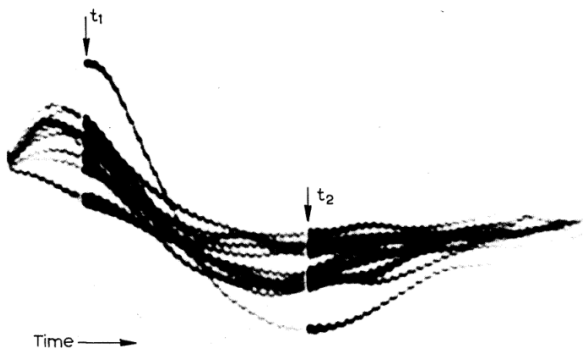


Fig. 2
The computer marks the times at which samples are taken by blanking points on the trace. The two points shown here were quite effective separators of the four groups. The sweep rate is twice that of Fig. 1.

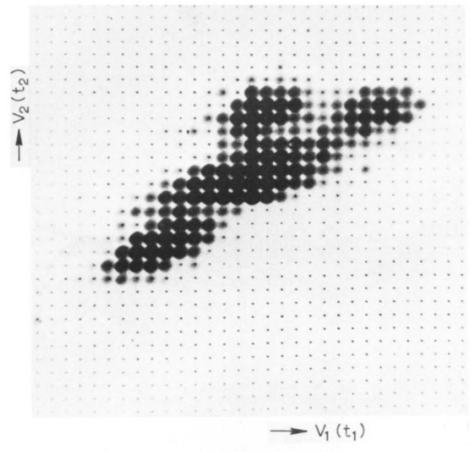
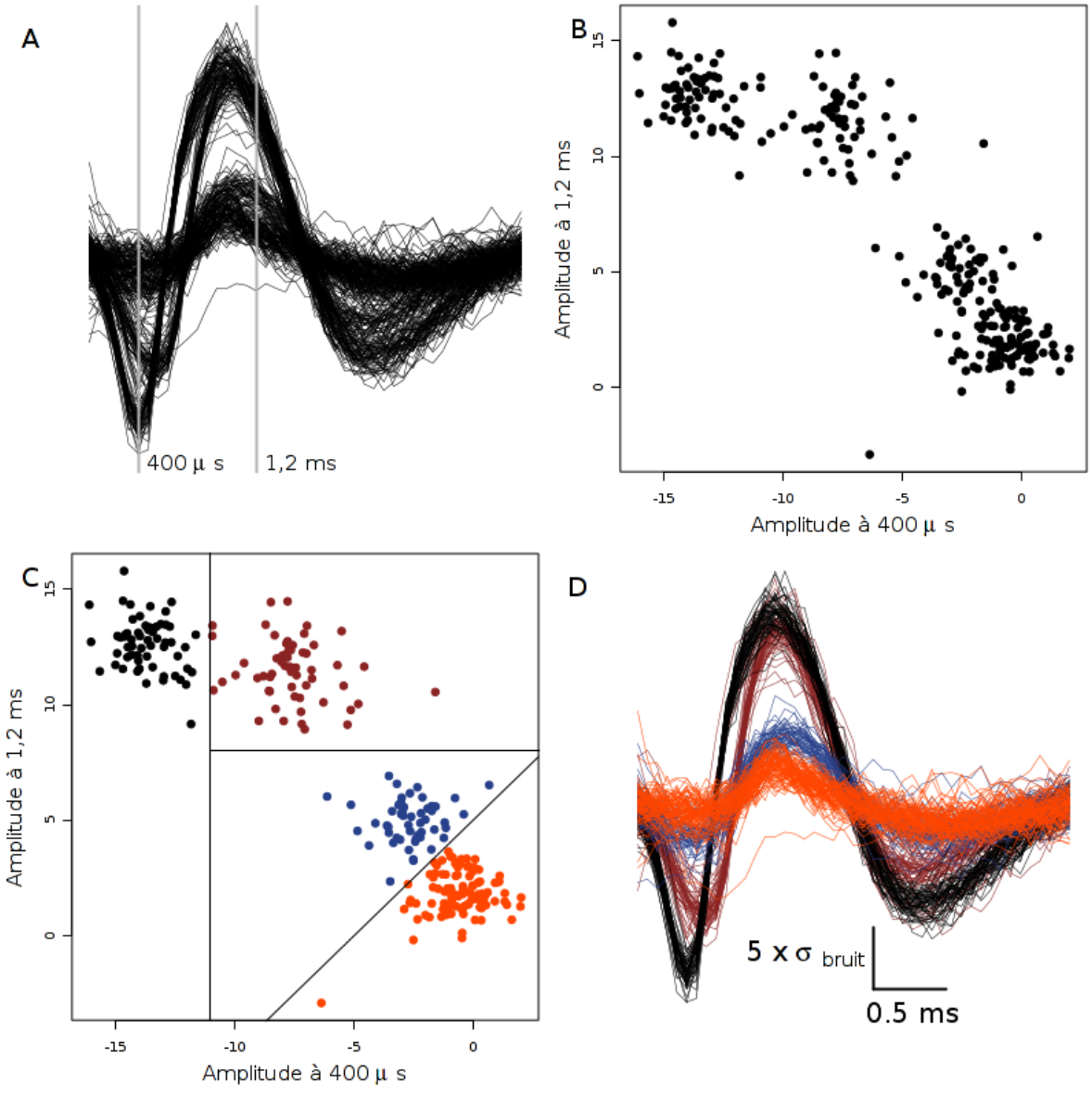


Fig. 1-3 of Simon (1965) *Electroenceph clin Neurophysiol* **18**:192.

A modern version of dimension reduction



Superposition resolution (1972)

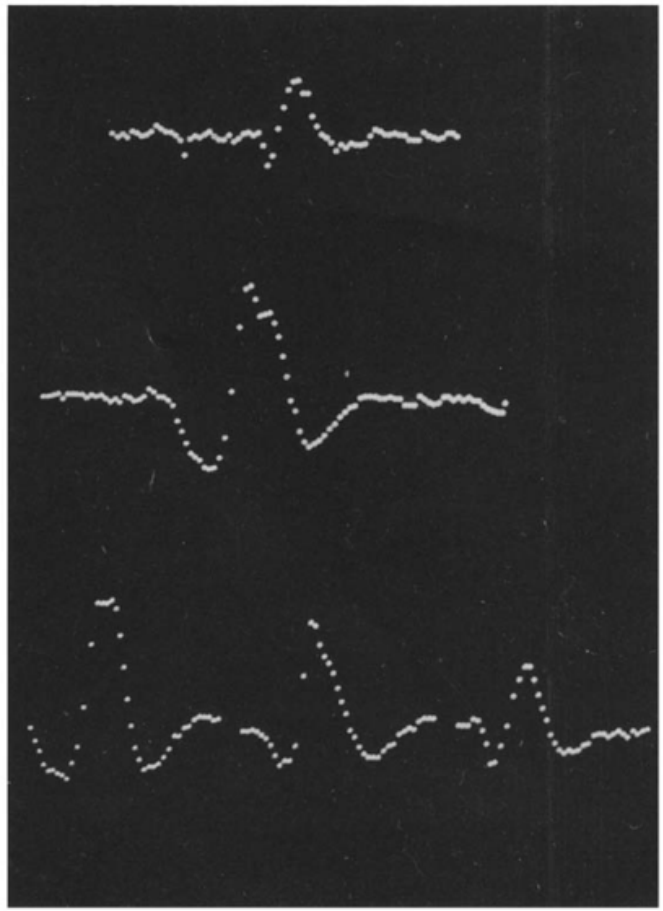
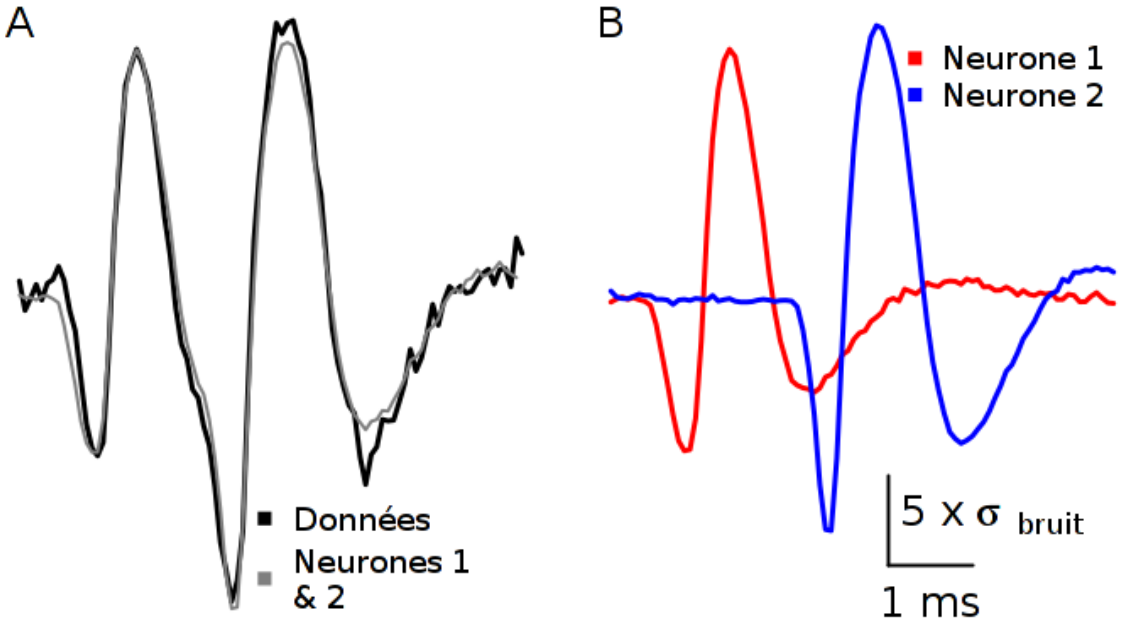


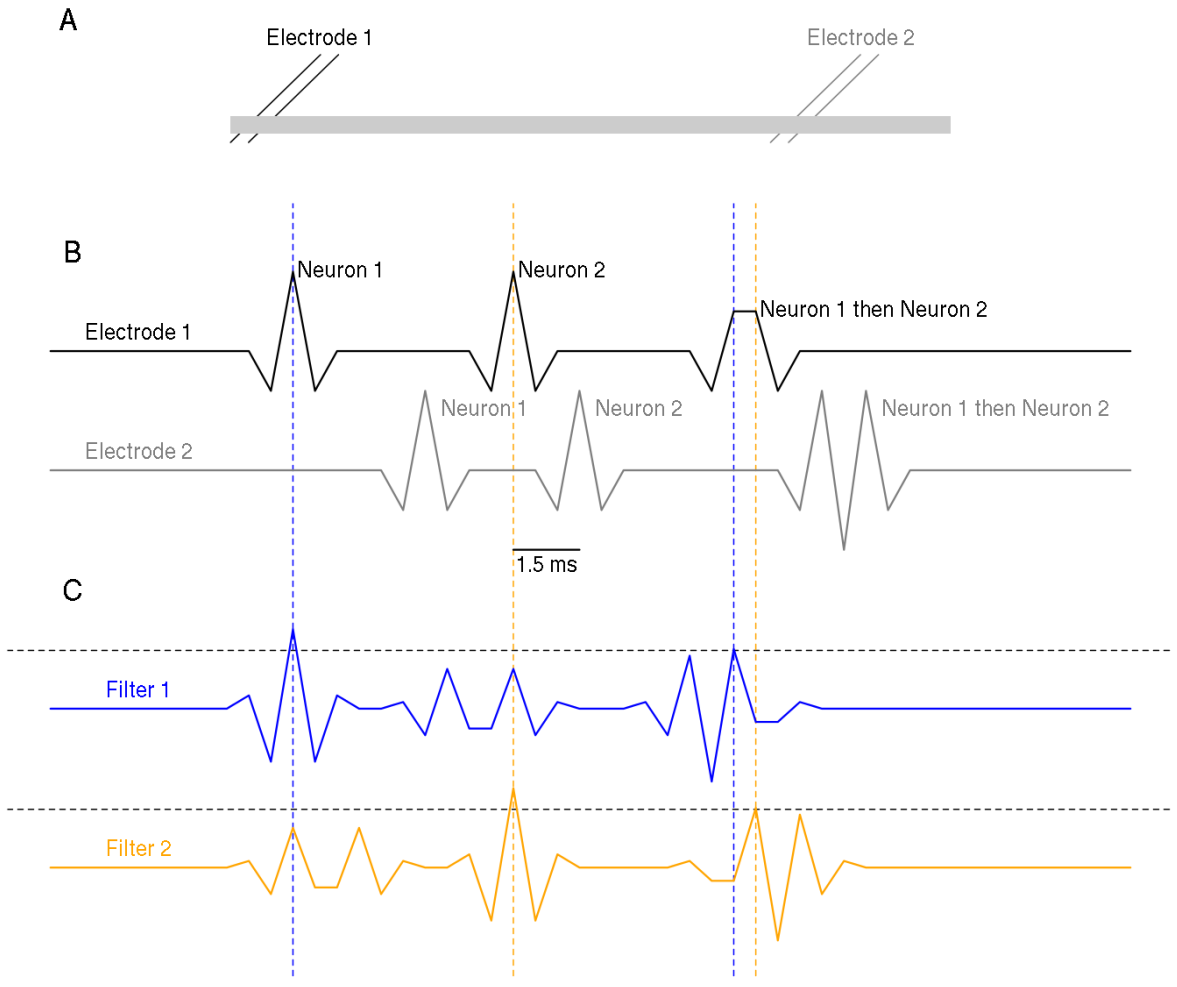
Fig. 3. Photograph of PDP-12 screen showing the display during an interference-potential analysis (all traces stationary and recalled from memory). *Upper trace*: result of subtraction of left template unit from the interference potential (*middle trace*). *Lower trace*: "contemporary" template units.

Fig. 3 of Prochazka et al (1972) *Electroenceph clin Neurophysiol* **32**:95.

A modern version of superposition resolution



Multi-channel linear filter: principle



You can reconstruct the figure with the following waveforms:

- Neuron 1: electrode 1 (-1,2,1,0,0,0,0,0,0) and electrode 2 (0,0,0,0,0,0,-1,2,-1).
- Neuron 2: electrode 1 (-1,2,1,0,0,0,0,0,0) and electrode 2 (0,0,0,-1,2,-1,0,0,0).

And the following filters:

- Neuron 1: electrode 1 (-1/2,1,1/2,0,0,0,0,0,0) and electrode 2 (0,0,0,0,0,0,-1/2,1,-1/2).
- Neuron 2: electrode 1 (-1/2,1,1/2,0,0,0,0,0,0) and electrode 2 (0,0,0,-1/2,1,-1/2,0,0,0).

Given signals $E_1(t)$ and $E_2(t)$ on electrode 1 and 2, the output of filter 1 (responding to Neuron 1) is:

$$F_1(t) = -E_1(t-1)/2+E_1(t)-E_1(t+1)/2-E_2(t+5)/2+E_2(t+6)-E_2(t+7)/2\,.$$

Multi-channel linear filter (1975)

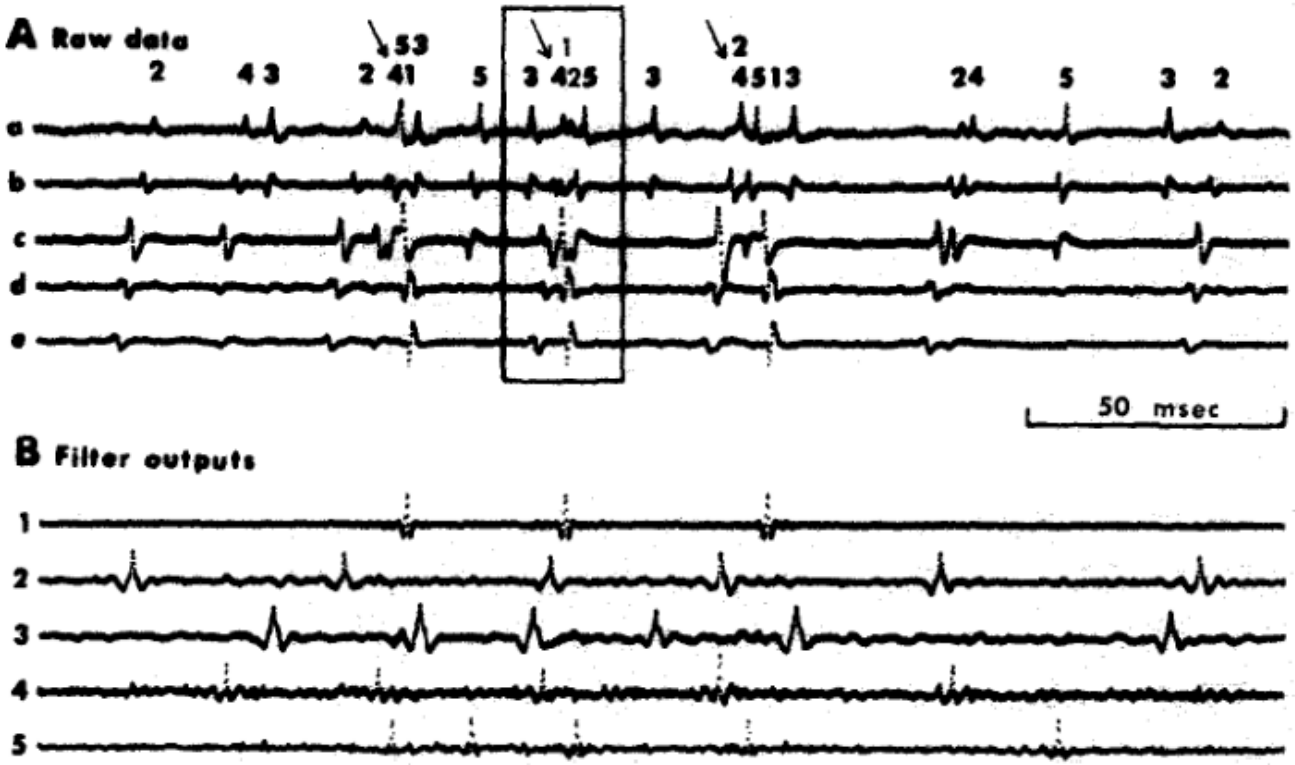


Fig. 2. A: a 200 msec portion of a single burst of neuronal activity from the cardiac ganglion of a spiny lobster (data provided by W. O. Friesen). Activity is monitored at 5 points along the ganglionic trunk with bipolar extracellular electrodes (traces from top to bottom successively more posterior positions). The numbers at the top show the identity of each impulse; they correspond to the numbers in Fig. 1. The portion of the record contained in the box is shown enlarged in Fig. 3. B: output from each of the 5 filters. Fast-rising 'pulses' correspond exactly to occurrences of the unit to which the filter is tuned. Unwanted units produce very little response or 'cross-talk'. Superposition of waveforms from two or more units does not affect filter performance (arrows). Ten similar bursts were analyzed in this run with no errors by the filters.

Fig. 2 of Roberts and Hartline (1975) *Brain Res* **94**:141.

Waveform changes during bursts (1973)

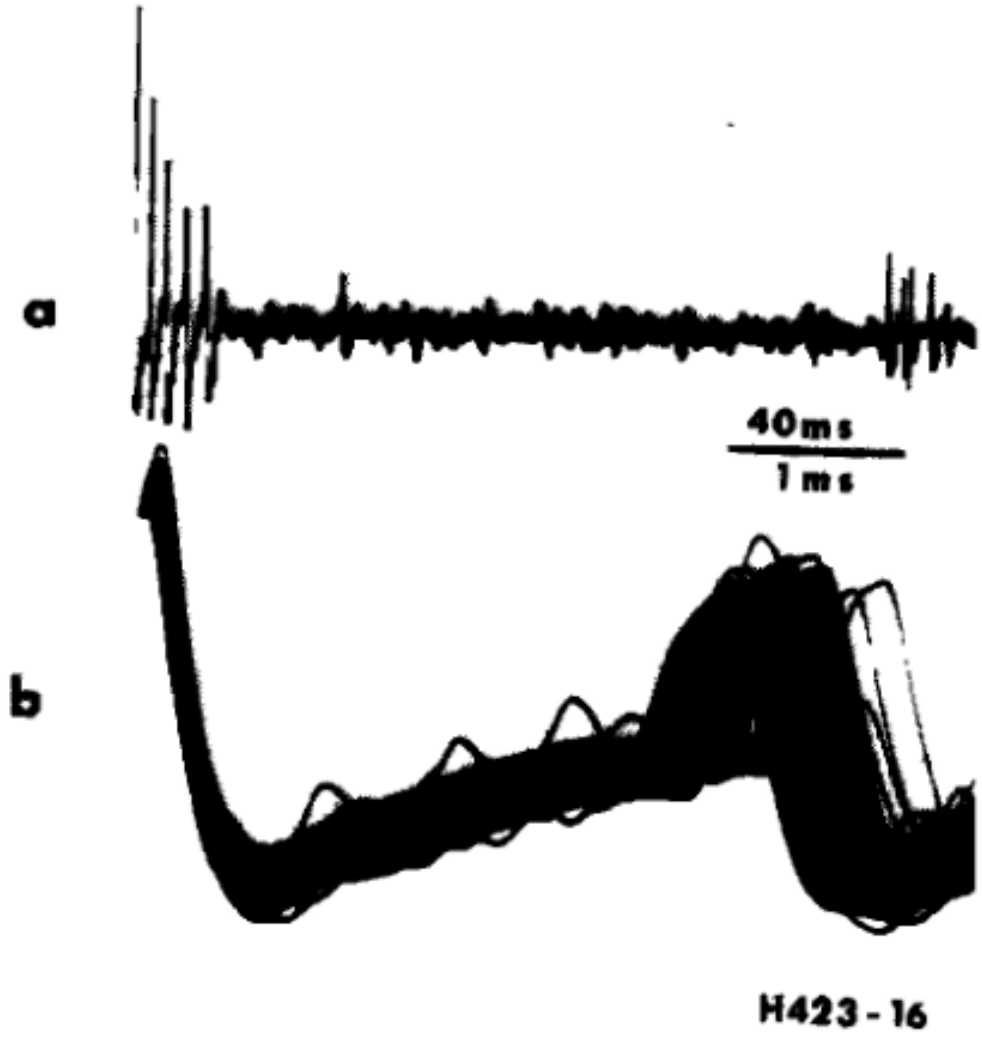


Fig. 2 of Calvin (1972) *Electroenceph clin Neurophysiol* **34**:94.

McNaughton, O'Keefe and Barnes solution (1983)

The method described in the present report is based on the fact that the size of the extracellular action potential varies inversely with the distance of the recording electrode from the current generator. In theory, a closely spaced tetrahedral array of recording electrodes with tips sufficiently close together to record signals from overlapping populations of neurons should permit the unique identification of all neuronal spikes which exceed the noise level. This is so since each cell would generate a unique point in the three-dimensional data space whose axes are defined by the spike height ratios of channels 1 and 2, 2 and 3, and 3 and 4. Note, that since the discrimination is based on amplitude ratios, the problem of intrinsic variation in spike amplitude such as occurs during the complex spike burst of hippocampal pyramidal cells is, in principle, solved.

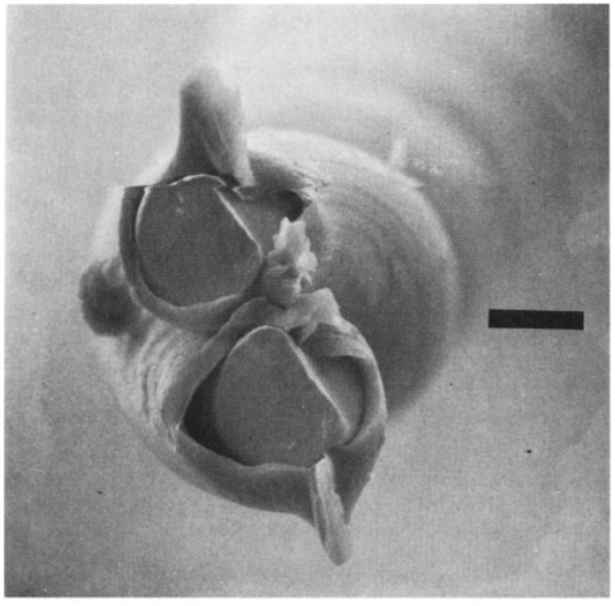


Fig. 1. Scanning electronmicrograph of a 'stereotrode' constructed by twisting together two lengths of 25 μm teflon-insulated wire, and cutting the ends with sharp scissors. Original magnification 240×. Calibration bar 20 μm.

Part of the Introduction and Fig. 1 of McNaughton et al (1983) *J Neurosci Methods* **8**:391.

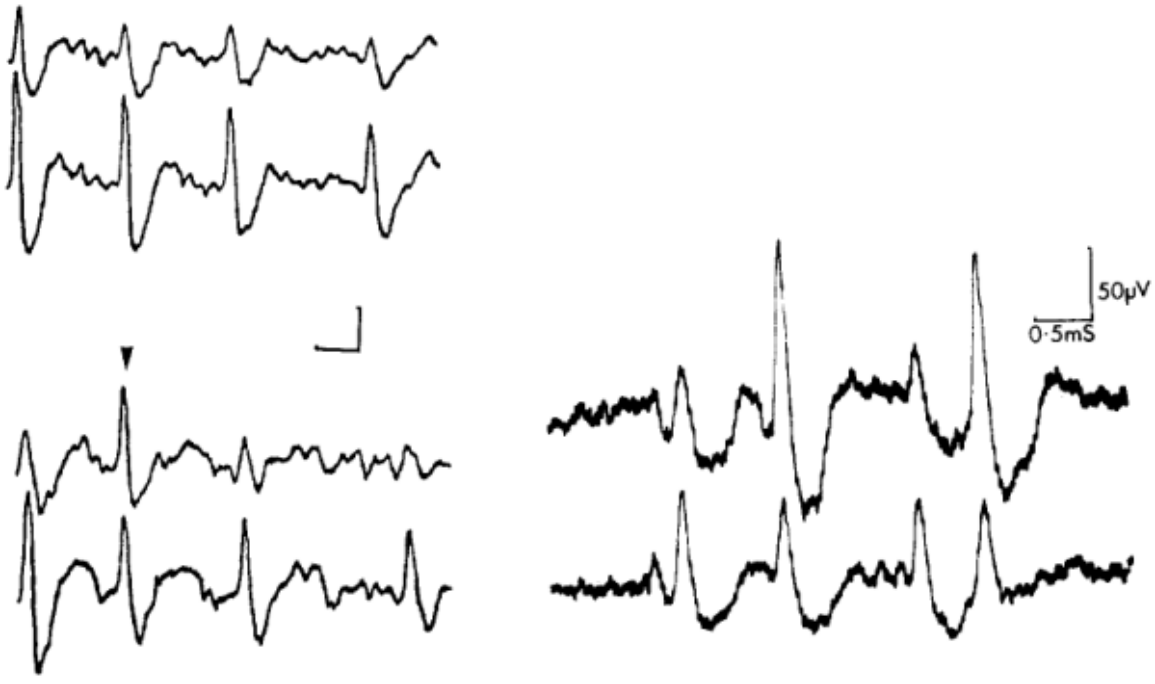


Fig. 2. Two stereotrode records selected from the same data set to illustrate the discrimination of a true complex-spike burst from a single generator (upper pair of traces), from a spurious 'burst' (lower pair of traces) which resembles a complex-spike on the Y channel but clearly contains a spike from a second generator (arrow).

Fig. 3. A second example of spikes which would be indiscriminable using spike height on a single channel, but clearly belong to two different cells when the two-channel amplitude ratio is considered.

Fig. 2 and 3 of McNaughton et al (1983)

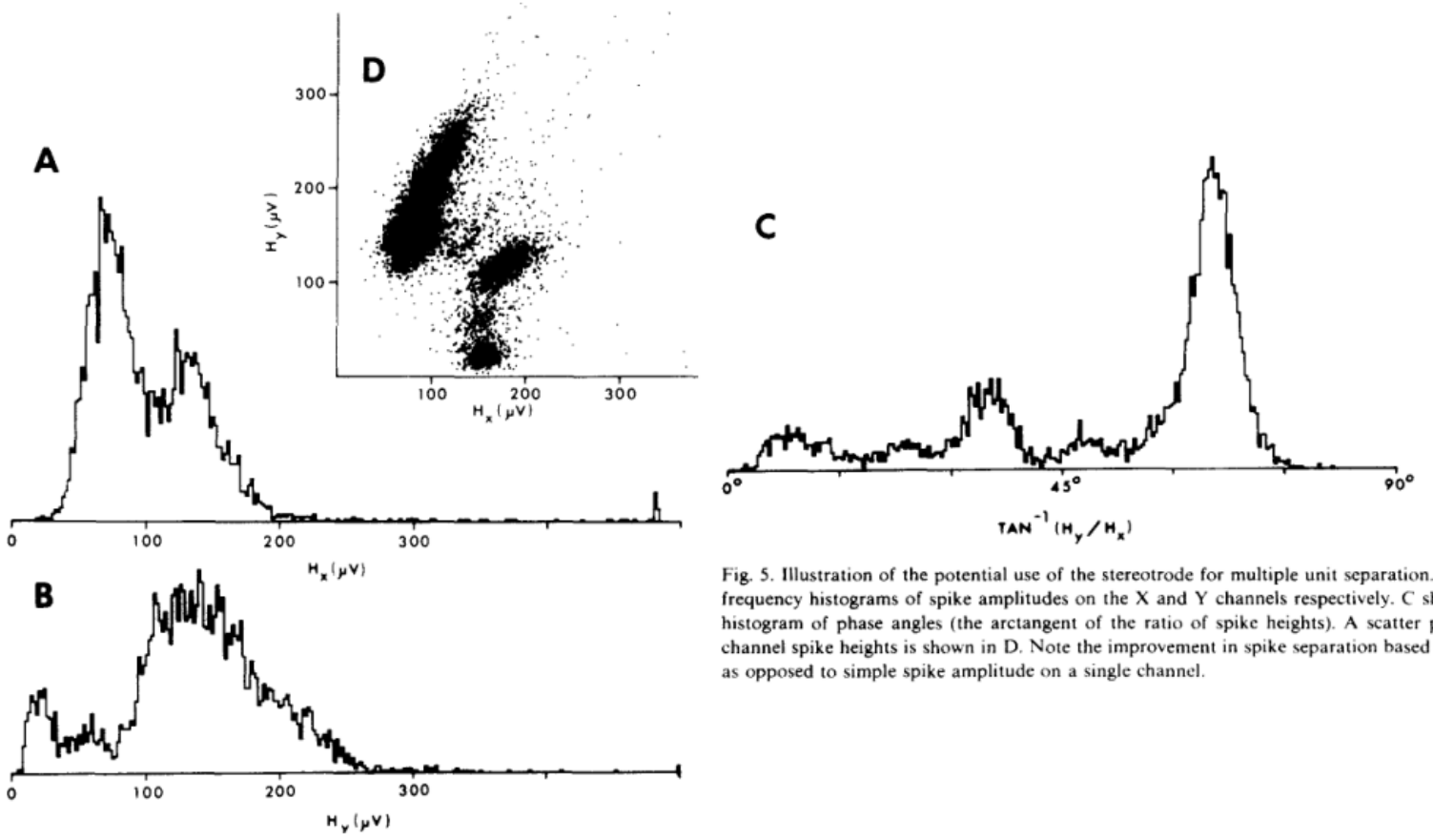


Fig. 5. Illustration of the potential use of the stereotrode for multiple unit separation. A and B show the frequency histograms of spike amplitudes on the X and Y channels respectively. C shows the frequency histogram of phase angles (the arctangent of the ratio of spike heights). A scatter plot of Y versus X channel spike heights is shown in D. Note the improvement in spike separation based on the phase angle as opposed to simple spike amplitude on a single channel.

Fig. 5 of McNaughton et al (1983)

Sampling jitter (1984)

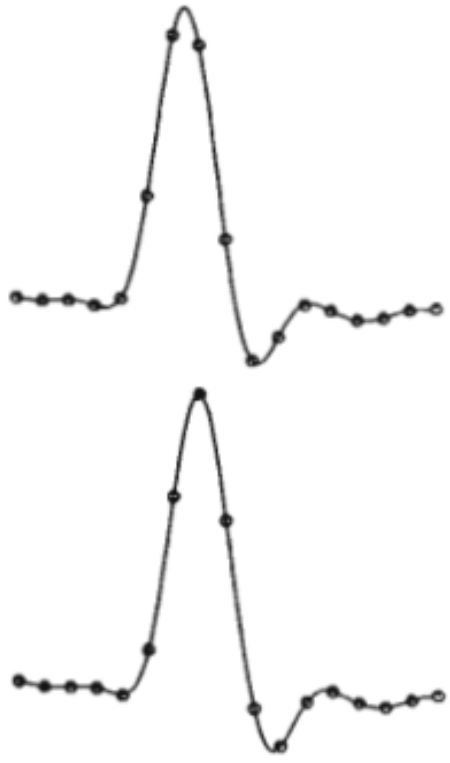


Fig. 1. Two copies of the same waveform, sampled near the Nyquist rate. Sampling at the Nyquist rate only sketches the underlying continuous waveform, and two renditions are apt to appear quite different if the samples are out of phase.

Fig. 1 of McGill and Dorfman (1984) *IEEE Trans Biomed Eng* **31**:462. EMG recordings.

Sampling jitter correction

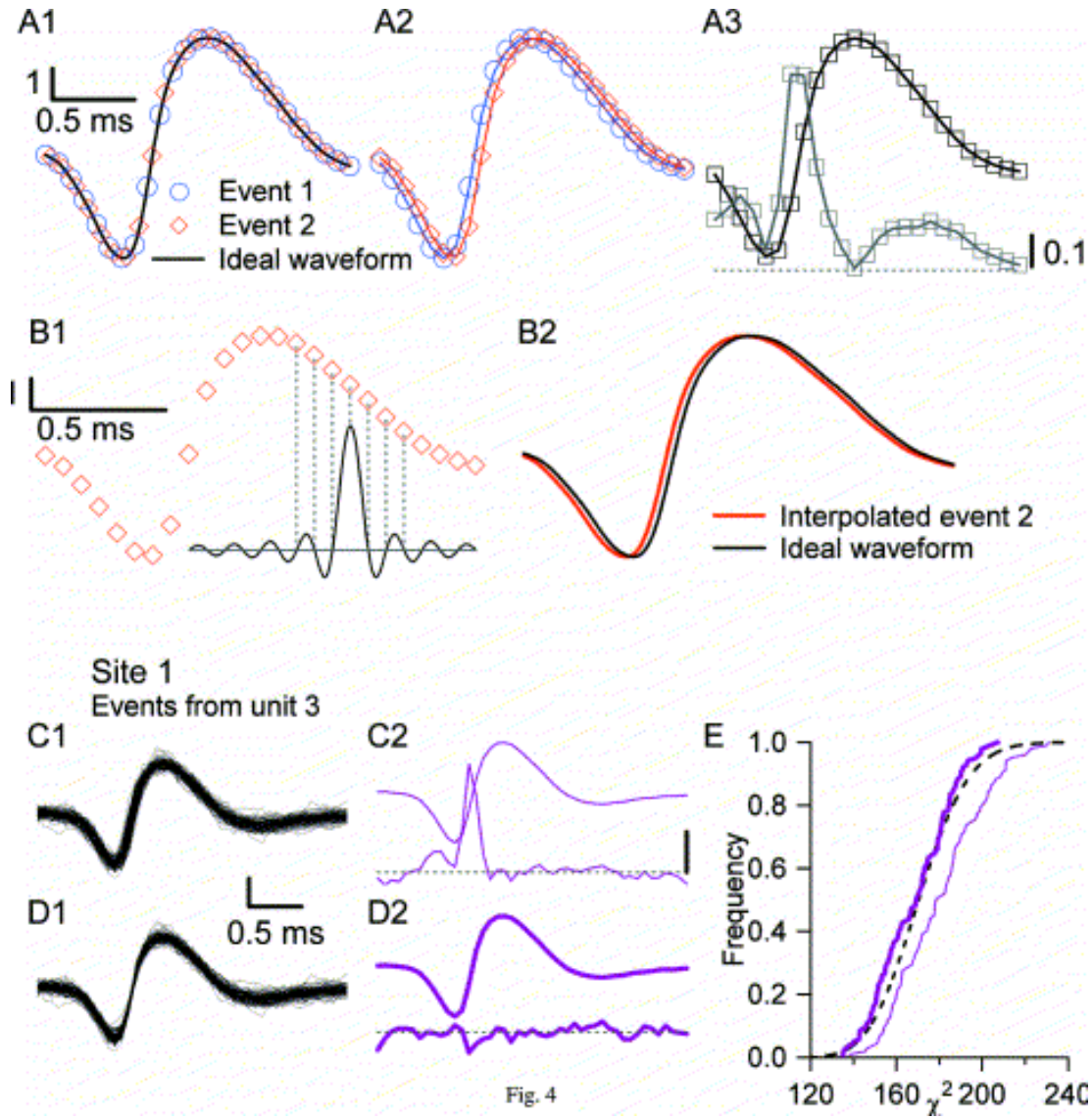
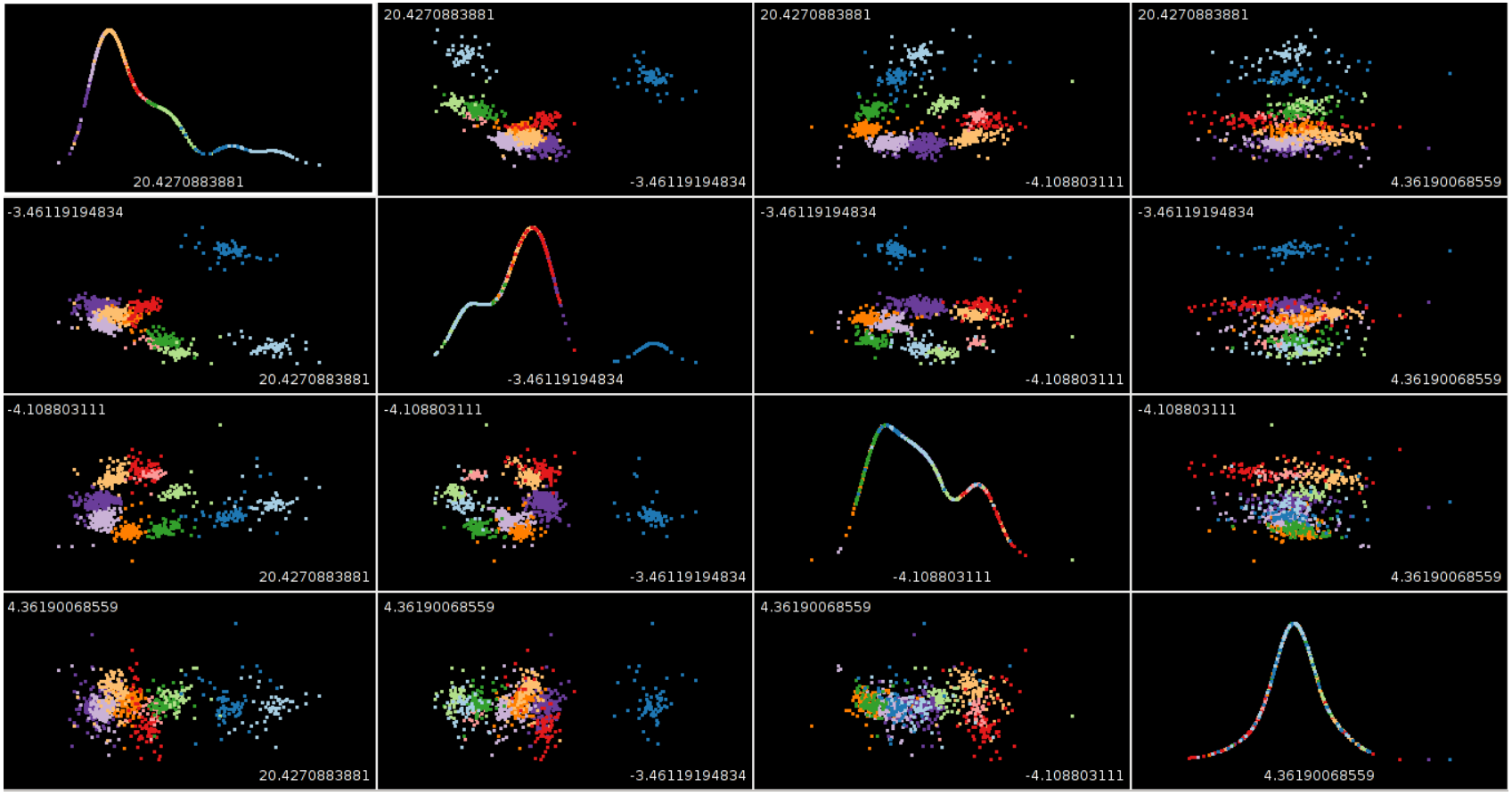


Fig. 4 of Pouzat et al (2002) *J Neurosci Methods* **122**:43.

Sophisticated visualization tools (late 80s early 90s)



A scatter plot matrix obtained with `GGobi` (www.ggobi.org).

Automatic clustering: K-means, Gaussian Mixture Model (GMM), etc.

During the last 25 years there has been a massive input from statistical methods for:

- dimension reduction (ICA, etc.)
- automatic clustering (K-means, Gaussian Mixture Model, etc.)
- choosing the number of mixture components / neurons (AIC, BIC, etc.)

This collection of methods is part of what is now commonly Machine Learning...
See, Hastie et al (2009) *The Elements of Statistical Learning*. Springer.

That's all folks

Thank you for listening!