Extracellular recordings and statistical analysis of data in neuronal networks

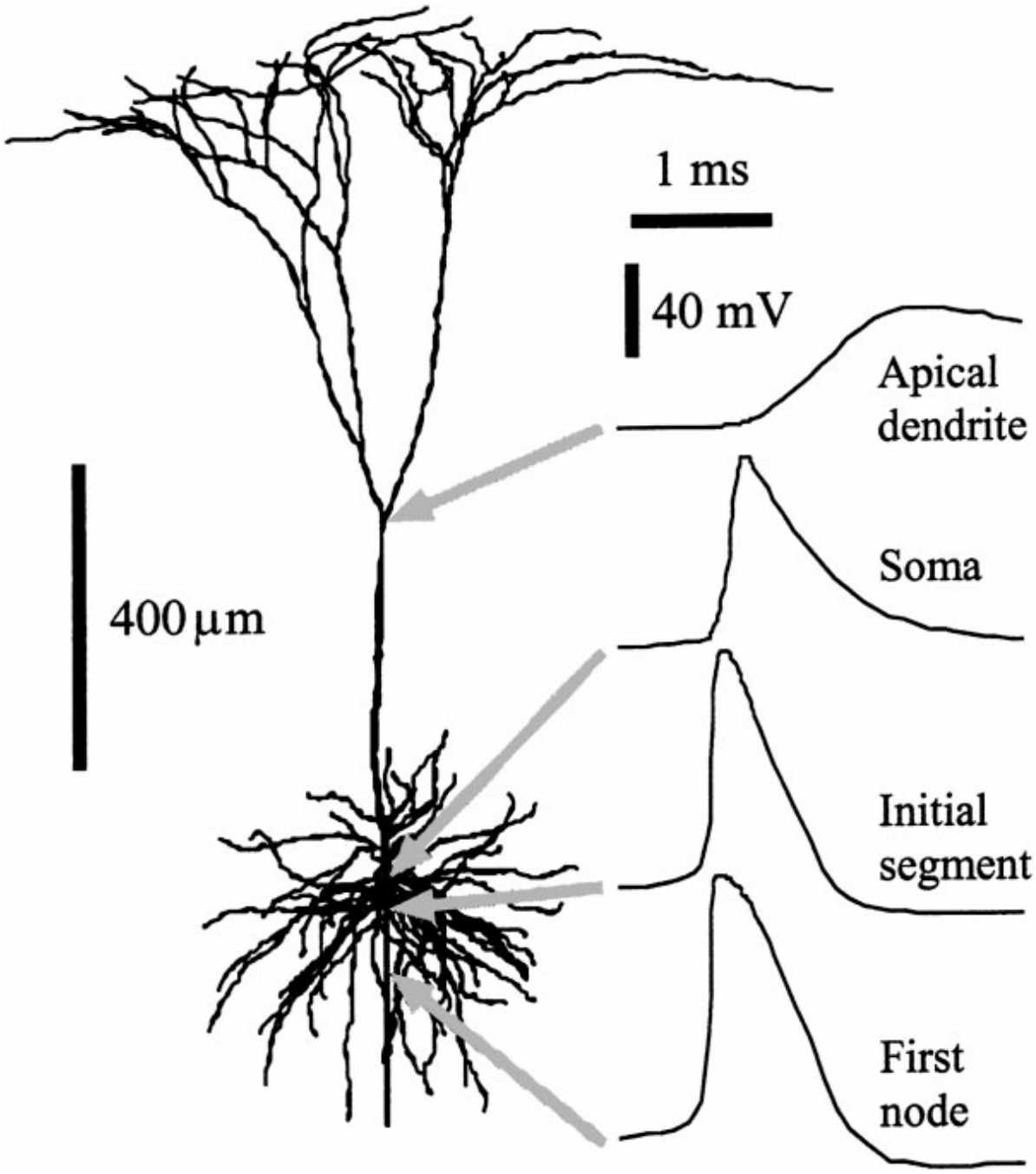
Please remember that sometimes we will use the following definitions:

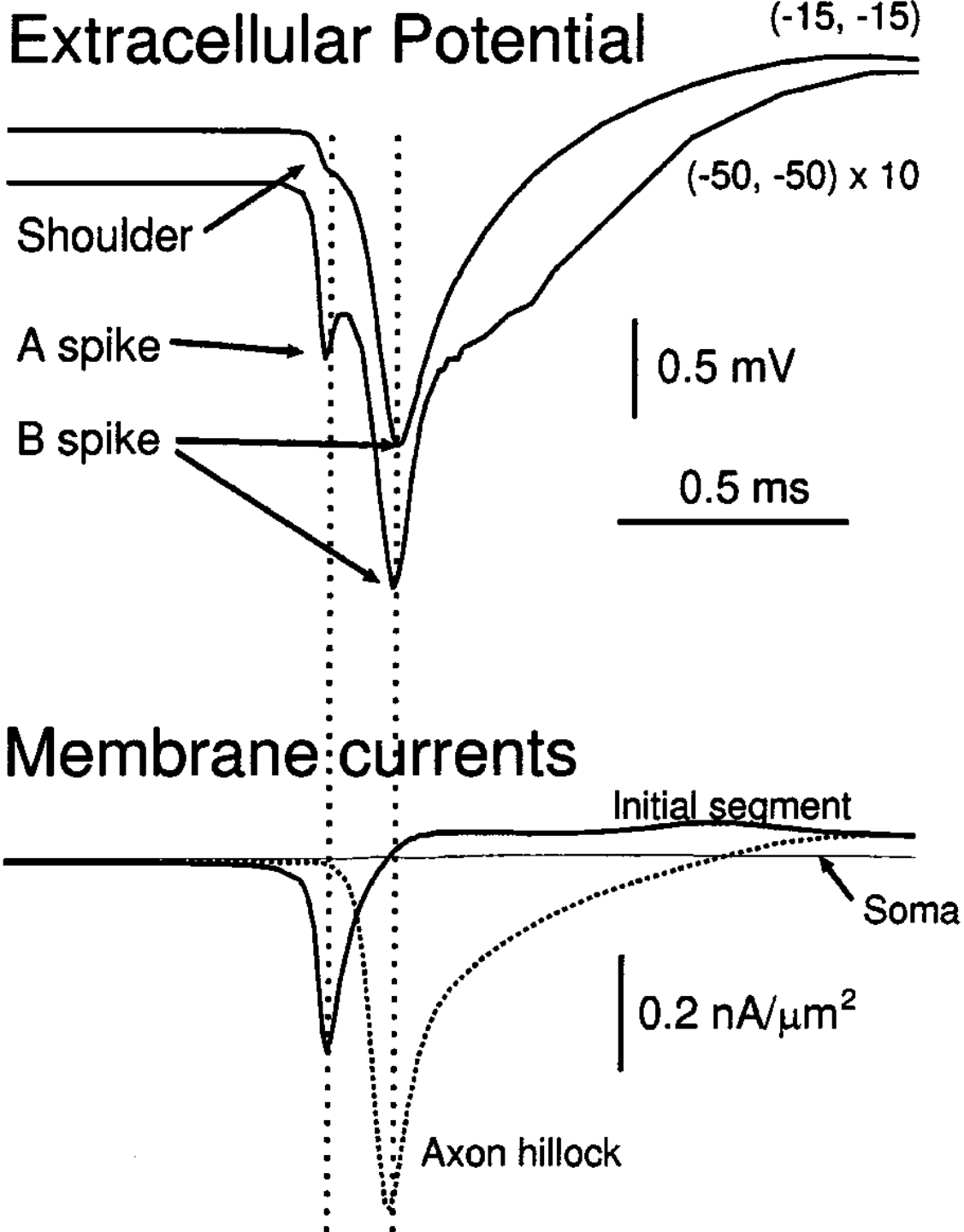
1) Fano factor = ratio between spike counts variance and mean

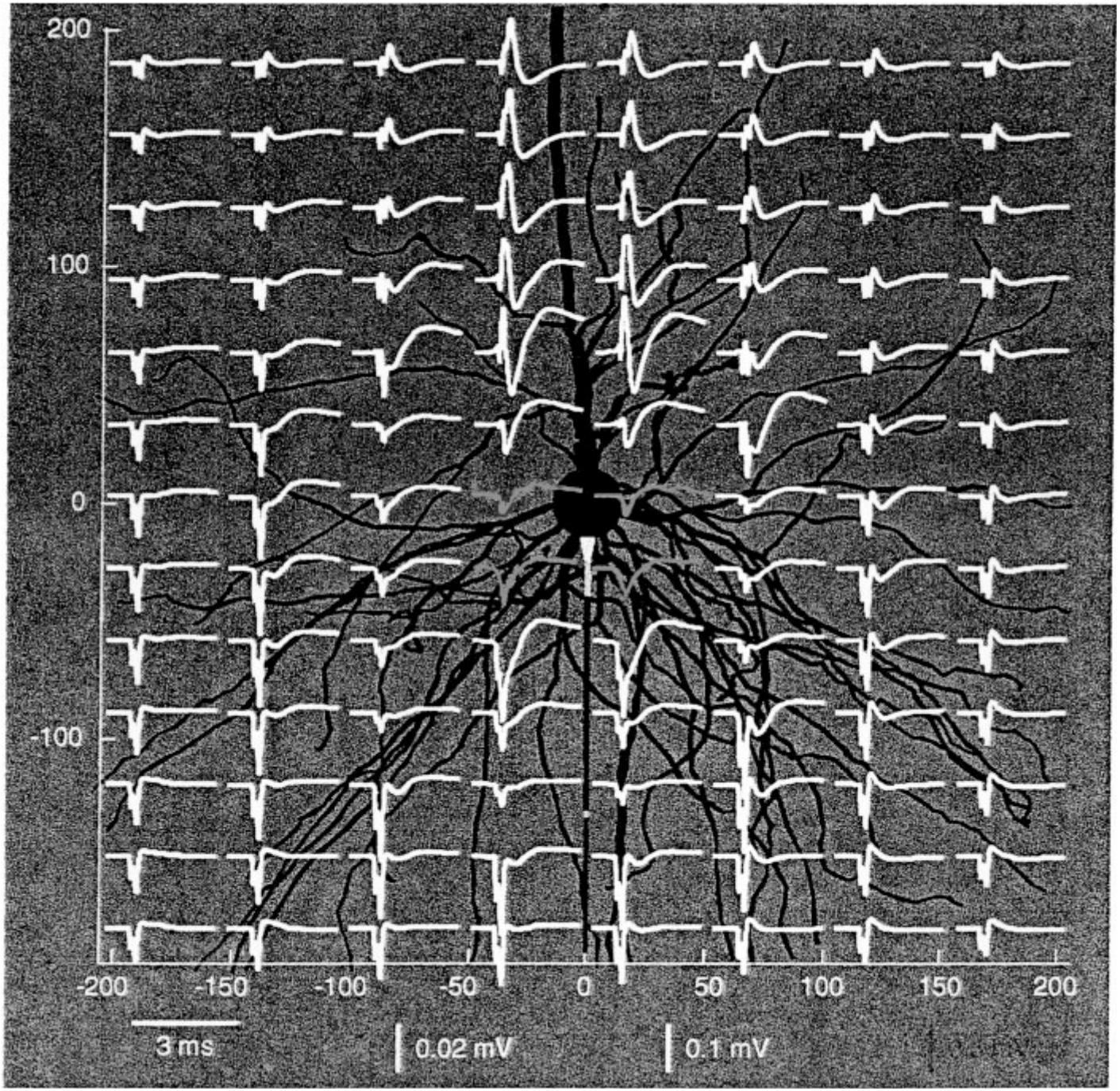
2) ACF = autocorrelation function

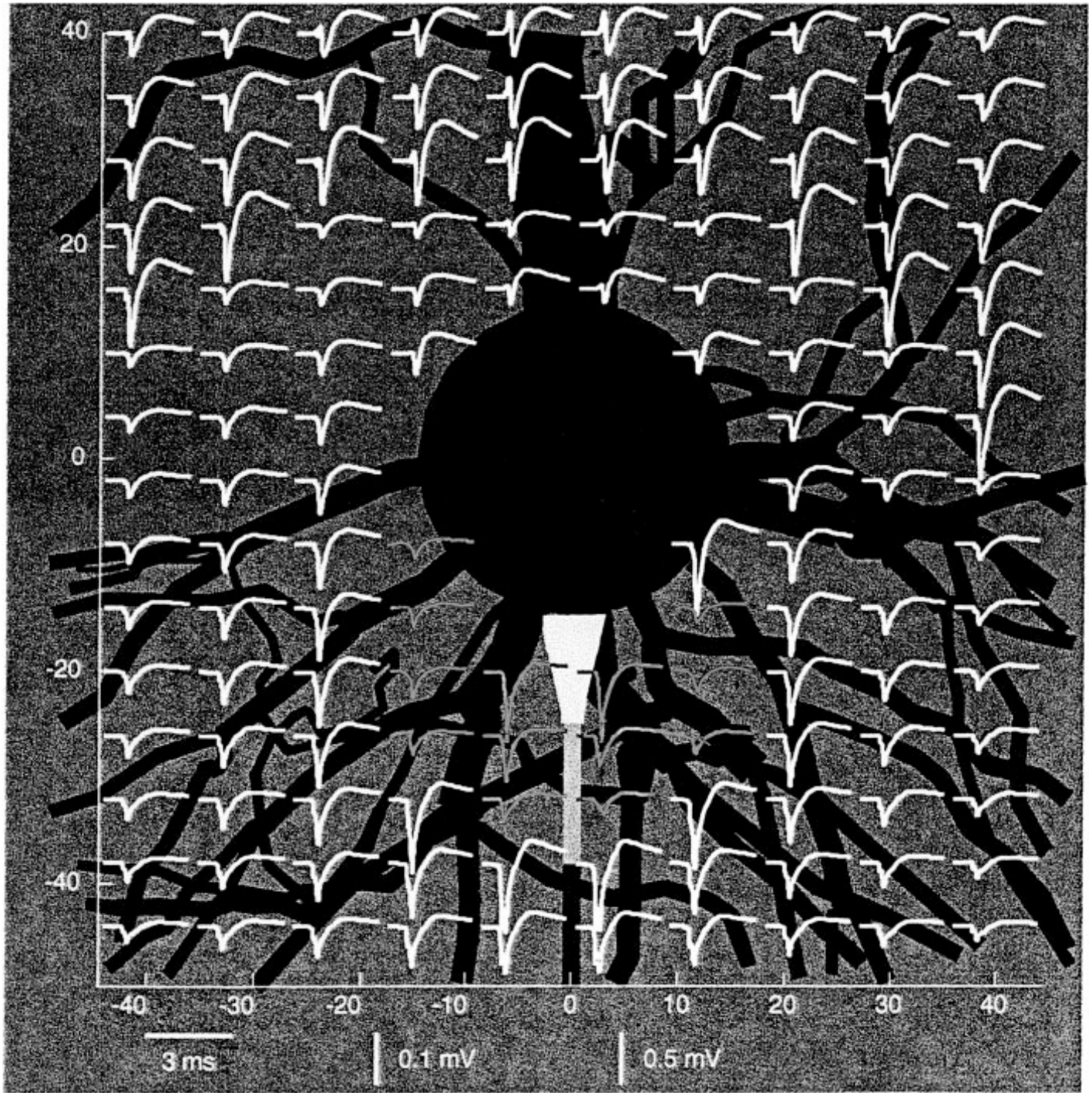
3) CV = coefficient of variation = standard deviation/mean

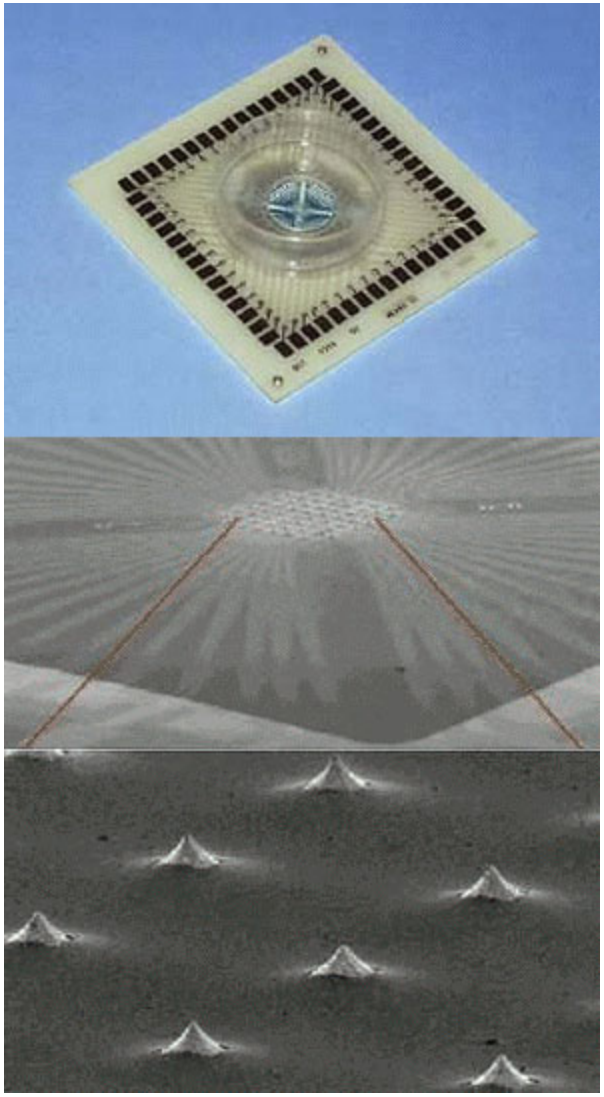
4) CV2 or CV^2 = squared CV

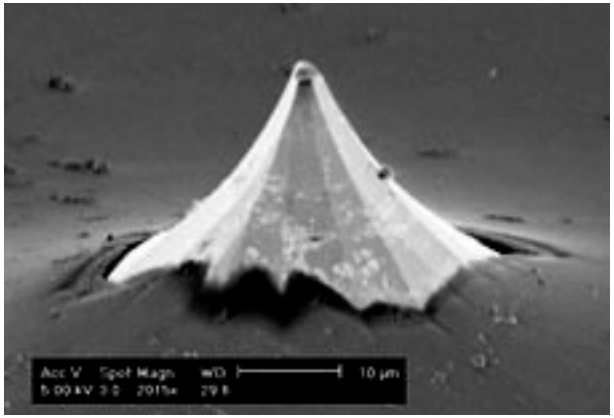

1 ms
40 mV
400 µm
Apical dendrite
Soma
Initial segment
First node

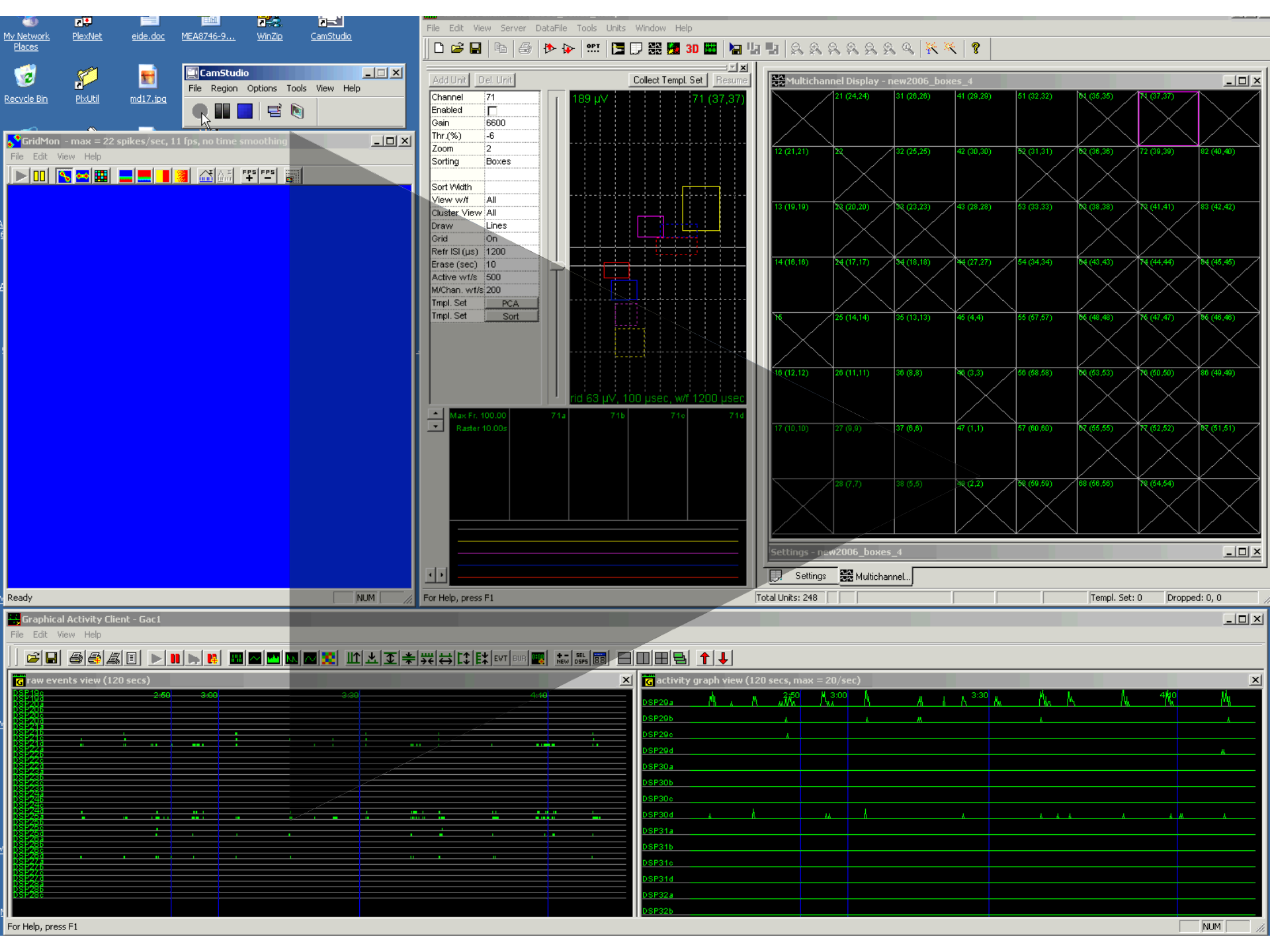

Extracellular Potential
(-15, -15)
(-50, -50) x 10
Shoulder
A spike
B spike
0.5 mV
0.5 ms
Membrane currents
Initial segment
Soma
0.2 nA/μm²
Axon hillock


200
100
0
-100
-200
-150
-100
-50
0
50
100
150
200
3 ms
0.02 mV
0.1 mV


40
20
0
-20
-40
-40
-30
-20
-10
0
10
20
30
40
3 ms
0.1 mV
0.5 mV




Acc.V Spot Magn WD 10 µm
5.00 kV 3.0 2015x 29.8


My Network Places
PlexNet
eide.doc
MEA8746-9...
WinZip
CamStudio
Recycle Bin
PlxUtil
md17.jpg
CamStudio
File Region Options Tools View Help
GridMon - max = 22 spikes/sec, 11 fps, no time smoothing
File Edit View Help
Ready
NUM
File Edit View Server DataFile Tools Units Window Help
Add Unit
Del. Unit
Collect Templ. Set
Resume
Channel 71
Enabled
Gain 6600
Thr.(%) -6
Zoom 2
Sorting Boxes
Sort Width
View w/f All
Cluster View All
Draw Lines
Grid On
Refr ISI (µs) 1200
Erase (sec) 10
Active wf/s 500
M/Chan. wf/s 200
Tmpl. Set PCA
Tmpl. Set Sort
189 µV
71 (37,37)
rid 63 µV, 100 µsec, w/f 1200 µsec
Max Fr. 100.00
Raster 10.00s
71a
71b
71c
71d
For Help, press F1
Multichannel Display - new2006_boxes_4
Settings - new2006_boxes_4
Settings
Multichannel...
Total Units: 248
Templ. Set: 0
Dropped: 0, 0
Graphical Activity Client - Gac1
File Edit View Help
raw events view (120 secs)
activity graph view (120 secs, max = 20/sec)
DSP29a
DSP29b
DSP29c
DSP29d
DSP30a
DSP30b
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DSP31d
DSP32a
DSP32b
For Help, press F1
NUM

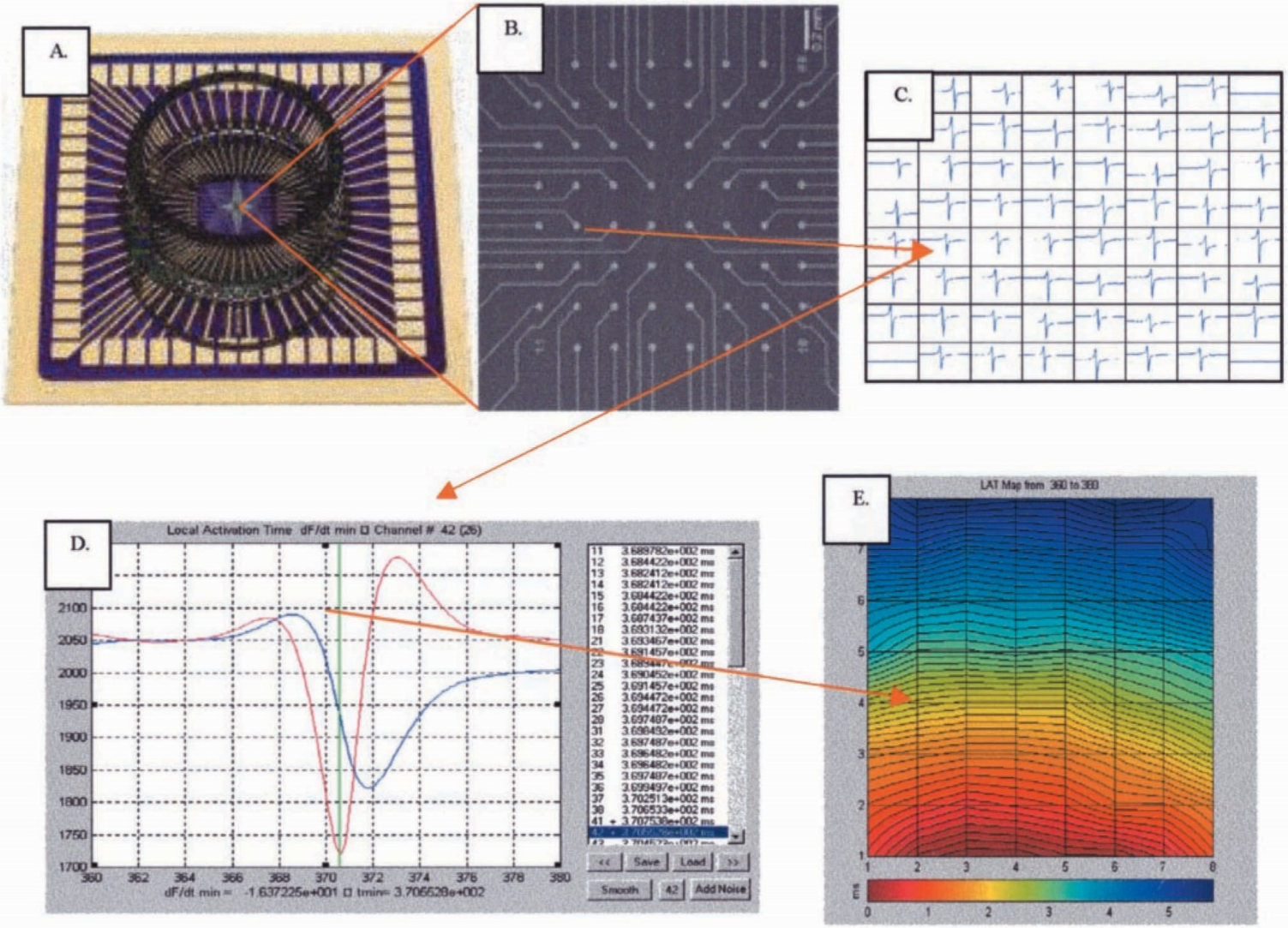


Figure 1. The MEA mapping technique uses MEA plates (A) consisting of 60 microelectrodes (B). Simultaneous extracellular recording from all 60 electrodes (C) and automatic calculation of all the LATs (D) allows construction of high-resolution activation maps (E) with activation propagating from early (red) to late (blue) sites.

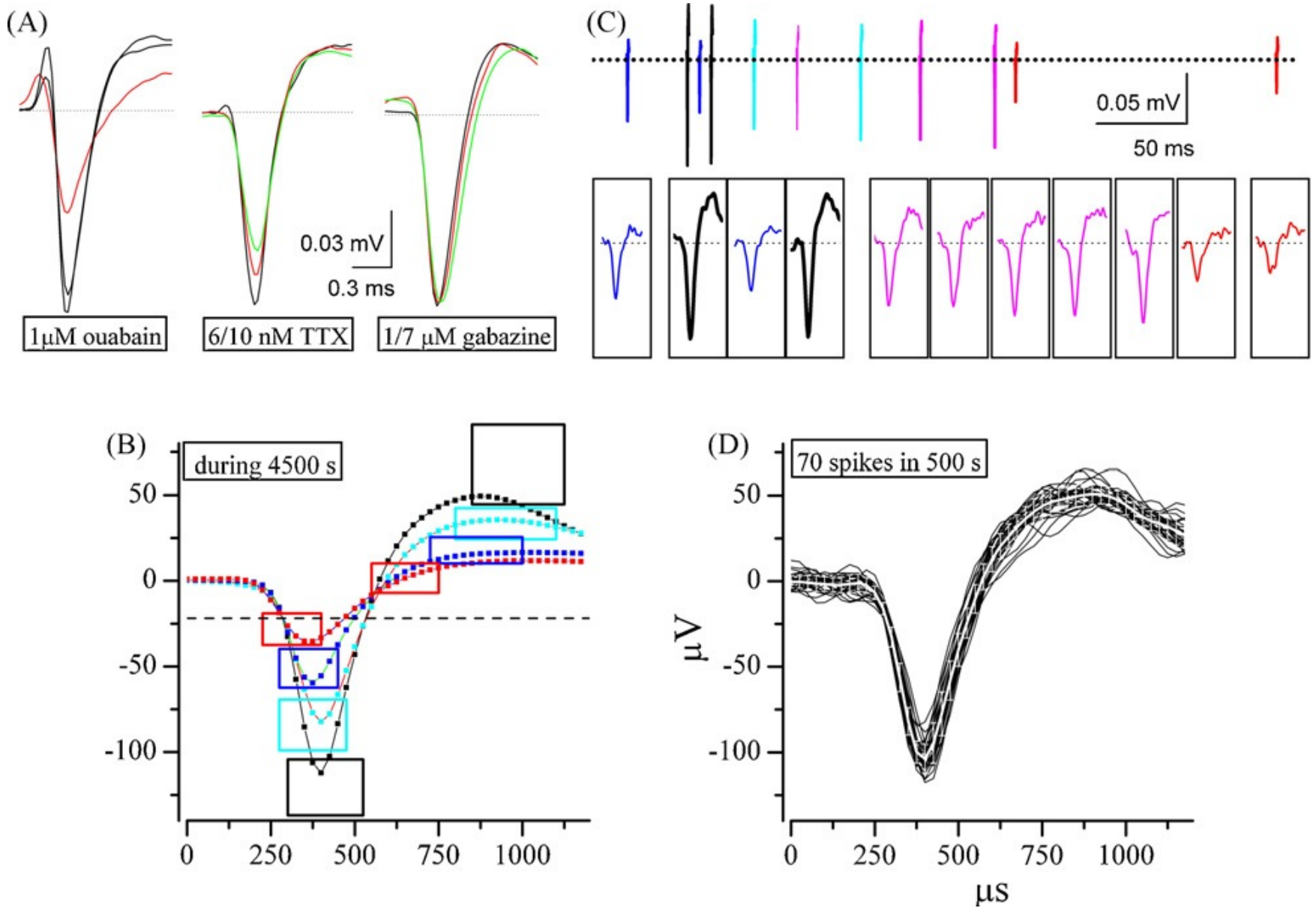
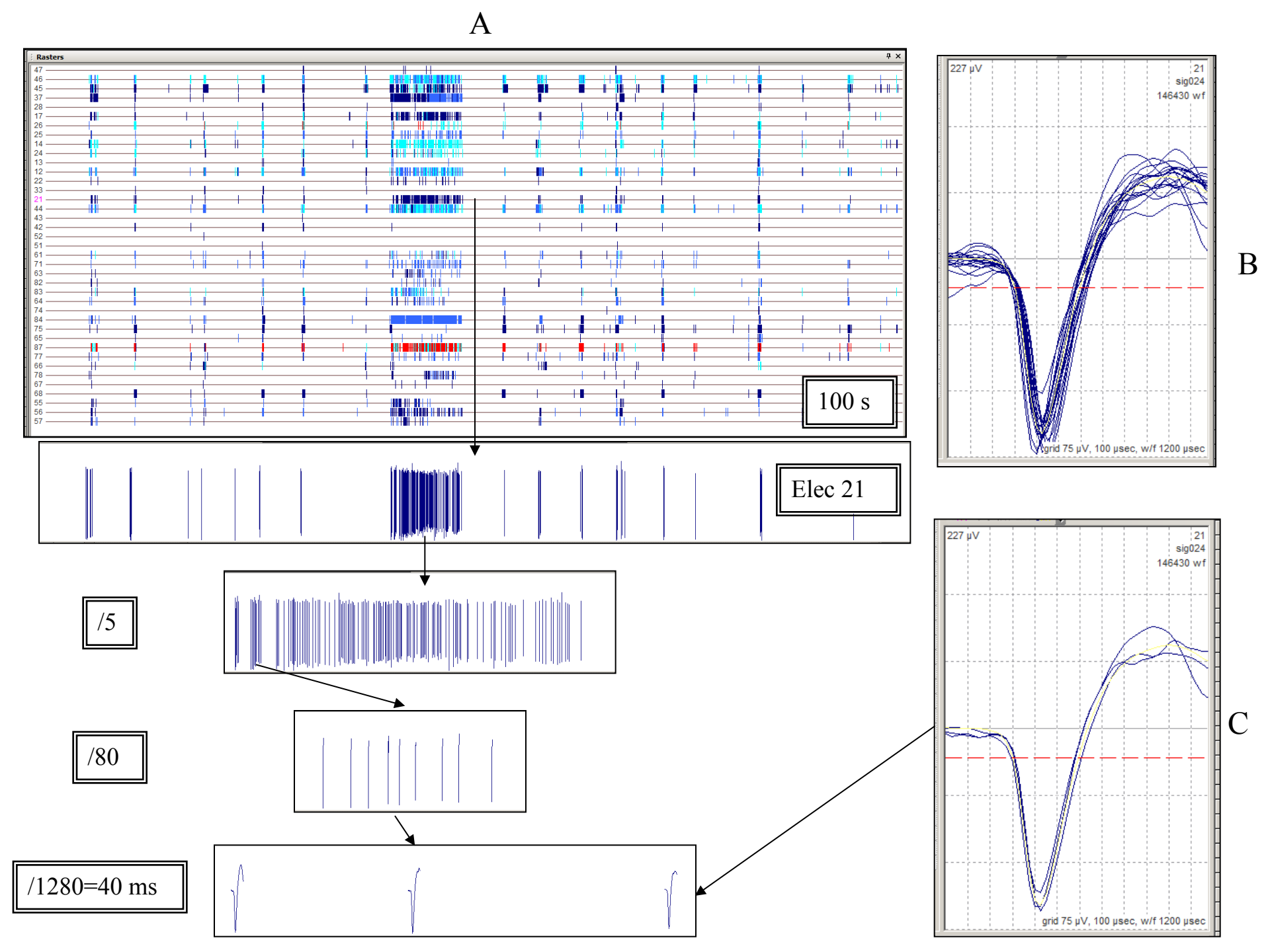
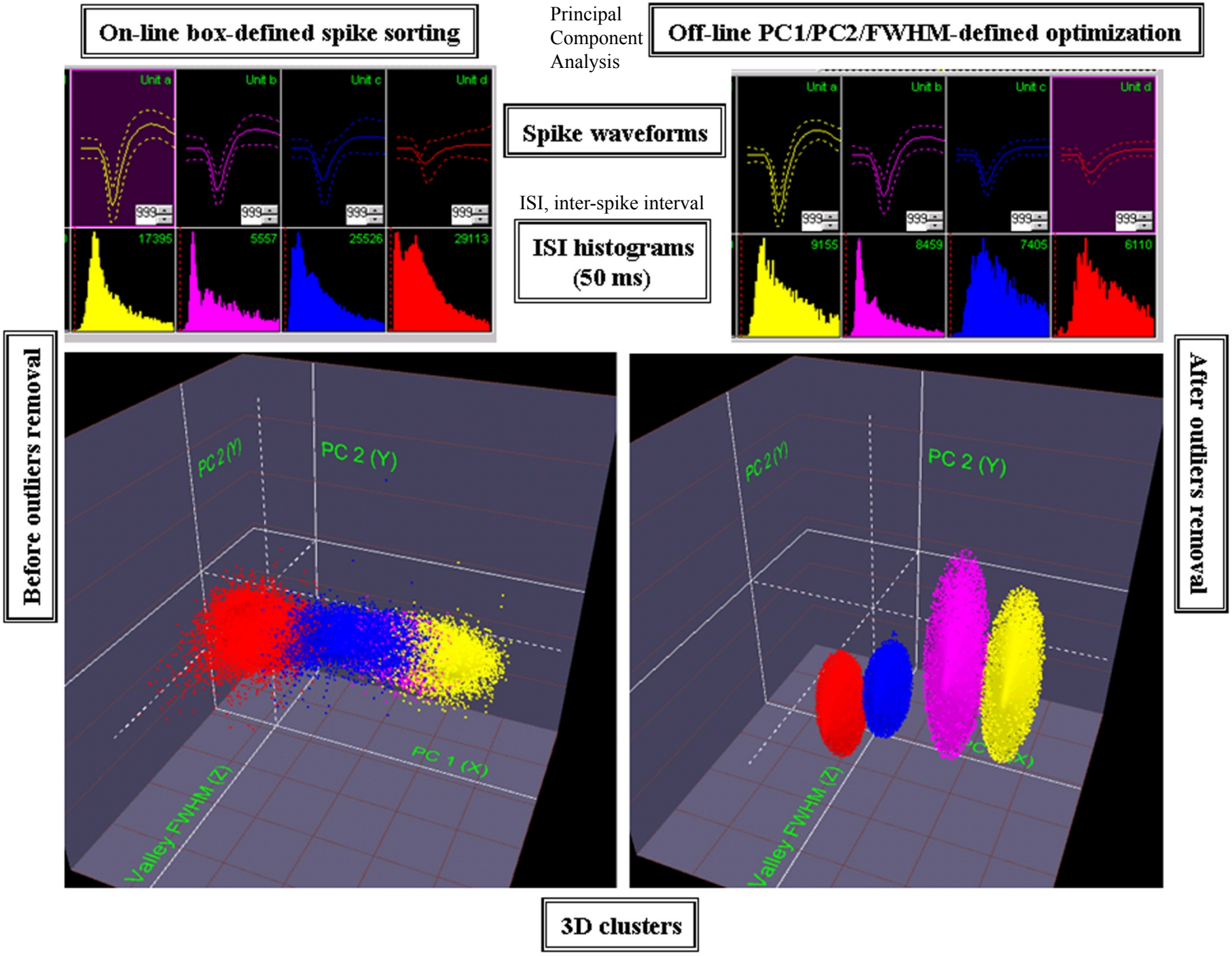
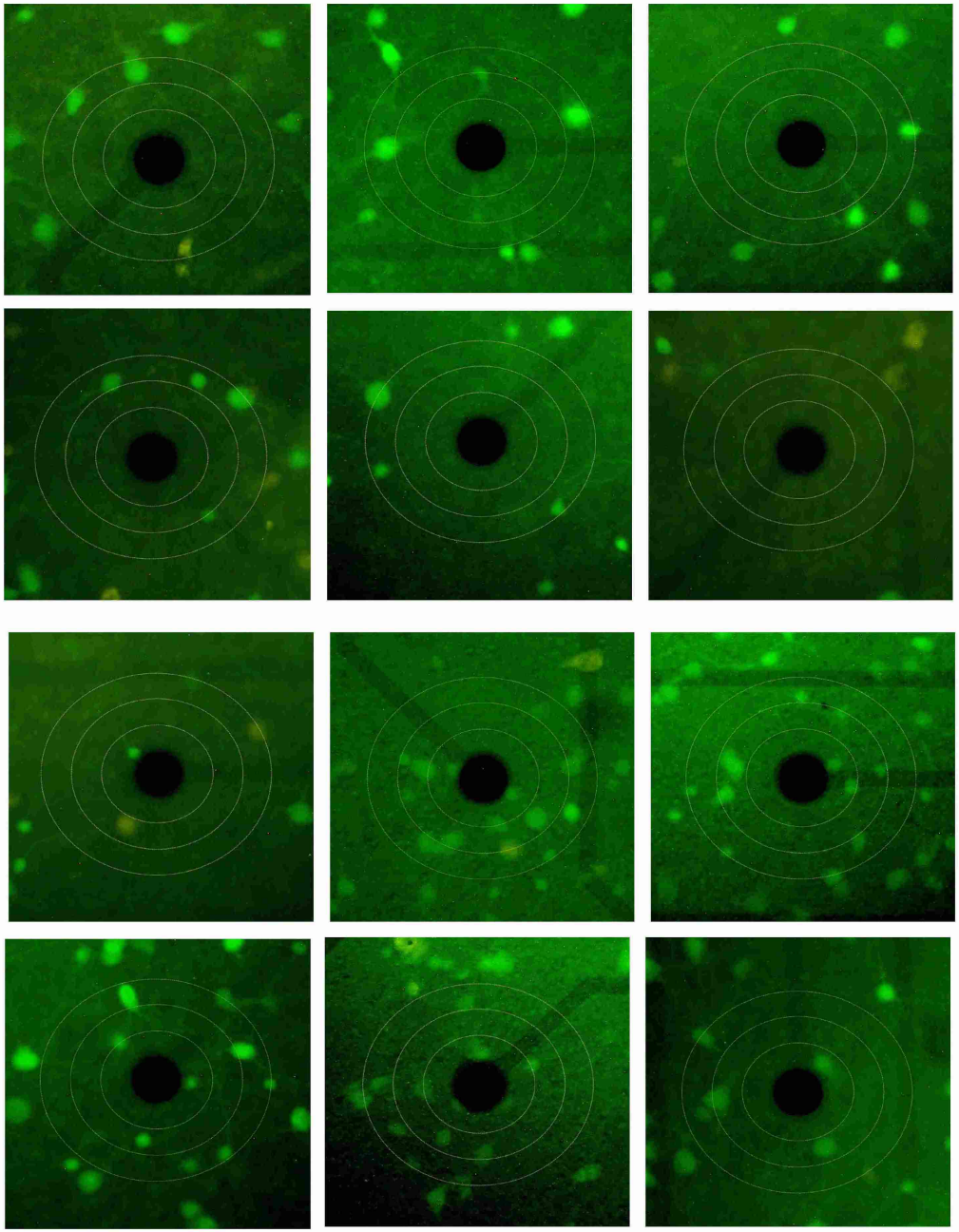


Fig. 1. Waveshapes and the sorting of waveforms into units. (A) (left) Superimposed spikes before (black line), during (red line) and after (black line) the perfusion with 1M ouabain; the smallest and the biggest traces were acquired 1 and 10 min before the firing block and the washout, respectively; (middle) superimposed spikes before (black line) and during the perfusion with 6 nM (red line) or 10nM (green line) TTX, respectively; (right) superimposed spikes before (black line) and during the perfusion with 1 (red line) or 7M (green line) gabazine, respectively. (B) A 400ms sequence of spikes recorded from the same electrode (the colors are related to those used in panel C). The horizontal line is the zero voltage. (C) The rectangular boxes for spike capturing are superimposed on the averages of the spikes sorted in the different units (closed squares-lines) during 4500 s. The dashed line is the threshold. (D) Average trace (white line and error bars, ±SD) and 70 spikes, superimposed, observed in 500 s from the same electrode (data obtained after removal of outliers, see Fig. 2). **From *F. Gullo et al. / Journal of Neuroscience Methods 181 (2009) 186–198***


A
Rasters
100 s
Elec 21
/5
/80
/1280=40 ms
227 µV
21
sig024
146430 wf
grid 75 µV, 100 µsec, w/f 1200 µsec
B
227 µV
21
sig024
146430 wf
grid 75 µV, 100 µsec, w/f 1200 µsec
C


On-line box-defined spike sorting
Principal Component Analysis
Off-line PC1/PC2/FWHM-defined optimization
Unit a
Unit b
Unit c
Unit d
Spike waveforms
17395
5557
25526
29113
ISI, inter-spike interval
ISI histograms (50 ms)
9155
8459
7405
6110
Before outliers removal
After outliers removal
PC 2 (Y)
PC 1 (X)
Valley FWHM (Z)
3D clusters

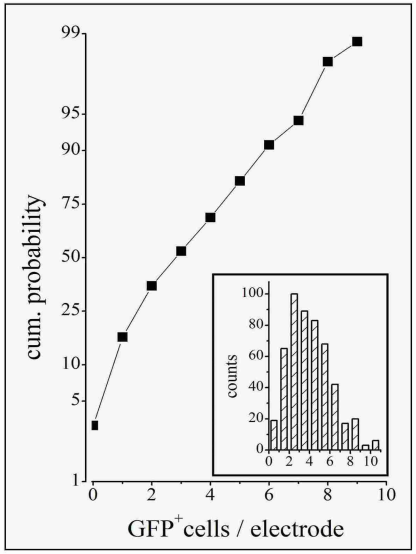
Identification of neuron type was obtained by using a mice expressing a fluorescent protein (GFP) in GABAergic cells (= inhibitory neurons)



Exemplary electrodes devoid of GFP^+ cells but with AEN spikes

AEN, authentic excitatory neuron

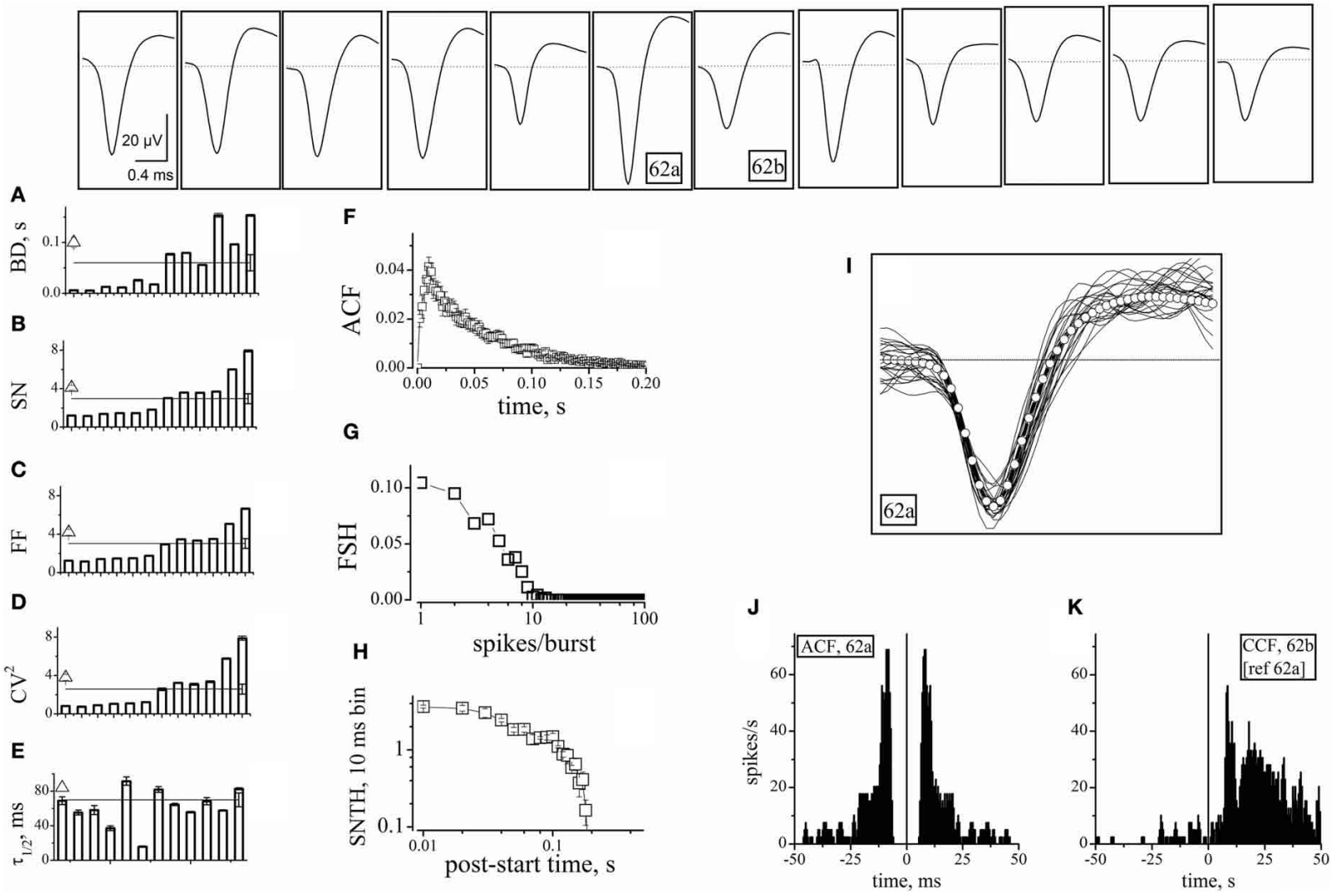
Moreover, we know that only cells staying into a 55 um-diameter area are those correctly identified



AIN , authentic inhibitory neuron

Exemplary electrodes devoid of AEN spikes but with GFP^+ cells and AIN spikes

Fig. 2



electrodes devoid of GFP+ cells, i.e. Authentic Excitatory Neurons

FIGURE3|Properties of units identified from electrodes devoid of GFP+ cells. The upper insets show the averaged (5400s) waveshapes of spikes assigned to units recorded from electrodes devoid of GFP+ cells. **(A–E)** Histograms of BD,SN,FF,CV2, and τ1/2, respectively, computed from data segments of 2 h recordings consisting in ~160 bursts, for each of the 12units whose spike template is shown in the upward insets. In the same plot, the lines (with error bars at the right end) represent the corresponding mean values. The open triangles shown on the left of panels A–E are average values obtained by using the software mentioned in methods. **(F)** The ACF of the 12 units mean. **(G)** The FSH of the 12 units mean. **(H)** The SNTH of the 12 units mean. **Becchetti A, Gullo F, Bruno G, Dossi E, Lecchi M and Wanke E (2012) Exact distinction of excitatory and inhibitory neurons in neural networks: a study with GFP-GAD67 neurons optically and electrophysiologically recognized on multielectrode arrays.** ***Front.Neural Circuits 6:63. doi: 10.3389/ fncir. 2012.00063***

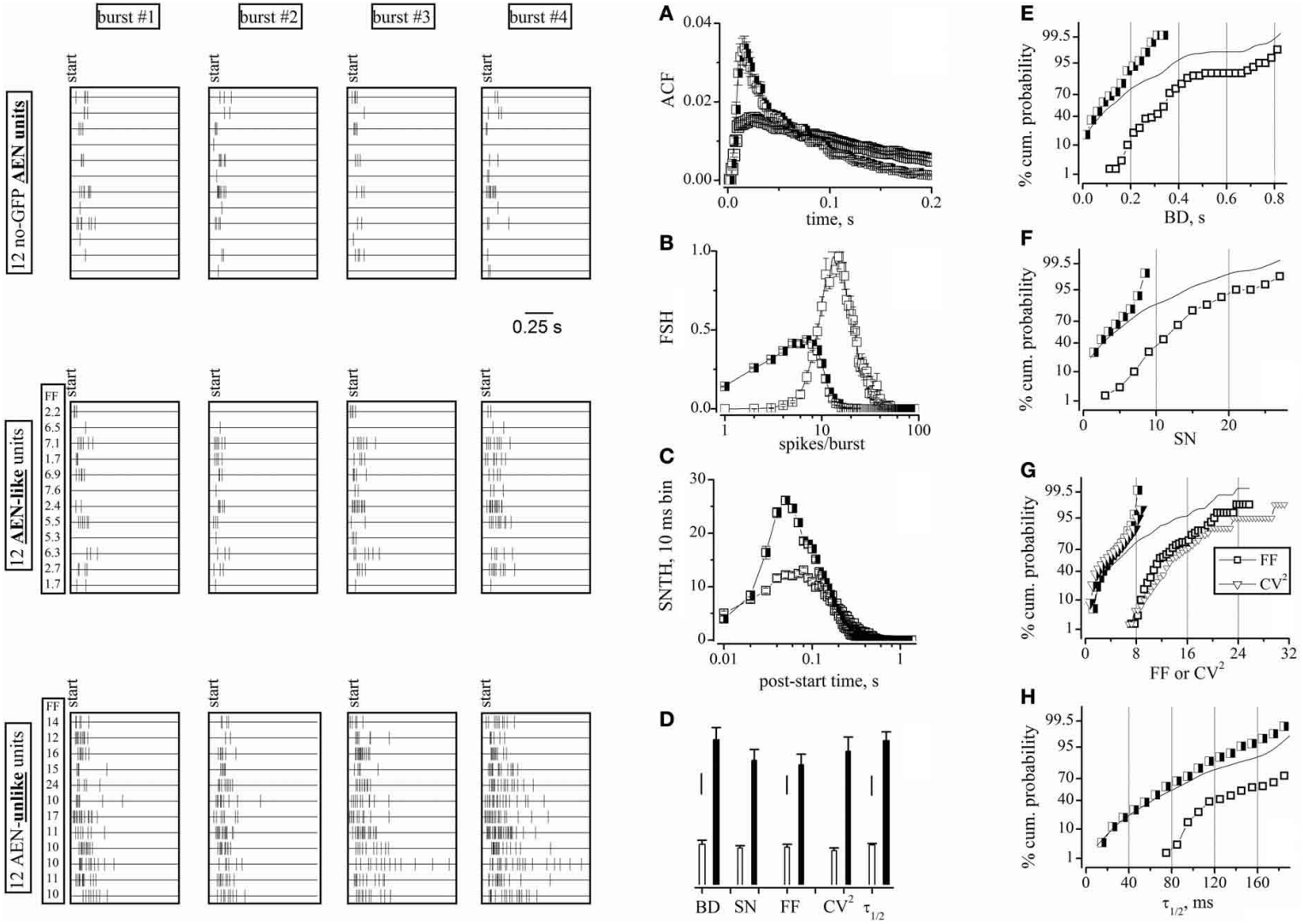


FIGURE4|Raster plots of cell activity and statistical properties of neuron clusters. Data were obtained from the same experiments and timesegments as shown in **Figure3**, but using all 98 units.Left panels:four-bursts raster plots for 12neurons (duration,1s). Upper left: spike bursts recorded from electrodes devoid of GFP+ cells (the2nd and 3° row from top correspond to units 62b and 62a,whose waveforms are shown in **Figure3**. Middle-left:spike bursts of the AEN-like type (see the main text). Lower-left: spike bursts of the AEN-unlike type.The left columns labeled FF in the middle and lower panels give the average FF values of each units. **(A–C)** ACF, FSH, and SNTH plots, respectively, obtained by using the software clusterization of the 78 and 20 units classified using FF (timewindow of 6s). Half-closed squares: AEN-like units; open squares:AEN-unlike units. **(D)** Bar plots of BD, SN, FF, CV2, and τ1/2 values computed for the AEN-like (78units; whitebars) and AEN-unlike (20 units; blackbars) clusters. Vertical scale bars: BD, 50 ms; SN, FF, and CV2, 2; τ1/2, 20ms. **(E–H)** Percentage cumulative histograms of BD, SN, FF, CV2 and τ1/2, as indicated, for all units (lines), for excitatory (half-closed squares) and inhibitory (open squares) neurons, after FF-based clusterization. The bin width in cumulative histogram were, respectively: 0.025s, 1, 0.5, 0.5 and 10 ms. For each variable, 3 independent data points were computed every 30min. Total number of excitatory and inhibitory cells was 234 and 60, respectively.

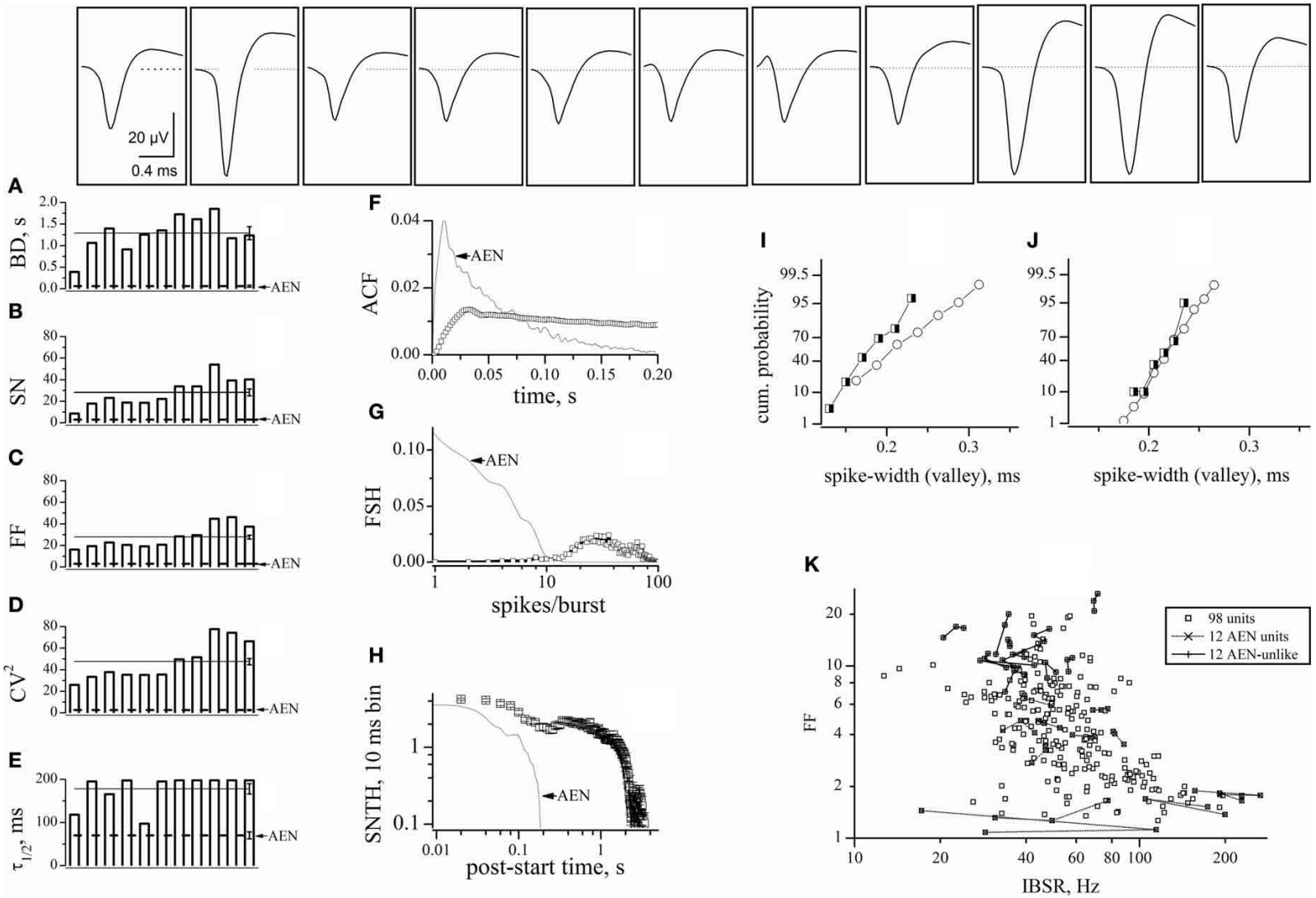
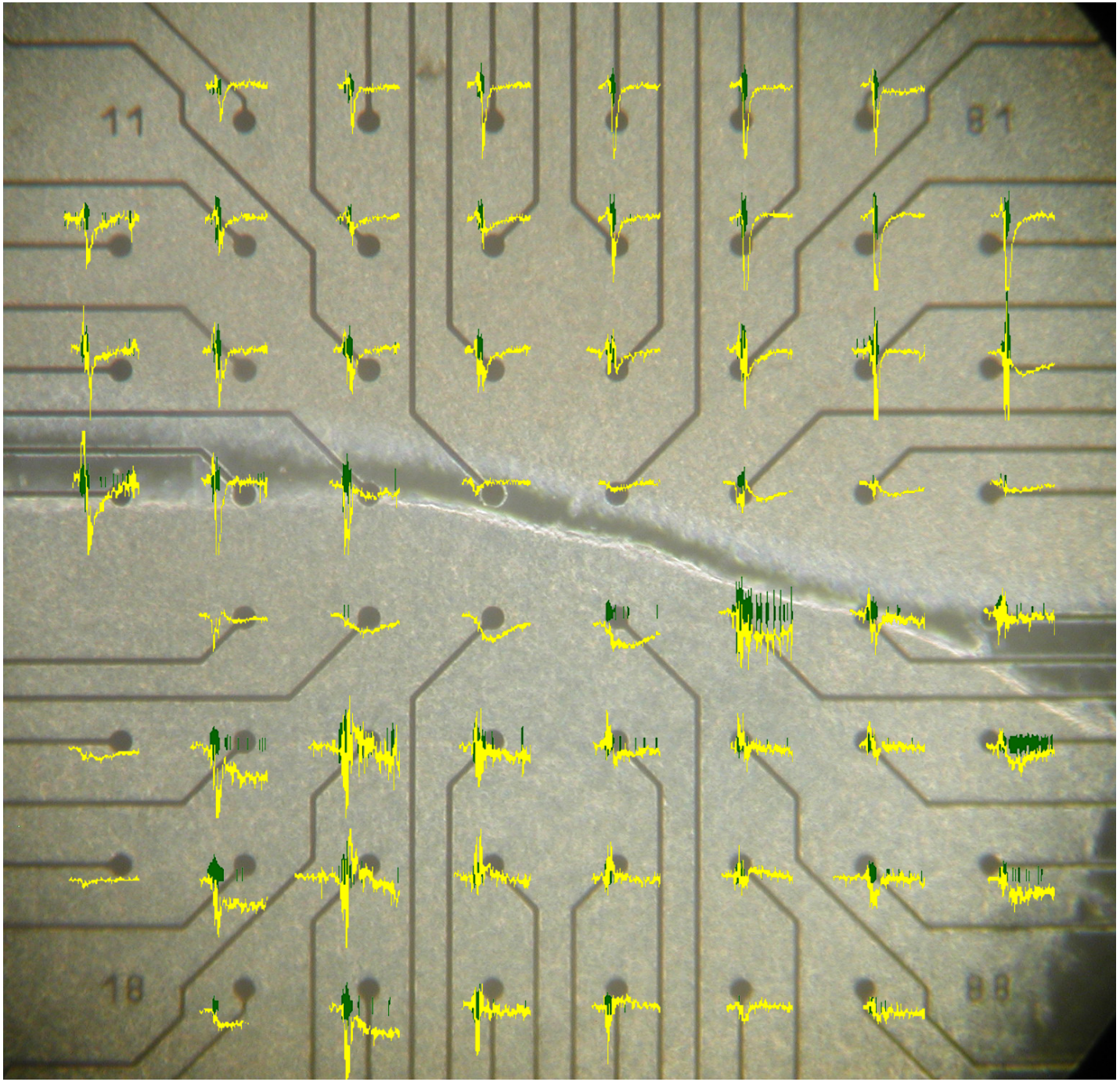


FIGURE5| Characterization of AIN-like units. Properties of units identified from electrodes sampling from a region containing at least oneGFP+ cell, but yielding no AEN-like spikes. The upper insets show typical waveforms of the spikes assigned to each unit. **(A–E)** plots of BD, SN, FF, CV2, and τ1/2, respectively. These were computed from 2h continuous recordings comprising approximately 160 bursts, for each of the 11 units whose spike templates are shown in the upper insets. In the same plot, the straight continuouslines(witherrorbarsontherightend)representthe correspondingaveragevaluesfortheseAINs.Forcomparison,thedotted lines represent the corresponding values for AEN units, reproduced from **Figure3**. **(F)** The ACF of the 11 units mean (open squares). **(G)** The FSH of the 11 units mean (open squares). **(H)** The SNTH of the 11 units mean(open squares; for clarity, only half of the data points are shown). For comparison, dot-lines report the corresponding data calculated from AEN units, reproduced from **Figure 3**. **(I–J)** Cumulative probability histograms obtained by analyzing the spike-width (at half-maximum amplitude) in the experiments shown in **Figures 5** and **3**, respectively. Open circles:excitatory neurons; half-filled squares:i nhibitory neurons. **(K)** Plot of FF versus IBSR of the 98 units used in the experiment of **Figures 3** and **4**. For each unit, 3 independentFF(IBSR)datapointswerecomputedoverconsecutive30min segments(*n* = 294). To illustratethedatafluctuationsduringatotal1.5h recording time, the three data points of each 30-min experiment were connected either by dots- or continuous lines for, respectively, the 12 AEN and AEN-unlike units (whose timestamps are shown in **Figure 4**).


11
81
18
88

Wirth, Corina and Hans-R. Lu¨ scher. Spatiotemporal evolution of excitation and inhibition in the rat barrel cortex investigated with multielectrode arrays. *J Neurophysiol* 91: 1635–1647, 2004. We investigated the spatiotemporal evolution of activity in the rat barrel cortex using multielectrode arrays (MEAs). In acute brain slices, field potentials were recorded simultaneously from 60 electrodes with high spatial and temporal resolution.

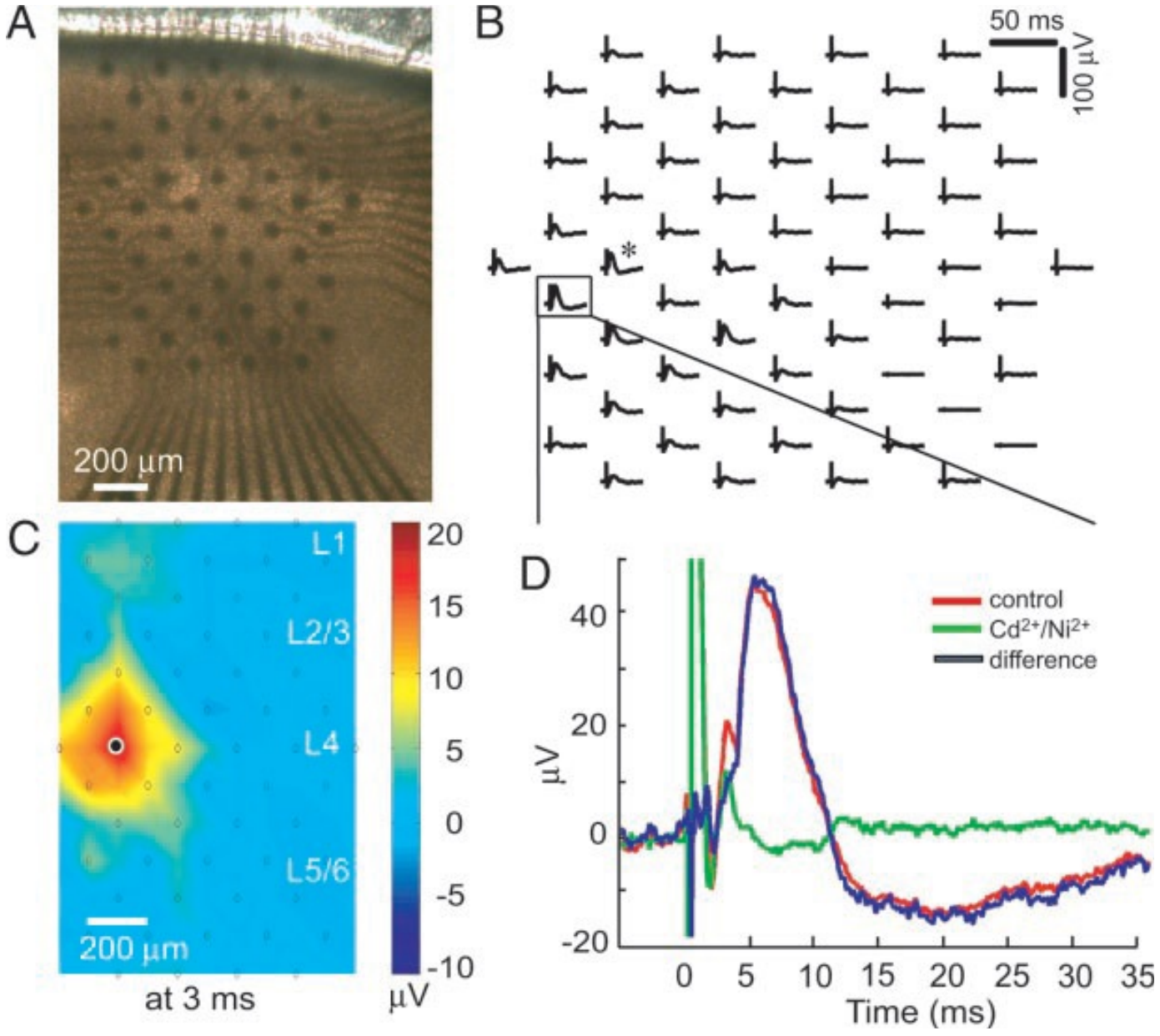
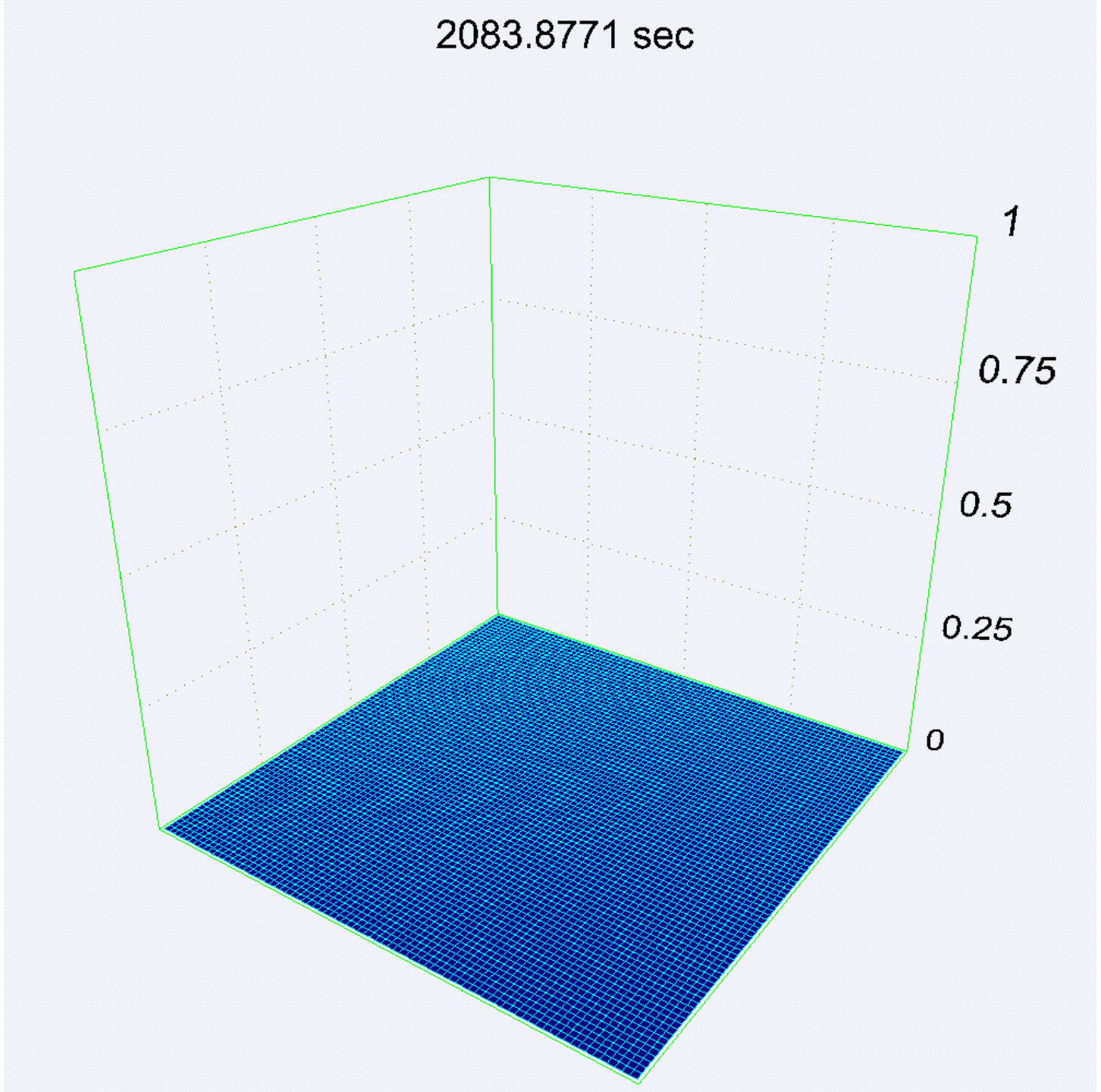


FIG. 1. Thalamocortical slices and multielectrode array (MEA) recordings. *A*: small piece of barrel field cortex mounted on the MEA. L1 covers the 1st MEA row. Row 13 of the MEA is located deep in layer 6 at the border to the white matter. The barrels are barely visible as bright clusters in rows 5–7 of the MEA. The leads connecting the individual electrodes to the connectors of the preamplifier can clearly be seen. *B*: stimulus induced (3V, 0.3 Hz, 150 s) local field potentials (LFPs) recorded with the MEA electrodes (**average over 20 sweeps**). The stimulus artifact indicates the timing of the stimulus, which was delivered via a theta-glass micropipette filled with ACSF at the location indicated by the asterisk. Three damaged electrodes in the right lower corner of the MEA were switched off. *C*: spatial activity map of the postsynaptic signals of the recording shown in *B,* 3 ms after stimulation (see METHODS). Black dot: stimulation location. *D*: enlarged recordings from the single electrode indicated in *B* (average over 20 sweeps). To extract the postsynaptic response from the LFP (blue trace), we subtracted the presynaptic component recorded in the presence of 200 M Cd2 and 100 M Ni2 (green trace) from the original trace (red). This procedure eliminated the fiber volleys and terminal potentials leaving the postsynaptically induced synaptic local field potentials (sLFPs) and was applied to all recordings prior to the analysis shown in this paper.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1
0.75
0.5
0.25
0