| 1 | Electrical Identification and Selective Microstimulation of Neuronal |
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| 2 | Compartments Based on Features of Extracellular Action Potentials |
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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Reliable detection of individual axonal APs triggered by extracellular stimulation

3 Exemplary waveforms of averaged and individual APs triggered consecutively from 4 different 4 stimulation sites and recorded at 3 distal axonal compartments are presented in panels (a) to (d). Colors 5 of averaged AP waveforms indicate occurrence times of negative AP peaks relative to the stimulation 6 time. Individual APs are colored in gray. Gray axonal contours serve as guide to the eye and are 7 estimated by observing relative changes in spatial position of the extracellular signal peaks across 8 consecutive 50-µs time intervals, frame by frame. The four complete stimulation-triggered footprints 9 are presented in Fig. 2a-d and in Supplementary Movie S2. The stimulation spots in Supplementary 10 Fig. S1a-d correspond to those in Fig. 2a-d.

Supplementary Figure S2. Correlations between features of the spontaneous AP footprint and site-specific stimulation thresholds of proximal neuronal compartments

(a) Correlations between stimulation threshold values and individual parameters extracted from
spontaneous AP footprints are presented in four graphs. Occurrence time of the negative peak in the
traces (1); occurrence time of the minimum in the 1st derivative of the traces (2); negative peak
amplitude of the extracellular traces (3) and peak-to-peak amplitudes (4) are correlated with the
corresponding stimulation threshold values extracted from stimulation maps of 14 neurons. Most
sensitive, second and third sensitive sites (Mss, 2ndss and 3rdss), are color-coded by red, orange and
yellow respectively.

20 (b) Correlations between stimulation threshold values and individual parameters extracted from

21 spontaneous AP footprints are presented in four graphs. Spatial distance of the stimulation site from the

22 most sensitive site (1); distance of the stimulation site from the electrode featuring the peak trace (2);

distance of the stimulation site from the footprint's center of mass (3) and distance of the stimulation

site from the location of the initial trace (4) are correlated against corresponding stimulation threshold

25 values extracted from stimulation maps of 14 neurons. Most sensitive, second and third sensitive sites

26 (Mss, 2ndss and 3rdss), are color-coded by red, orange and yellow respectively.

1 Supplementary Figure S3. Spatiotemporal distribution of extracellular AP waveforms with

2 regard to neuronal morphology

- 3 Spike-triggered footprint superimposed over a micrograph of the corresponding lipofected neuron.
- 4 Colors of AP waveforms indicate timing of their occurrence relative to the first recorded activity. In the
- 5 insets magnifying two axonal portions the distribution of axonal AP waveforms is displayed. The same
- 6 neuron is presented in Fig. 5, 8, Supplementary Fig. S4, and Supplementary Movie S5.

7 Supplementary Figure S4. Influence of suprathreshold stimulation voltages on neuron's

8 activation reliability

9 (a) (Left) Activation reliability map of threshold stimulation. Circle sizes indicate relative activation 10 reliability (%), and the colors represent activation failing points (no triggering of an AP, see Fig. 8). 11 Activation reliability represents the percentage of successfully evoked responses, whereas the 12 activation failing point represents the number of stimulations after which activation reliability dropped 13 below 95%. Three electrodes (numbered as 3, 4 and 8) were stimulated at 100 Hz at threshold voltages. 14 (Right) Reliability profiles for electrodes 3, 4 and 8 in the activation reliability map. Red dashed lines 15 indicate the activation failing thresholds and red circles projected on the x-axes indicate activation 16 failing points. 17 (b) (Left) Activation reliability map of suprathreshold stimulation. Circle sizes indicate relative 18 activation reliability (%), and the colors represent activation failing points (no triggering of an AP, see 19 Fig. 8). Activation reliability represents the percentage of successfully evoked responses, whereas the 20 activation failing point represents the number of stimulations after which activation reliability dropped 21 below 95%. Three electrodes (numbered as 3, 4 and 8) were stimulated at 100 Hz at suprathreshold

voltages. (Right) Reliability profiles for electrodes 3, 4 and 8 in the activation reliability map. Red

23 dashed lines indicate activation failing thresholds and red circles projected on the x-axes indicate

24 activation failing points.

Supplementary Figure S1











Supplementary Figure S2



Supplementary Figure S3





Threshold stimulation at 100Hz





Suprathreshold stimulation at 100Hz





b