Supplementary Figure 1

Activity in turtle dorsal cortex is sparse.

a. Probability distribution of firing rates across the population (notice log scale) in our data. The range of firing rates is wide but rates are strongly biased towards low values with an average of 0.04 spikes/s.

b. Average autocorrelation function normalized by the total number of spikes fired by each neuron. Notice low values for all lags indicating rare bursting activity.

c. Average cross-correlation between neurons. For every neuron we calculated the cross-correlation with all simultaneously recorded neurons, normalized to the total number of spikes fired by the neuron. These normalized functions were averaged over the population. Notice low correlation values across all lags.

For all plots, neurons with low SNR (<0.5), average spike amplitude (<10µV) or low number of spikes (<200) were rejected from this analysis.
Supplementary Figure 2

Scalability to large, high-density CMOS electrode arrays.

a. Bright-field image of a cortical slab on a CMOS electrode array. The large size of the active area (2.1mm x 3.85mm), enables recording from very large slabs of intact cortex with high spatial resolution (pitch = 17.5 μm).

b. High-pass filtered spike template (10ms window starting 1.5ms before spike time) over 1024 simultaneously recorded electrodes. Template was averaged over 500 consecutive spikes identified in one channel as negative peaks exceeding 5.5 median absolute deviations.

c. Spike-triggered average of raw voltage traces over the spikes and electrodes in b (200ms window starting 100ms before spike time). The positive amplitude of each trace is color-coded. Note the dominant positive slow SIFs following the spike waveform.
Supplementary Figure 3

Simultaneous patch-MEA recordings.

a. Schematic drawing of recording configuration with combining intracellular whole-cell patch-clamp (black pipette) and extracellular MEA recording (grid). Cortical slabs are typically placed on the MEA ventricular face down.

b. Simultaneous whole-cell patch-clamp (upper black trace) and MEA (lower red trace) recordings of current-induced action potentials in one interneuron. Lower trace: step current injection.

c. Correspondence between average intracellular and extracellular spike waveforms.
Supplementary Figure 4

Robustness of triangulation of soma position and comparison of exponential to power-law decay model.

a. Spatial spread of extracellular spike signal from a sorted unit. Each box represents the mean (red) and individual traces (grey; see look-up-table at right; P = probability of occurrence over 250 consecutive spikes) originating from source location (blue dot at center) in x,y plane. Vertical gray line indicates spike peak time (t=0). Electrode pitch: 40µm. The somatic and axonal signals differ both in shape (bi or tri-phasic for axonal, see top row) and timing (note delays in the axonal negative peak. Note that soma triangulation is not affected by the bias introduced by the axon.

b. Spatial exponential decay (λ = 42µm) of peak spike amplitude (data from neuron in a). Distance measured in x,y,z from position of source, itself computed by triangulation. Red curve fitted to points in black. Data points corresponding to the axonal signal (negative potentials) do not affect the fitting parameters and the resulting exponential curve.

c. Comparison of fitting error between exponential (Exp; \( V = V_0 e^{-x/\lambda} \)) and power law (Pow; \( V = V_0 x^{-(\lambda)} \)) models for spatial decay of spike amplitude from the soma. The central mark in the box plot indicates the median, and the bottom and top edges of the box indicate the 1st (Q1) and 3rd (Q3) quartiles, respectively (148 neurons; 3 preparations). Whiskers at [Q1-1.5*(Q3-Q1), Q3+1.5*(Q3-Q1)].
Supplementary Figure 5

Propagation of axonal spikes.

a. Left: An example of the spatial spread of the averaged (unfiltered) spike waveform across the different electrodes. Notice the triphasic axonal-like signal near top right. A negative field is visible following the spike. Right: timing of spike at different electrodes (red circles) is determined from the negative peak of the highpass (>200Hz) waveform after smoothing with a median filter of 0.35ms. Only significant peaks with peak prominence > 1 (findpeaks, Matlab), width at half prominence > 0.5ms and absolute peak height > 5*std are shown (std is the standard deviation during 1.5-4ms before spike peak). The temporal shift in spike peak is clearly visible.

b. Estimation of axonal propagation velocity. The distance of every electrode from the triangulated soma position is plotted as a function of the corresponding spike-peak delay for all significant peaks. To calculate the velocity, a least square fit is performed and Cook’s distances are calculated. The final speed is calculated by refitting over data points not rejected. Rejected (red circles) are points with a Cook distance larger than median + 0.5*MAD of the Cook distances.

c. Left: scanning electron microscope image from a block scan of turtle cortex. Inset shows an enlargement of area marked by the red square. One of the axons in the image (white arrow) was traced from its cell body by serial block-face scanning, and its diameter calculated as a function of distance from the cell body (right: blue) and converges to a diameter of <500nm. The axon of a different cell is shown in red.

d. Distribution of propagation velocities calculated (as in b) across neurons. To select only neurons for which propagation can be accurately calculated we considered only those with enough (>20) significant peaks at distances >300µm from the triangulated cell body position and with a p-value < 10^-4 for the speed term in the linear regression (in total, 821 neurons). Red line marks the average value (153µm/ms).
Fine characterization of SIF sub-types.

a. Ordered correlation matrix of SIF waveforms from 1411 separated units (see methods) obtained from seven cortex preparations. The blue rectangle corresponds to excitatory cells; the red, purple and green rectangles correspond to inhibitory cells with SIFs of different amplitudes and delays (as shown in b).

b. Average waveforms of each one of the clusters in a. Initial negativity at t = 0 represents the action potential. Templates with positive SIFs differ in their amplitude as well as kinetics, possibly due, in some cases, to contamination by polysynaptic effects.

c. Correspondence between SIF amplitude, polarity and spike width for different SIF templates. Spike width was measured as the width at half the maximal amplitude on the electrode with the maximal spike amplitude on the array.

d. SIFs yield better type separation than spike width. Red and blue correspond to inhibitory and excitatory neurons classified as in Fig 3. SIF score was determined by first extracting, for each electrode, the maximal correlation value between all templates and all electrodes with distances of up to 290µm from the spike peak electrode. Finally the score was calculated as the sum over the correlation values to inhibitory templates minus the correlation values to excitatory templates.

e. Frequency (probability) distribution of correlation between each spike waveform and I1 template waveform (red in b) after removing cluster I2 (purple in a-c, <18% of all neurons). Two clear modes can be identified, corresponding to the E and I populations. Only a small fraction of the waveforms (1.9%, grey band) show low correlation values that preclude clear classification.
Supplementary Figure 7

Intrinsic electrical properties, morphology and postsynaptic effect identify neuron types.

Patched neurons in turtle cortex could be classified as excitatory or inhibitory using electrophysiological criteria, such as spike width, firing frequency and firing frequency adaptation, as well as morphological parameters such as the presence (in excitatory neurons) or absence (in inhibitory neurons) of dendritic spines. As ground truth we used evoked monosynaptic postsynaptic potentials/currents in paired recordings. The ground truth data suggests a very good match with the morphological feature of spiny vs. aspiny dendrites, which were thus used when no paired recordings were performed.

a. Excitatory neurons. a1: Current-induced spike train in an excitatory neuron. Note the typical adaptation of spike frequency, long action potential half-width and spikelets. a2: Morphology of the neuron in a1, stained with biocytin and viewed from the ventricular side of cortex. Arrows point to segments shown in insets. Insets: zoom-ins on dendritic (top) and axonal (bottom) segments. a3: Images of another stained excitatory cell with clear spiny dendrites (right).

b. Inhibitory neurons. Same as in a but for two cortical interneurons (b2: layer 3; b3: layer 1). Note facilitation of spike frequency and short AP half-width. Dendrites appear aspiny and axonal bouton density is higher than in pyramidal neurons.

c. Spiny pyramidal neurons have excitatory postsynaptic effects. c1: Current induced spiking of a pyramidal neuron elicited excitatory postsynaptic potentials (epsps) in a simultaneously recorded spiny pyramidal neuron. Six trials of the presynaptic Vm were aligned to the peak of the first action potential (vertical broken grey line), overlaid and are shown together with the average Vm (grey). Postsynaptic responses and the average (blue) epsp are shown below. c2: Schematic connectivity (left) and morphologies of the two cells recorded in c1 (tangential view).

d. Scatterplot of the action potential half-width versus adaptation ratio for 282 cortical neurons (grey dots). Red dots and blue triangles show neurons with inhibitory and excitatory postsynaptic effect, respectively. Neurons with excitatory effect had spiny dendrites, while neurons with inhibitory effects had aspiny smooth dendrites.

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Supplementary Figure 8

Suppression of all spatial SIF components following synaptic block.

a. A pyramidal neuron was patched and its extracellular action potential and SIF waveforms were recorded on a planar MEA before (gray) and after (red) application of synaptic blockers. A top view of the reconstructed axons is superimposed in magenta. Note that, whereas the SIF is abolished following synaptic block, the spike waveform is unaffected.

b. Same as (a) for a different pyramidal neuron.
Supplementary Figure 9

Correspondence between spatial distribution of SIFs and axonal projections.

Pyramidal neurons were patched and their extracellular action potential and SIF waveforms (black traces, right column) were recorded on a planar MEA (120 electrodes and 100μm pitch in a and c, 59 electrodes and 200μm pitch in b). The neuron was stained by intracellular injection of biocytin, reconstructed, and its morphology aligned to the MEA. Top: perspective from one side to see depth of axonal projections. Bottom: top view of the neuron on the array’s x,y plane. Dendrite shown in grey. Axon collaterals shown in color code, where hue represents distance from electrode array. In all cases, SIF amplitudes are larger where axon collaterals run close to the MEA electrodes.
Supplementary Figure 10

SIF dynamics.

Current-source density analysis applied to spike-sorted data in the x-y plane (details in Methods) for 16 neurons (8 interneurons, top; 8 excitatory cells, bottom) recorded simultaneously. Blue shading: negative or inward ("sink"); red shading: positive or outward ("source"). 0ms is time of action potential’s negative peak. Cross-hair on triangulated soma position.
**Supplementary Figure 11**

Dependence of SIF spatial distribution on network firing rates.

a. Identification of high and low network firing epochs. The high-frequency activity intensity (AI) was extracted and high-activity events were detected (grey, see Methods) and separated from low-activity events (white).

b. Examples of SIFs of two neurons (top and bottom rows) calculated from spikes fired during low (left) and high (right) activity epochs. SIF morphology can be either insensitive (top row) or sensitive (bottom row) to activity levels, as evident by the high (0.99) or low (0.21) spatial correlation value between SIFs, respectively. Intersection of red lines marks the triangulated position of the cell body in x,y plane.

c. The distribution of the relative (absolute value normalized to the maximal amplitude) peak amplitude difference between low and high activity epochs. For most (more than half) neurons, the relative difference is below 20%.

d. The distribution of the spatial correlation between SIFs during high and low activity levels. For most neurons (more than half), the correlation value is above 0.7.

In c and d, only neurons (n=808) for which SIFs could be accurately estimated (fired at least 250 spikes during both high and low activity epochs) were considered.
Supplementary Figure 12

Reliability of orientation-bias detection.

The reliability of SIF orientation-bias detection was tested against potential measurement or analytical artifacts.

In a and b, we rejected all neurons contained in a 300µm-wide inner ring starting at the edge of the MEA, thus precluding artifacts linked to biased sampling of neurons at the edges (N=147 excitatory neurons, 531 interneurons).

In c and d, we rejected all neurons with spike amplitude <10µV, nspikes<200 and SNR<0.5 (see methods) (N=151 excitatory neurons, 646 interneurons).
In e and f, we rejected all neurons with positive, low amplitude SIFs (cluster I2 in Suppl. Fig. 6b, purple) (N=150 excitatory neurons, 651 interneurons from the 1411 neurons analyzed in Suppl Fig 6).

In g and h, we extracted SIFs only from spikes fired during low network activity epochs. Note the remaining bias in excitatory projections, and the reduction of inhibitory projection fields, presumably linked to the absence of compound polysynaptic SIFs. Neurons with low SNR (<0.5), average spike amplitude (<5µV) or low number of spikes (<100) were rejected. (N=108 excitatory neurons, 114 interneurons).

a,c,e,g: Density plot of SIF vector distribution in Cartesian coordinates (as in Fig. 5c) pooled over 7 preparations for inhibitory (left) and excitatory (right) neurons.

b,d,f,h: Average (interpolated) SIFs over 7 preparations for inhibitory (left) and excitatory (right) neurons (as in Fig. 5d).