Cell type- and activity-dependent extracellular correlates of intracellular spiking

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Submitted 21 August 2014; accepted in final form 15 May 2015

Anastassiou CA, Perin R, Buzsáki G, Markram H, Koch C. Cell type- and activity-dependent extracellular correlates of intracellular spiking. J Neurophysiol 114: 608–623, 2015. First published May 20, 2015; doi:10.1152/jn.00628.2014.—Despite decades of extracellular action potential (EAP) recordings monitoring brain activity, the physical origin and inherent variability of these signals remain enigmatic. We performed whole cell patch recordings of excitatory and inhibitory neurons in rat somatosensory cortex slice while positioning a silicon probe in their vicinity to concurrently record intra- and extracellular voltages for spike frequencies under 20 Hz. We characterize biophysical events and properties (intracellular spiking, extracellular resistivity, temporal jitter, etc.) related to EAP recordings at the single-neuron level in a layer-specific manner. Notably, EAP amplitude was found to decay as the inverse of distance between the soma and the recording electrode with similar (but not identical) resistivity across layers. Furthermore, we assessed a number of EAP features and their variability with spike activity: amplitude (but not temporal) features varied substantially (~30–50% compared with mean) and nonmonotonically as a function of spike frequency and spike order. Such EAP variation only partly reflects intracellular somatic spike variability and points to the plethora of processes contributing to the EAP. Also, we show that the shape of the EAP waveform is qualitatively similar to the negative of the temporal derivative to the intracellular somatic voltage, as expected from theory. Finally, we tested to what extent EAPs can impact the lowpass-filtered part of extracellular recordings, the local field potential (LFP), typically associated with synaptic activity. We found that spiking of excitatory neurons can significantly impact the LFP at frequencies as low as 20 Hz. Our results question the common assertion that the LFP acts as proxy for synaptic activity.

Clustering; extracellular recordings; intracellular spikes; LFP; spike waveform

Monitoring activity in the living brain in vivo has traditionally been performed via extracellular voltage (Vₑ) recordings through single wires, wire bundles (for example, tetrodes; Gray et al. 1995), and, more recently, high-density silicon probes (Csicsvari et al. 2003; Buzsáki 2004; Patel et al. 2006; Du et al. 2009) used to detect action potential (spike) signals originating from transmembrane currents of neurons mediated through the conductive extracellular medium. While such Vₑ recordings have played a pivotal role in our understanding of circuit processing and computation, many questions still remain about events and processes related to these signals. For example, how do spatiotemporal features of the extracellular action potential (EAP) waveform change as a function of distance to the recording site, spike pattern, or cell type? What are the physical properties of the medium separating a neuron from an extracellular site? How do space- and time-dependent features of the EAP signature manifest themselves in the Vₑ recording? In the past, it has been difficult to address such questions mainly due to methodological challenges: voltage recording within, across, and outside neural compartments within a few tens of micrometers from multiple recording sites is a nontrivial task. The few pioneering studies that did so typically recorded Vₑ from a proximal wire (Amzica and Steriade 2000) or wire-bundle electrode (Henze et al. 2000), limitations that largely reduced analyses to temporal aspects of spiking and EAPs.

Understanding the spatial scale of events such as EAP initiation and propagation across the extracellular medium is crucial towards understanding Vₑ signals and what these represent. For example, what is the sampling volume of an extracellular electrode? In the past, various distances have been suggested ranging from a few tens to hundreds of micrometers (Henze et al. 2000; Gold et al. 2006; Pettersen and Einevoll 2008; Schomburg et al. 2012). Beyond spatial sampling, Vₑ recordings, and EAP waveforms in particular, are highly space- and time-dependent, posing significant challenges to physiologists attempting to attribute specific EAP shapes and features to individual neurons (so-called units), a process often referred to as spike sorting. One way to increase sorting reliability is through multiple, spatially proximal, recording sites recording from the same neuron with single-neuron recorded EAP waveforms being different (but temporally tightly coherent) across sites (Gray et al. 1995). Hitherto the origin and degree of EAP waveform variability from a single neuron as function of experiment-, cell type-, and spike history-specific parameters remain largely unknown (Hill et al. 2011). In summary, it has been hard to bridge single-neuron, single-compartment intracellular and transmembrane physiology with extracellular signals as these are typically measured in vivo.

Traditionally, biophysical studies of EAP generation and recording are separated in two groups: first, the ones characterizing the neural origin of EAPs in terms of intracellular and transmembrane conductances contributing to these signals, and second, those characterizing the physical properties of the extracellular medium that crucially impacts EAP features. To characterize the origin of EAPs, intracellular in vivo recordings have been performed (Buzsáki et al. 1996; Henze et al. 2000) (using intracellular sharp electrodes) while computational modeling has played a crucial role in bridging the gap between transmembrane events and extracellular signals (Gold et al. 2006; Pettersen and Einevoll 2008; Schomburg et al. 2012)....
2006; Pettersen and Enevoll 2008; Schomburg et al. 2012; Reimann et al. 2013; Anastassiou et al. 2013). Notably, the wide majority of computational models studying EAPs typically assume a homogeneous, purely resistive extracellular medium. Regarding characterization of the physical properties of the extracellular medium, hitherto, measurements of extracellular conductivity have been performed with various experimental setups such as metal wires (Ranck 1963), microstimulation electrodes (Logothetis et al. 2007; Goto et al. 2010), glass pipettes (Histed et al. 2009), parallel plates (Deans et al. 2007), etc. While these pioneering studies have provided insights into the conductivity of the extracellular space, very few studies have attempted to address this question using the main generator of electrogensis in the brain, the neuron itself. The neuron as a current source for such measurements poses a unique challenge: physiologically, neural signals can vary substantially so that the current source is not as stereotyped as current injections from specialized hardware and, technically, because intracellular and extracellular stimulation and recordings need to be performed in close proximity as EAP signals rapidly decay within a few tens of micrometers from the soma (Henze et al. 2000; Buzsáki 2004). Yet, neurons give rise to inherently nonlinear, local, and transient subthreshold and spike responses, and EAP waveforms naturally follow that pattern casting EAP and extracellular medium characterization in the absence of actual neurons problematic.

MATERIALS AND METHODS

Slice preparation and cell identification. Fourteen- to eighteen-day-old Wistar rats were quickly decapitated according to the Swiss Welfare Act and the Swiss National Institutional Guidelines on Animal Experimentation for the ethical use of animals. The project was approved by the Swiss Cantonal Veterinary office following its ethical review by the State Committee for Animal Experimentation. The brain was carefully removed and placed in iced artificial cerebrospinal fluid (ACSF). The 300-μm-thick parasagittal slices of the primary somatosensory cortex (hindlimb area) were cut on a HR2 vibratome (Sigmann Elektronik, Heidelberg, Germany). Experiments were performed at room temperature (to minimize electromagnetic interference). Cells were visualized by infrared differential interference contrast video microscopy utilizing a VX55 camera (Till Photonics, Gräfelfing, Germany) mounted on an upright BX51WI microscope (Olympus, Tokyo, Japan). Care was taken to use only “parallel” slices, i.e., slices that had a cutting plane parallel to the course of the apical dendrites and the primary axonal trunk. This ensured a sufficient preservation of both the neuron’s axonal and dendritic arborizations.

Chemicals and solutions. Slices were continuously superfused with ACSF containing the following (in mM): 125 NaCl, 25 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, and 25 p-glucose, bubbled with 95% O₂–5% CO₂. The intracellular pipette solution contained the following (in mM): 110 K-glucuronate, 10 KCl, 4 ATP-Mg, 10 phosphocreatine, 0.3 GTP, 10 HEPES, and 13 biocytin, adjusted to a pH 7.3–7.4 with 5 M KOH. Osmolarity was adjusted to 290–300 mosM with t-mannitol (25–35 mM). The membrane potential values given were not corrected for the liquid junction potential, which was approximately −14 mV. Some experiments were performed under 20 μM SR 95531 hydrobromide (Gabazine; Tocris) to block inhibition and give rise to more spiking in the slice, yet we observed no impact in terms of EAP waveform features. Thus results presented herein are from experiments without any synaptic receptor blockers.

Intracellular whole cell patch-clamp stimulation and recordings. Multiple somatic whole cell stimulation and recordings (1–4 cells simultaneously) were performed with Multiclamp 700B amplifiers (Molecular Devices, Union City, CA) in the current-clamp mode. The reference electrode in all experiments was positioned ∼1 cm from the slice in the ACSF bath in order not to be affected by the extracellular stimulus. Bridge balance compensation was continuously performed during all recordings. Data acquisition was performed via an ITC-1600 board (Instrutech, Port Washington, NY), connected to a personal computer running a custom written routine under IgorPro (WaveMetrics, Portland, OR). Sampling rate was 10 kHz. Patch pipettes were pulled with a Flaming/Brown micropipette puller (DMZ Universal Puller; Zeitz-Instrumente, Munich, Germany) and had an initial resistance of 4–8 M. Three-dimensional morphological reconstruction of biocytin-labeled cells was done under an Olympus BX 51W microscope fitted with a water-immersion ×60 [numerical aperture (NA) 0.9] or an oil-immersion ×100 (NA 1.35) objective using Neurolucida software (MicroBrightField, Magdeburg, Germany).

Extracellular recordings. The 4-shank, 32-site (B32) NeuroNexus (Ann Arbor, MI) silicon probe was attached to one of the micromanipulators and moved slowly to the target. Each shank had eight recording sites (150 μm² each site, 1–3 MΩ impedance), and inter-shank distance was 200 μm. Recordings sites were staggered to provide a two-dimensional arrangement (20-μm vertical staggered separation). Before experiments, the silicon probe was inserted in a 5% tripsoon solution for ~60–120 min for cleaning purposes. Upon insertion of the silicon probe into the slice, a waiting period of ~30–45 min allowed for the neurons closest to the shanks to settle in position. Whole cell patch-clamp recordings were then performed around the probe. Data were acquired using a 32-channel Plexon (Houston, TX) wideband amplifier with sampling frequency of 10 kHz and 1,000-fold amplification. Distance along the tissue depth axis between soma (intracellular site) and extracellular sites along the shank was determined via z-stacks (spacing: 1 μm). Euclidean distance between soma and each shank site was then calculated based on the distance from two landmarks, the silicon probe tip and the top edge. The soma location was taken as the center of the soma of the patched neuron in the x–y plane of the depth where the soma appeared largest.

Theory and modeling. The compartment model used (see Fig. 3) was developed by Hay et al. (2011), and we adopted it from the NEURON database. Briefly, the authors used an automated feature-based parameter search to faithfully replicate elicitation of somatic Na⁺ spikes in response to a prolonged step current, the generation of a Ca²⁺ spike at the distal apical dendrites, as well as the interaction between the two spiking zones via the backpropagating firing. For a given modeled L5b pyramidal neuron morphology, the optimization-algorithm provided a set of acceptable models (and consequently a range of model parameters) that faithfully replicate the target experimental results, as well as exhibiting the experimental variability. Experimental studies have shown that numerous such combinations of ion channel densities can result in similar firing behavior. Our simulations were performed using the NEURON software package (version 7.3). To account for the extracellular recordings sites we assumed a purely resistive extracellular medium of 2.5-m resistivity with the location of the extracellular sites emulating that of an actual H32 shank (confirmed via visual inspection).

Simulated extracellular activity (see Fig. 7, C–E) was generated using 37-ms-long EAP signatures around spike initiation time (determined intracellularly) from recorded cells summed with a lognormal spiking time distribution with a coefficient of variation (COV) of 0.5 and mean-firing frequencies of 1, 8, and 30 Hz for individual cells. Simulations involving eight cells simultaneously active (see Fig. 7, I–K) used mean-firing frequencies of 1, 4, and 12 Hz per cell to obtain multiunit activity (MUA) of 8, 32, and 96 Hz, respectively. Extracellular recordings from channels that did not record spiking [assessed by a flat spike-triggered average (STA) response] were used to test if extracellularly recorded action potentials produced significant changes to the extracellular signals. Two sample paired t-tests were
performed for 30 different frequencies at 5-Hz intervals from 2 to 147 Hz, and Bonferroni correction for multiple comparisons was applied to 1% significance levels.

RESULTS

Intra- and extracellular action potentials. To simultaneously monitor intracellular and extracellular voltages at multiple locations around identified neurons we modified a 12-pipette experimental setup (Anastassiou et al. 2011; Perin et al. 2011) by doing away with 1 of the pipettes and mounting a 4-shank/32-electrode silicon probe (8 electrodes per shank with staggered layout and interelectrode distance of 20 μm; see Materials and Methods) on one of the manipulators (Fig. 1). Such silicon probes have typically been used in hippocampal and neocortical recordings in vivo (Mizuseki et al. 2009, 2011; Mizuseki and Buzsáki 2013). In principle, this arrangement allowed us to position the silicon probe inside the slice at various orientations, i.e., horizontal or perpendicular to the somatodendritic axis of individual neurons (for example, compare Fig. 1, A with, B–D). Yet, for the majority of experiments we positioned the silicon probe parallel to the somatodendritic axis as typically done in vivo. Subsequently, using up to six pipettes, we approached neurons in close proximity to the silicon probe shanks and performed whole cell patch-clamp experiments. At the beginning of each experiment we applied a cell characterization protocol to determine cell properties such as input resistance and capacitance (Druckmann et al. 2011). Upon completing cell characterization, we injected 9-s intracellular DC current stimuli Iinj in steps of varying strength, from weak (“weak stimulation” in Fig. 1) to strong (“strong stimulation” in Fig. 1), to elicit spiking at different frequencies while simultaneously recording the extracellular voltage from the somatic patch-clamp pipette (blue traces in Fig. 1) and the extracellular voltage (red traces in Fig. 1) from different locations along the silicon probe (wideband signal was recorded, no filtering was performed). We did so for different neurons and layers of rat somatosensory cortex (hindlimb area): herein we present our findings for L23, L4, and L5 pyramidal neurons as well as basket cells. The cell type was established by combining cell-characterization findings and morphological reconstruction (Fig. 1; black lines: dendritic arbor; red lines: axon; location of the silicon probe shanks is also designated).

Spatial scaling of EAPs. What are the physical properties of extracellular space and how are intracellular and transmembrane signals reflected and propagated in it? We calculated the STA from spikes as measured intracellularly by aligning the intracellular action potential waveform at the time of spike tspike defined as the maximum of the second-order temporal derivative of Vj before the characteristic positivity (Fig. 2, A–D, 1st column; solid blue line: mean intracellular STA; dashed blue lines: SD; dashed red line: spike time). We show the results from four individual neurons, a L23 (Fig. 2A), a L4 (Fig. 2B), and a L5 (Fig. 2C) pyramidal neuron as well as a L3 (Fig. 2D) basket cell. Using the intracellular spike times, we temporally aligned the EAP waveforms recorded along the shank closest to the patch-clamped soma for the four electrodes along the left and right side of this one shank, respectively, for the four neurons (Fig. 2, A–D, left shank side: 2nd column; right shank side: 3rd column; black lines: mean Vj). The current source density (CSD), approximated by the negative second spatial derivative of Vj along the shank (Nicholson 1973), is plotted in color (location of the cell body along the depth axis is indicated by a triangle). The CSD represents the volume density of the net current entering or leaving the extracellular space and has been typically used to determine spatiotemporal patterns of synaptic activity (Buzsáki et al. 2012). Our measurements show the EAP and CSD contribution of spiking currents of individual neurons.

A frequently made assumption is that the extracellular milieu is described by a purely homogeneous and isotropic ohmic conductivity (Ranck 1963; Logothetis et al. 2007; Anastassiou et al. 2011 but see also Gabriel et al. 1996; Bédard et al. 2004),
with $V_e$ governed by Laplace’s equation $\nabla^2 V_e = 0$. The boundary condition along a cable-like source is given by $(1/\rho)\nabla V_e \cdot n = J$ with $J$ being the transmembrane current density and $\rho$ the extracellular resistivity (Holt 1998). In the special case for a single point source in an unbounded isotropic volume conductor, the solution becomes $V_e = I\rho/4\pi r$, in which $I$ (unit, A) is the current amplitude of the point source and $r$ (unit, m) is the distance from the current source to the measurement site, i.e., the spatial scaling of $V_e$ is inversely proportional to the distance from the current source $I$. We tested the conductivity characteristics of the extracellular milieu at length scales relevant to neurons (i.e., tens of $\mu$m) and realistic...
current sinks and sources (i.e., membrane currents of identified neurons during spiking) by plotting the mean EAP amplitude measured from the eight electrodes along the closest shank at $t_{\text{spike}}$ as a function of distance from the cell body (see MATERIALS AND METHODS). As expected, EAP amplitude decreases for increasing distance (Fig. 2, A–D, 4th column; circles: mean EAP amplitude).

As observed, at $t_{\text{spike}}$, EAP amplitude decays as a monopole (Fig. 2, 4th column; broken line: least squares fit of $1/r$ function). To quantify $\rho$, we fit $V_e(t_{\text{spike}}) = I(t_{\text{spike}})\rho/4\pi r$ where


I(t_{\text{spike}}) = -C \frac{dV(t_{\text{spike}})}{dt} with C (unit, F) being the cell capacitance and V(t) (unit, V) the intracellular somatic voltage as measured by the patching pipette. The cell capacitance C and resistance R were measured for each cell by injecting an intracellular subthreshold current and fitting the membrane response to an RC response. It has been shown that \(-C \frac{dV(t_{\text{spike}})}{dt}\) satisfactorily approximates spike-related currents \(I(t_{\text{spike}})\) (Harris et al. 2000) and we found this to hold for the first 0.5–1 ms after t_{\text{spike}} (data not shown). Based on the aforementioned, we determined the extracellular resistivity \(\rho\) (unit, \(\Omega m\)) for 9 L23 pyramidal neurons (red), 4 L4 pyramidal neurons (blue), and 12 L5 pyramidal neurons (black) and found it to be \(\rho_{L23PC} = 2.9 \pm 2.5 \Omega m\), \(\rho_{L4PC} = 1.4 \pm 1.1 \Omega m\), and \(\rho_{L5PC} = 2.3 \pm 4.2 \Omega m\) (Fig. 2E, left; means \(\pm SD\)). (The main reason for the large SD is the problematic estimation of C rather than poor EAP distance scaling.) A statistically significant difference in resistivity was measured between \(\rho_{L23PC}\) and \(\rho_{L4PC}\) (ANOVA, \(P < 0.05\)) with the rest of comparisons being statistically insignificant.

We also report the Pearson correlation as a measure for the quality of fit (0: no correlation; 1: perfect fit; mean \(\pm SD\)) of the spatial scaling of the EAP amplitude to the point-source approximation. As observed, there is good agreement between the measured spatial scaling of EAP amplitude and point-source approximation (Fig. 2E, right). Based on the point-source approximation, ratio \(R = V/f = \rho/4\pi\) can be calculated as well as distances \(r_{50}\) and \(r_{10}\) from the soma where the EAP amplitude is reduced to half and a tenth, respectively, the EAP amplitude at 10 \(\mu m\) from the cell body (Fig. 2F). (Notably, this depiction assumes identical current source \(I\) for all cell types.)

**Temporal features of EAPs.** So far, our analysis relied on extracellular signals measured at one instant, \(t_{\text{spike}}\). Yet, the EAP waveform does not only have spatial features, it also has temporal ones. What are the temporal EAP characteristics and, importantly, what is their variation? For example, while the most salient feature of the intracellular (extracellular) spike waveform is the transient, \(\sim 0.5\) to 1 ms-long, Na- and K-dependent positivity (negativity), analyzing the recorded waveforms reveals that spectral content of spikes is significantly stronger at lower frequencies than in the 500- to 2,000-Hz range of the spectrum. Furthermore, pyramidal neurons have consistently slower action potential and EAP waveforms than basket cell interneurons (Fig. 3A; blue: mean \(V_1\); red: strongest EAP signals from the left and right part of the shank closest to the soma), an observation in agreement with previous studies (Pettersen and Einevoll 2008; Schomburg et al. 2012, although see also Vigneswaran et al. 2011).

To study temporal EAP features, we focus on the EAP signature as recorded along each side of the shank nearest to the patched neuron. Specifically, we normalize the EAP amplitude across sites and study the time differences in EAP minima along each shank side (Fig. 3B; black line designates EAP minimum for recorded signals). We have two definitions of time delay: the first uses \(t_{\text{spike}}\) as measured intracellularly as time reference (Fig. 3C, black) while the second uses the time \(t_{\text{EAP}}\) of the minimum of the largest EAP deflection (Fig. 3C, cyan). The temporal delay between EAPs along the shank is typically smaller than 0.4–0.5 ms. Since the location and interelectrode distances along a shank are known, we can calculate the velocity \(v\) of the EAP signal propagation by dividing the interelectrode distance \(\Delta x\) by the (absolute) time delay \(\Delta t\) of EAP negativities, \(v = \Delta x/\Delta t\) (Fig. 3D). Once more, we have two definitions of velocity: the first takes \(\Delta t\) to be the time difference between EAP negativities across successive electrodes (Fig. 3D, black) while the second takes \(\Delta t\) to be the time difference between the EAP negativity of each electrode and the one of the strongest EAP (Fig. 3D, cyan). Both velocity definitions result in similar outcomes, i.e., the EAP signal propagation velocity of spikes along the shank for all cells is \(\sim 0.5–2\) mm/ms.

How do such temporal EAP delays and propagation arise? One hypothesis is that the extracellular medium has both a resistive and a capacitive component with the latter giving rise to temporal delays in the propagation of the EAP signal from the soma to the extracellular site (Ranck 1963; Gabriel et al. 1996; Bédard et al. 2004; Bédard and Destexhe 2009). Given the measured extracellular resistivity, the extracellular medium would need to have a capacitance of \(\sim 1.012 F/m\) to give rise to the time delays typically observed along a shank, which is orders of magnitude greater than estimates of extracellular capacitance (see DISCUSSION for details) (Koch 1999). Furthermore, such large capacitances would show up in a loss of high-frequency components in the EAP, which is not the case as seen in Fig. 3A. While we cannot exclude the possibility of some extracellular capacitive components, at least for spiking, our experiments suggest that such effects are small.

An alternative hypothesis is that action potentials elicited at the axon initial segment propagate back into the dendrites (Stuart et al. 1997; Shai et al. 2014, 2015; Koch 1999). In this case, extracellular sites closer to dendrites than the soma (especially sites closer to the thick main apical dendrite; Henze and Buzsáki 2001) record backpropagating action potentials rather than directly somatic ones. A first indication in support of this hypothesis is that the time delay definition with reference to the minimum of the strongest downward EAP deflec-

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Fig. 3. Temporal characteristics of EAP signals from identified single neurons. A: frequency spectra of intracellular spikes (blue lines) and the 2 largest EAPs (red lines) of the 4 neurons shown in the figure. B: alignment of the mean EAP signals from the 8 sites along the silicon shank closest to the cell body to the spike initiation time \(t_{\text{spike}}\) (determined in intracellularly) reveals temporal differences between the EAP signals (left to right). C: time difference between the EAP minimum at different sites along the same shank and the intracellular spike onset (black) or the time of the EAP negativity of the electrode recording the strongest EAP (cyan). The 2 shank sides are considered separately (see Fig. 2, A–D, 2nd and 3rd columns), hence the multiple lines (x-axis: electrode number as defined in Fig. 2A). D: signal-propagation velocity \(v\) calculated from the interelectrode distances (x-axis: electrodes involved in calculation of \(v\)). AP minima delays are attributed to somatic APs traveling back along the apical dendrites (see text). E: same intracellular input as in Fig. 1 delivered to the soma of a L5 pyramidal neuron simulation (see MATERIALS and METHODS) extracellular recording sites positioned at the same locations as for the silicon probe (left) and the resulting intra (top-) and extracellular responses (middle and bottom). \(V_1\) at a site 30 \(\mu m\) from the soma is either computed by taking into consideration the entire neuron (middle) or only the soma (bottom). F: if the same analysis as in B is carried out for the simulated data, the temporal differences between the EAP signals along the same shank can be attributed to membrane currents along the whole neural morphology (top). (Notably, the EAP delay and propagation speed are very similar to the ones measured experimentally for the L5 pyramidal neuron in B and C.) An identical simulation with only somatic compartments contributing to the EAP reveals no temporal differences (bottom).
tion (Fig. 3C, cyan) is in the vast majority positive, suggesting the site closest to the cell body precedes the EAP deflection in other sites. Moreover, the same delay definition gives rise to fairly monotonic trends, which, given the sites of the shank typically closest to the cell body, are the ones on the edge (sites 4 and 5 in Fig. 2, B–D). This points to the traveling nature of the signal. An additional indication is that the point-source approximation quantitatively fits the EAP amplitude vs. distance relationship only for the spike initiation time. When the same relationship is plotted for the overall EAP amplitude, i.e., the EAP amplitude for all times (and not specifically the spike initiation time) along the shank, the quality of fit of the point-source approximation attenuates, suggesting EAP delays are predominantly attributed to spike backpropagation.
To further assess this hypothesis, we performed simulations using a biophysically and anatomically faithful model of rat somatosensory L5 pyramidal neurons that supports backpropagating action potentials (Hay et al. 2011) (see materials and methods). As in our experimental setup, we inject current steps into the somatic compartment of the model (Fig. 3E; blue: \( V_i \) response to current injection) and calculate \( V_e \) under the assumption of a purely resistive medium (Holt and Koch 1999; Gold et al. 2006; Pettersen and Einevoll 2008; Reimann et al. 2013, \( \rho = 2.5 \, \text{M} \)) at locations very similar to the position of the silicon probe shank in an experiment with the L5 pyramidal neuron shown in Fig. 1C (Fig. 3E, middle red trace). In another approach, we compute \( V_e \) by only considering the contribution of the somatic compartments (Fig. 3E, bottom red trace). Following Fig. 3B, we calculate the mean EAP waveform for the eight recordings based on the intracellular spike time \( t_{\text{spike}} \), four for each side of the shank, and normalize the EAP amplitude (Fig. 3F; red: mean; black line designates EAP minimum for all recorded signals). While for the EAP signal contributed from the whole neural morphology, time delays in the EAP minimum appear, this is not the case for the EAP signal contributed from the soma only. Moreover, the time delays observed in the simulation are very similar to the ones measured in our experiments (compare Fig. 3B, case L5PC with Fig. 3F, top).

Changes in the EAP waveform as a function of firing rate and spiking order. Beyond temporal delays of EAP negativities along a single shank, extracellular spike waveforms show considerable variability even when recordings are performed from a single spiking cell and the same extracellular recording site (Hill et al. 2011), with such variability particularly enhanced during bursting, i.e., high-frequency (>200 Hz) spiking, of cortical neurons (Buzsáki et al. 1996; Henze et al. 2000; Harris et al. 2003). Yet, bursts are comparatively rare events. What is the inherent variability of the EAP waveform as a function of neural activity? This question has been difficult to address in vivo given there is no obvious way of discriminating “natural” EAP variability from other occurrences such as electrode displacement, motion artifacts and coupling to electric fields associated with muscular activity (Schomburg et al. 2014).

We investigated the extent to which the EAP waveform changes as a function of spike rate. That is, we ascribed an interspike interval (ISI) to every spike (ISI definition: time difference between the previous and the current spike) with spike times determined from the intracellular somatic recording. Stimulation of the whole cell patched neurons with DC intracellular stimuli of varying strength resulted in Gaussian-like spike frequency histograms with spike frequency defined as 1/ISI (Fig. 4A; blue: intracellular voltage; red: extracellular trace from the closest electrode to the soma; black: ISI histogram of a L5 pyramidal neuron).

We assessed both intracellular and extracellular spike waveforms from the same cell for different spike frequencies. We focus on five EAP characteristics: 1) the initial capacitive positivity (\( V_{e,cap} \)) amplitude; 2) the EAP negativity amplitude (\( V_{e,extr} \)); 3) the EAP repolarization positivity (\( V_{e,repol} \)) amplitude; 4) the EAP repolarization time \( \tau_r \); and 5) the EAP half-width \( \Delta t_{\text{hw}} \) at half height (Fig. 4B; all features designated in red). Concurrently, we report the correlates of intracellular spike waveforms of the aforementioned features: 1) the change in intracellular spike voltage threshold compared with the mean; 2) the intracellular spike amplitude (\( V_{i,extr} \)); 3) the intracellular postspike negativity (\( V_{i,repol} \)) amplitude (compared to baseline); 4) the intracellular spike repolarization time \( \tau_r \); and 5) the intracellular spike half-width \( \Delta t_{i,hw} \) (Fig. 4B; all features designated in blue). In Fig. 4C we show how these spike-waveform characteristics change with spike frequency for a single L5 pyramidal neuron (spike frequency histogram shown in Fig. 4A). Note how EAP amplitude characteristics (in red) vary more with spike frequency than their intracellular correlates (in blue). For example, while \( V_{e,extr} = 102 \pm 14 \, \text{µV} \) (mean \pm SD), \( V_{i,extr} = 61 \pm 3 \, \text{mV} \) with the COV (defined as the ratio between the SD and mean) being 0.14 vs. 0.05, i.e., almost three times larger for \( V_{e,extr} \) than for \( V_{i,extr} \). On the other hand, variability in extracellular temporal characteristics is broadly similar to their intracellular correlates. For example, for \( \Delta t_{i,hw} \) and \( \Delta t_{e,hw} \), the COV is 0.16 vs. 0.24, revealing larger variability in the temporal characteristics of intracellular than extracellular spikes. Interestingly, it was recently shown that intrinsic biophysical mechanisms are activated along the dendrites of CA1 pyramidal neurons in theta and gamma bandwidths (Vaida and Johnston 2013). In our experiments we observed consistent changes in EAP features (most visible in \( V_{e,extr} \) and \( V_{e,repol} \)) in the theta-frequency band width (4–12 Hz).

In Fig. 4, D–F, we report how spike waveform characteristics change as a function of spike frequency with reference to the mean waveform across all spikes from an individual neuron, irrespective of spike frequency for 8 L23 (D) and 12 L5 (E) pyramidal neuron recordings as well as 4 basket cell (F) recordings. We report the relative error for each feature. For example, for the EAP amplitude, we report ratio \( V_{e,extr} - V_{e,extr} \) with \( V_{e,extr} \) being the EAP amplitude.

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**Fig. 4.** Cell type-specific intra- and extracellular spike-waveform characteristics as a function of spike frequency. A: intracellular somatic DC current injection of varying strength results in intracellularly (blue) and extracellularly (red) recorded spikes. Spike frequency is defined for each spike as the inverse of its ISI resulting in a Gaussian-like spike frequency histogram (right). B: 5 extracellular spike-waveform characteristics were studied (left to right): the initial capacitive positivity (\( V_{e,cap} \)) amplitude, the EAP negativity amplitude (\( V_{e,extr} \)), the EAP repolarization positive (\( V_{e,repol} \)) amplitude, the EAP repolarization time \( \tau_r \), and the EAP half-width \( \Delta t_{e,hw} \) (features designated in red). Likewise, 5 intracellular spike waveform characteristics were analyzed (left to right): change in voltage spike threshold compared with the mean, the intracellular spike amplitude (\( V_{i,cap} \)), the intracellular postspike negativity (\( V_{i,repol} \)) amplitude (compared to baseline), the intracellular spike repolarization time \( \tau_r \), and the intracellular spike half-width \( \Delta t_{i,hw} \) (features designated in blue). C: intra- (solid blue) and extracellular (solid red) spike-waveform characteristics as a function of spike frequency for the L5 pyramidal neuron in A. Broken lines indicate the mean of each intra- and extracellular spike-feature across spike frequencies. D–F: differences in intra- and extracellular spike waveform characteristics (C) as a function of spike frequency with reference to the mean waveform across all spikes irrespective of spike frequency for 8 L23 (D) and 12 L5 (E) pyramidal neuron recordings as well as 4 basket cell (F) recordings (red: extracellular feature; blue: intracellular feature; circles: mean; error bars: SD). Differences are expressed as the relative error (for more thorough explanation, see text). \( V_{e,extr} \) variability is consistently larger than \( V_{i,extr} \) variability for all cell types (2nd and 3rd columns; basket cell variability in the 3rd column, in blue, is attributed to repolarization being very close to baseline) while temporal feature variability much less so (4th and 5th column).

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in a particular spike frequency bin and \(< V_{c,ampl} \) > the mean EAP amplitude across all spikes. We express the ratio in form of percentual change (line: mean; error bar: SD; red: extracellular feature; blue: intracellular feature). As observed, there is substantial variation in the two most salient EAP features, the EAP amplitude \(V_{e,extr} \) (Fig. 4, D–F, 2nd column) and the EAP repolarization amplitude \(V_{e,repol} \) (Fig. 4, D–F, 3rd column) for all cell types. Importantly, the relative error in these features is consistently larger in the extracellular rather than the intracellular waveforms (compare red to blue) and nonmonotonic as a function of spike frequency. On the other hand, the relative error in repolarization time shows larger variability in intracellular than extracellular spike waveforms while being similar for the half-width time. We did not find any distinguishing differences in relative error between cell types.

Transient membrane conductances such as sodium inactivation and slower calcium-dependent currents can alter spike shape (Buzsáki et al. 1996; Henze et al. 2000). In Fig. 5A, intracellular stimulation of varying strength (top: weak; bottom: strong) yields spike trains of \(-3 \) (top) and \(10 \) Hz (bottom) for a L5 pyramidal neuron. While for weak stimuli there is little variation in intra- and extracellular spike waveforms (dashed lines: amplitude-range), for strong stimulation the amplitude of the spike waveform (especially the extracellular) changes substantially between the first and subsequent spikes despite their similar ISIs. To quantify such intra- and extracellular spike waveform variability, we repeated the same somatic injection multiple times, calculated the mean waveforms of the first, second, third, etc. spike, and subtracted them. Using this procedure, we calculate the mean difference between intra- and extracellular spike waveforms as a function of spike occurrence for weak and strong stimulation (Fig. 5B). In Fig. 5C we report the change (relative error) in (left) intra \((V_{i,extr}, \text{blue})\) vs. extracellular amplitude \((V_{e,extr}, \text{red})\) and (right) half-width \((\Delta t_{e,low} \text{ vs. } \Delta t_{e,high})\) as a function of spike order comparing the first 10 spikes to the 11th for weak (top) and strong (bottom) stimulation. For example, to calculate the relative error in \(V_{e,extr}\) amplitude for a particular stimulation strength, we calculate \((V_{e,extr} - V_{e,11th}^{i,extr})/V_{e,11th}^{i,extr}\) with \(V_{e,extr}\) being the EAP negativity (as defined per Fig. 4) for each of the spike order bins and \(V_{e,11th}^{i,extr}\) being the EAP negativity of the 11th spike bin. It is observed that for increasing spike order, EAP amplitude decreases and EAP width increases, that is, EAPs becomes smaller and wider. Moreover, compared with the intracellular waveform, the decrease in amplitude and increase in width are much enhanced. In Fig. 5D, we assess this observation for the spike amplitude by fitting the data shown in Fig. 5C, left, with a linear function (least-squares fit with \(y = ax + b\) where \(a\) is the slope and \(b\) the offset) and report the normalized slope \(\alpha / b\) for L23 (top) and L5 (bottom) pyramids for intra (blue)- and extracellular (red) spike waveforms as a function of stimulus amplitude. For both cell types the rate of amplitude change is negative, i.e., intra- and extracellular spike amplitude decreases as a function of spike order. Moreover, for L23 pyramids, for weak and strong stimulation, the rate of decrease (slope) does not differ significantly between intra- and extracellular spikes. This is not the case for L5 pyramids where for strong stimulation, the intra- and extracellular spike amplitude decrease is significantly (ANOVA, \(P < 0.05\)) different, suggesting that the decrease in EAP amplitude does not proportionally reflect the intracellular one. Such observations are partly attributed to the fact that EAPs reflect both somatic and dendritic, i.e., distributed and inhomogeneous, electrogenesis (Koch 1999).

What is the spatial dependence of these observations? In Fig. 5E (left and middle) we show the mean CSD of the 2nd vs. 11th spike for weak (top) and strong (bottom) stimulation (same L5 pyramid as in Fig. 5, A and B; see right in Fig. 5B for EAP differences) and show their difference in Fig. 5E, right. By subtracting CSDs of the same neuron for different spike occurrences it becomes evident that, for the particular L5 pyramid, strong stimulation and fast spiking results in a different spatiotemporal CSD constellation compared with weak stimulation and slow spiking. To quantify these differences, we fit a two-dimensional Gaussian function \(G(x,t) = A_{\text{CSD}} \exp(-\frac{(t-t_0)^2}{2\sigma_1^2} - \frac{(x-x_0)^2}{2\sigma_2^2})\) \(1\text{ ms around } t_{\text{spike}} \) (Fig. 5E, time = 0) and plot the relative error (Fig. 5F, left to right) in CSD amplitude (\(A_{\text{CSD}}\)), space (\(\sigma_x\)), and time (\(\sigma_t\)) constant as a function of spike occurrence with reference to the 11th spike (Fig. 5F, black: strong stimulation; cyan: weak stimulation; same colored lines: results for both sides of the shank closest to soma; data from same cell as in Fig. 5A–C). While changes in spatial and temporal constant broadly existed, the most sensitive parameter to the spike order for weak vs. strong stimulation is the CSD amplitude that, in the case of strong stimulation, changed by \(-60\%\) for that neuron. To assess this effect quantitatively, we follow the method introduced in Fig. 5, C and D, and fit the CSD amplitude vs. spike order data with a linear function for a set of L23 and L5 pyramidal neurons and report the normalized slope (Fig. 5G). We do so for the CSD amplitude change (red) as a function of cell type (L23 vs. L5 pyramids), shank side (CSD calculated from the 4 electrodes on the same vs. the opposite side the cell body), stimulation amplitude (weak vs. strong) and compare it with the \(V_{i,extr}\) change (blue; same data as in D). The negative value of the slopes illustrates that also CSD amplitude decreases with spike order. Yet, unlike in Fig. 5D, the comparison between \(V_{i,extr}\) amplitude slope and CSD-amplitude slope is statistically insignificant, suggesting that (unlike for \(V_{i,extr}\) of L5 pyramids shown in Fig. 5D) CSD-amplitude decrease reflects the measured \(V_{i,extr}\) decrease. These observations remain broadly similar for L23 and L4 pyramids.

Figures 4 and 5 suggest that the relationship between the EAP waveform and \(V_i\) is complicated. In fact, biophysical considerations support that the negative derivative of the intracellular potential is proportional to the EAP waveform (Koch 1999). To assess this, in Fig. 6 we plotted the amplitude normalized \(-dV_i/dt\) and the EAP waveform for (A–C) a L23 and a L4 pyramid as well as for a basket cell (same cells as in Fig. 2). We show this relationship for increasing (left to right) spike rates (with the spike rate assessed the same as in Fig. 4). Indeed, the two waveforms are qualitatively similar in shape (importantly, not in absolute amplitude) during the initial Na and K component of the spike. The comparison becomes poorer after the EAP negativity when slower, repolarizing currents are activated that cannot be captured by simply differentiating \(V_i\). Concurrently, during such later stages of spike initiation, charge transfer is no more confined perisomatically but has propagated along the morphology casting the entire neuron a spatially distributed sink/source contributing to the extracellular potential.
Low-frequency contributions of EAPs. Recent computational (Denker et al. 2011; Schomburg et al. 2012; Reimann et al. 2013) and experimental in vivo (Ray and Maunsell 2011; Belluscio et al. 2012) studies support the notion that extracellular spike waveforms not only contribute to the higher frequency portion of $V_e$ recordings but also impact lower bandwidths. Addressing and quantifying such effects has proven difficult, especially since capturing the slower, mostly repolarizing, spike currents of smaller amplitude is challenging.

We used the STA EAP waveforms from identified cells (Fig. 7A, red) to construct fictitious extracellular traces and analyze their power with and without spikes. Nine-second-long $V_e$ recordings from an extracellular site far from the patched neuron that did not detect any spiking (as assessed from the STA) was the baseline. To this, EAP waveforms at ISIs drawn from a log-normal distribution (Mizuseki and Buzsáki 2013; Buzsáki and Mizuseki 2014) with mean spike frequency $f_0$ were added at three spike rates, $f_0 = 1, 8, \text{and } 30 \text{ Hz (Fig. 7B).}$ In a next step, we calculated 50 realizations for each $f_0$, which resulted in 50 unique (Fig. 7C–E) 9-s-long extracellular traces. We then calculated their spectral content (Fig. 7F; line: mean spectral power from 50 traces using the EAP of a L5 pyramidal neuron shown in Fig. 7A1; green: no spiking; red:
As observed, there is substantial deviation in spectral power between the spiking and no- or low-spiking traces at frequencies as low as 20 Hz. What part of the EAP waveform can impact power at such low frequencies? To address this question, we performed so-called “de-spiking,” i.e., we substituted a window of 0.6 ms before and after the spike initiation time with a spline fit (Belluscio et al. 2012). This resulted in EAP waveforms lacking the typical spike negativity but containing the repolarization (Fig. 7A; de-spiked EAP waveform in black). We calculated the spectral power for the same $f_0$ using the de-spiked waveforms (Fig. 7G) and confirmed our previous conclusion: reasonably high spiking impacts spectral power as low as 20 Hz.

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$f_0 = 1$-Hz spiking; black: $f_0 = 8$ Hz; blue: $f_0 = 30$ Hz.

Fig. 6. Comparison between EAP waveform with the negative 1st-order time-derivative of the intracellular AP waveform, $-dV/dt$, for different cell types (A–C: a L23 pyramid, a L5 pyramid, and a basket cell). Intracellular stimuli of varying strength are applied (see Fig. 1) resulting in spikes of varying interspike interval (ISI). Spike frequency is then defined as 1/ISI and spikes are grouped. The figure shows the mean extracellular (red) and the mean $-dV/dt$ (blue) for difference spike frequencies. (Waveforms are scaled so as to have the same amplitude.) As observed, the EAP and $-dV/dt$ waveforms are in close agreement (width, etc.) near the EAP negativity. Comparison between the EAP and $-dV/dt$ waveform becomes poorer ~1 ms after the EAP negativity when slower, repolarizing currents are activated.

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power of the extracellular recording as low as 20 Hz for \( f_0 \) greater than 1 Hz (Fig. 7I), an observation mainly attributed to repolarization currents as the difference remains when EAP negativities have been removed (Fig. 7J).

Of course, extracellular electrodes usually pick up EAPs from multiple neurons. Indeed, they typically record larger amplitude EAPs (larger than 50-µV amplitude; right: from site recording the 2nd largest EAP waveform) from multiple neurons. Indeed, they typically record larger amplitude EAPs from many more located in the greater vicinity (Buzsáki 2004; Holt and Koch 1999; Gold et al. 2006; Schomburg et al. 2012) (see also Fig. 2). To account for this, in Fig. 7, L–N, we performed the same analyses as in Fig. 7, I and J, but instead of using the EAP waveform from a single neuron we used EAP waveforms from multiple cells. To do so, we define the overall spike frequency \( f_{\text{MUA}} \) attributed to spiking from all cells (equivalent to multunit activity or MUA) and at each ISI (drawn from a log-normal distribution; Fig. 7, C–E) we sum the EAP waveform of a randomly chosen neuron (of the same cell type) from 8 L23 pyramidal neurons (Fig. 7, L–N, left), 4 L4 pyramidal neurons (Fig. 7, L–N, middle), and 12 L5 pyramidal neurons (Fig. 7, L–N, right) with a control trace (recorded from far away) for spike rate \( f_{\text{MUA}} = 8, 32, \) and 96 Hz (in each of Fig. 7, L–N, from left to right). The resulting \( V_e \) traces (similar to the ones shown in Fig. 7B) contain EAP waveforms from many neurons of a particular type. We show the statistical significance (t-test, dashed line: \( P < 10^{-3} \),
Bonferroni corrected for multiple comparisons) of the difference in spectral power between control (no spiking) and spiking traces at different frequencies (steps of 5 Hz; null hypothesis: spectral power between control and spiking traces are drawn from the same distribution) for intact (red) and de-spiked (black) EAP waveforms. As observed, while for $f_{MUA} = 8$ Hz (equivalent to 8 neurons spiking at 1 Hz) spiking does not substantially impact spectral power below 100 Hz, for faster (but still very realistic) rates of $f_{MUA} = 32$ and 96 Hz, spiking profoundly impacts spectral power at frequencies as low as 20 Hz. This is especially true for L4 and L5 pyramidal neurons. We also studied the effect of different EAP waveforms as measured by different sites along the shank by considering the strongest and second and third strongest EAP signals, both for the intact and de-spiked EAP waveforms (Fig. 7, L–N, 3 lines of the same color). While the strongest EAP waveforms (cell type: mean EAP amplitude ± SD; L23PC: 39 ± 10 μV; L4PC: 57 ± 35 μV; L5PC: 51 ± 61 μV) impact spectral density at low frequencies already for $f_{MUA} = 32$ Hz, broadly the same effects hold even when considering the second strongest EAP waveforms of the same neurons (cell type: mean EAP amplitude ± SD; L23PC: 32 ± 7 μV; L4PC: 30 ± 4 μV; L5PC: 40 ± 10 μV).

**DISCUSSION**

Assessing the neural origins of electric signals has remained problematic even if the biophysical laws governing the initiation and propagation of these electric events are well understood (Mitzdorf 1985; Logothetis and Wandell 2004; Buzsáki et al. 2012; Einevoll et al. 2013). Our experimental setup allows us to access intracellularly and whole cell patch-clamp somata of identified neurons located a few tens of micrometers away from extracellular silicon probes of known spatial constellations. By stimulating these identified neurons through intracellular DC current injections and eliciting spikes, we recorded intracellular and extracellular spiking signals. Notably, access to the intracellular voltage allows precise detection of spike times, alignment of intracellular and extracellular voltage traces, and reliable intracellular and extracellular STA. The latter is essential to our study as it allows experiment (distance to extracellular recording sites)- and cell type-specific (extracellular resistivity, slow vs. fast spiking) comparisons while doing away with the ambiguity of thresholding and origin (single vs. multiple neurons) of these signals. Using these experiments we addressed three questions: the spatial EAP propagation in cortical matter, temporal effects of EAPs, and spike contribution to the LFP.

**Spatial EAP propagation.** To assess the physical properties of the extracellular medium such as the extracellular resistivity and characterize the EAP spread, we used a phenomenological model treating electrogenesis along the entire morphology at $t_{\text{spike}}$ as a point source. While this way a number of processes and features are lumped into a single parameter/point, such an approximation offers many advantages over alternative experiments using microwires or parallel plates (Ranck 1963; Gabriel et al. 1996; Logothetis et al. 2007; Anastassiou et al. 2011) that lack spike-related electrogenesis. The use of the silicon probe allowed us to quantify the spatial spread of EAP signals at the microscopic level. We found that, at spike initiation time, the point-source approximation in a resistive medium (Holt and Koch 1999) captures the spatial decay of the EAP amplitude. Furthermore, by assuming current generation as the negative product of a cell’s capacitance and the intracellular somatic derivative (Koch 1999), we calculated the microscopic, layer-specific extracellular resistivity and found it to be in agreement to studies quantifying it at the meso- and macroscopic level (Logothetis et al. 2007; Goto et al. 2010).

**Temporal effects of EAPs.** We sought to understand variations in the extracellular spike negativity. We measured propagation delay and velocity along the silicon probe and found them to be in agreement with measurements of backpropagating action potentials (Stuart et al. 1997; Buzsáki et al. 1996; Buzsáki and Kandel 1998; Henze et al. 2000; Blanche et al. 2005). Thus we attribute such temporal variations to the contribution of dendritic compartments to the extracellular spike signal. This finding is seemingly in variance with the biophysical simulations of Gold et al. (2006), who suggested that it is mainly the cell body and proximal thick dendrites that affect the recorded EAP waveform. While true that the main contributor of the EAP waveform are perisomatic compartments of a neuron, more distal dendritic compartments also contribute to the recorded signal. In fact, our simulations suggest that only accounting for perisomatic compartments does not reproduce the observed time delays while strongly overestimating EAP amplitude close to the soma. Our findings are in line with computational (Pettersen and Einevoll 2008; Schomburg et al. 2012) and experimental (Buzsáki et al. 1996; Buzsáki and Kandel 1998; Henze et al. 2000; Blanche et al. 2005) work supporting the contribution of dendritic transmembrane currents to EAPs.

It has been hypothesized that temporal delays of EAPs can also appear if the extracellular medium has a capacitative component (in addition to its resistive nature) (Gabriel et al. 1996; Bédard et al. 2004; Bédard and Destexhe 2009). The main argument in favor of such a hypothesis is that the gray matter of cerebral cortex is filled with cellular membranes with the extracellular fluid taking up a fairly small volume (0.05–0.15) (Lopez-Aguado et al. 2001). Given the measured extracellular resistivity (~2.5 Ωm) and the measured temporal delay (~0.3 ms) observed in spike-negativity along a shank, assuming simple RC circuit formalism the extracellular medium would need to have a capacitance of ~1.012 F/m. This is orders of magnitude larger than the membrane capacitance (Koch 1999). In fact, our data support that local, compartmental electrogenesis such as backpropagating action potentials through dendrites impacts EAP features such as amplitude and time delays rather than extracellular capacitance filtering. Yet, we cannot exclude that for larger distances and different/stronger current sources, there might be a capacitative feature (Bédard et al. 2004).

Yet, is the fact that the point-source approximation is a good fit for EAP distance scaling at $t_{\text{spike}}$ not at odds with our observation that the whole cell morphology contributes to the EAP waveform? The short answer is no. Importantly, the point-source approximation (as presented in Fig. 2) is an apparent or phenomenological model of EAP distance scaling. Following the same argument, the extracellular resistivity as the negative product of a cell's capacitance and the intracellular somatic derivative (Koch 1999), we calculated the microscopic, layer-specific extracellular resistivity and found it to be in agreement to studies quantifying it at the meso- and macroscopic level (Logothetis et al. 2007; Goto et al. 2010).
question: given a certain distance between the cell body and the electrode, what is the EAP spread? Clearly, to address such a question in its entirety, a sophisticated three-dimensional model would be required that can fully simulate all electrogensis (and its heterogeneity) in the intracellular, transmembrane, and extracellular space as well as in/around the recording electrode. As such, our phenomenological point-source model based on measured properties provides a useful first-order approximation, for example, for EAP spread (Fig. 2F).

An important aspect of extracellular recordings is their inherent, activity-dependent variability. Indeed, the EAP amplitude of hippocampal pyramidal neurons can vary as much as 60% during a high-frequency (>200 Hz) burst (Buzsáki et al. 1996), for example, within a place field (Harris et al. 2001). These features, together with artifactual sources of variability from electromyogram contamination or hardware sources, pose challenges for spike waveform-based clustering and classification of neurons in vivo. Our in vitro measurements present advantages over in vivo recordings, since variability of the EAP waveform is not affected by fluctuations of the membrane potential and spiking activity of other neurons. We chose spike frequency as an independent variable as in vivo measurements indicate that slow spiking gives rise to more stereotypical EAP waveforms than fast rates, e.g., during burst firing (Buzsáki et al. 1996; Harris et al. 2001). We report that the most salient features of EAPs, the EAP negativity attributed to fast sodium- and potassium-dependent currents and the immediately proceeding EAP positivity attributed to slower potassium (but also calcium) currents, can substantially vary as a function of spike frequency. In general, we found that spike variability of the EAP amplitude is more pronounced compared with intracellular spike variability (Henze et al. 2000), is nonmonotonic as a function of spike frequency, and is present in all cell types we studied. The temporal EAP features, such as halfwidth or decay time of spike repolarization, varied more reliably with the intracellular waveform than the amplitude (Barthó et al. 2004).

We also analyzed EAP features as a function of spike order (1st vs. 2nd vs. 3rd spike) and found that EAP amplitude of L5 pyramids decreased with spike order significantly more than the intracellular spike amplitude, an observation already established in vivo for CA1 pyramidal neurons (although for much higher spike rates) (Buzsáki et al. 1996). Such observations support the notion that perisomatic and, in fact, dendritic electrogenesis can vary and that a cascade of time-dependent and spatially distributed events is reflected in the EAP, much more so than in the intracellular somatic recordings of spikes (Gold et al. 2007; Anastassiou et al. 2013). Additionally, while EAP amplitude decrease cannot account for intracellular spike amplitude change (even if only for L5 pyramids), CSD amplitude decrease can, suggesting that single neuron CSDs or, alternatively, detection of spikes of individual neurons from multiple extracellular electrodes at known locations yields more robust spike measurements.

Finally, based on passive cable theory (Rall 1977), assuming an intracellular current injection $i_i \text{(x,t)}$ at a specific location along a patch of membrane, the membrane current per unit length of cable, $i_m(x,t)$, is given

$$i_m(x,t) = \frac{V_m(x,t) - V_{\text{rest}}}{r_m} + c_m \frac{\partial V_m(x,t)}{\partial t} - i_i(x,t)$$

where $V_m$ is the membrane potential at location $x$ and time $t$, $V_{\text{rest}}$ the resting potential, $r_m$ the membrane resistance of unit length cable, and $c_m$ the membrane capacitance per unit length. We observe that $i_m$, i.e., the source term of extracellular signals such as the EAP (Holt and Koch 1999; Gold et al. 2006; Anastassiou et al. 2013), is proportional to the temporal derivative of $V_m$, which, in this case, can be approximated by the intracellular potential, $V_i$. One important implication is that the timing of the positivity of the intracellular spike does not coincide with the EAP negativity. Assuming resistive extracellular conductivity, it follows that $i_m$ is the source of the $V_e$ signal so that $i_m$ and $V_e$ are completely in phase. It also follows that $V_i$ ($V_m$) and $i_m$ have an $RC$-type relationship, i.e., there is a phase between them. Thus, in terms of timing, $V_i$ (or $V_m$) and $V_e$ will not be in phase (e.g., see Figs. 2 and 4B). In fact, the EAP negativity from a signal recorded close to the soma broadly corresponds to the maximum rate of rise of the intracellular spike and thus typically precedes the intracellular spike positivity (see Fig. 6 and Buzsáki et al. 1996; Henze et al. 2000). Further away from the soma, electrogenesis of local membranes can impact the EAP waveform so that the time correspondence between EAP and intracellular somatic spike features may become further obscured. In addition, experimentally, the similarity between the EAP waveform and the temporal derivative of $V_i$ remains qualitative especially after the initial phase of the action potential generation casting the $V_i$ derivative as a rather coarse approximation of the EAP waveform.

Spike contribution to the LFP. Unit activity is traditionally thought to impact the high-frequency (above 500 Hz) spectrum of extracellular recordings. The low-pass-filtered part of the extracellular signal, the so-called LFP, has historically been considered to mainly reflect a combination of postsynaptic activity and associated currents such as return currents as well as other processes such as oscillatory membrane and calcium-dependent currents (Mitzdorf 1985; Logothetis et al. 2007; Buzsáki et al. 2012; Einevoll et al. 2013). Recent computational and experimental studies have challenged this picture by showing that spike currents can impact bandwidths of the extracellular signal in the traditional LFP band (below 100 Hz; Rasch et al. 2009; Ray and Maunsell 2011; Belluscio et al. 2012; Zanos et al. 2011; Schomburg et al. 2012; Reimann et al. 2013; Waldert et al. 2013). Although interpretation of single-neuron CSD analyses is somewhat ambiguous, our experiments are in agreement with simulations (Petersen and Einevoll 2008; Schomburg et al. 2012) showing that extracellular spikes can impact bandwidths lower than 200 Hz. Yet, the extent to which spiking can impact LFP characteristics has remained speculative. Our intracellularly induced spikes show that EAPs and spike afterpotentials of L4 and L5 pyramids can contribute spectral power as low as 20 Hz compared with nonspiking $V_e$ traces. The effect of EAPs in such low frequencies is attributed to the slower, smaller amplitude repolarization typically difficult to distinguish in vivo although synaptic currents may also influence the EAP waveform in such time scales (Glickfeld et al. 2009).

What are the functional and computational ramifications of this observation? Spike-field coherence (with certain LFP
bandwidths) is often used to infer the relationship between synaptic input (considered to be reflected in the lower LFP bands) and neural output (spiking) (Fries et al. 1997; Womelsdorf et al. 2006; Montgomery et al. 2008; Rutishauser et al. 2010; Anastassiou et al. 2011). Yet, if spiking itself can impact bands as low as beta or gamma, i.e., LFPs in these bandwidths are also shaped by spiking, then the causal relationship between LFPs (as a proxy to synaptic input) and spiking becomes questionable (Billeh et al. 2014; Anastassiou and Koch 2015; Schaub et al. 2015). On the other hand, clearly, synaptic currents are required to elicit spiking and will impact LFPs. The extent to which spiking and LFP (even in low bands) are independent is thus state dependent (for example, slow vs. fast background spiking) and measures such as spike-field coherence need to be assessed accordingly.

ACKNOWLEDGMENTS

We thank Aleena Garner, Kenji Mizuseki, Adam Shai, and Tim Blanche for comments and discussions.

GRANTS

This work was funded by the Swiss National Science Foundation (to C. A. Anastassiou), the Human Frontier Sciences Program (to C. Koch, G. Buzsáki, and H. Markram), the National Institute of Neurological Disorders and Stroke (to C. Koch), and the Mathers Foundation (to C. Koch). C. A. Anastassiou and C. Koch thank the Allen Institute founders, P. G. Allen and J. Allen, for support.

DISCLOSURES

Conflict of interest statement: No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.A.A. and R.P. conception and design of research; C.A.A. and R.P. performed experiments; C.A.A. and R.P. analyzed data; C.A.A. and R.P. drafted manuscript; C.A.A. and R.P. conceived and designed the experiments; R.P. prepared figures; C.A.A. and R.P. performed experiments; C.A.A. and R.P. analyzed data; R.P. prepared figures; C.A.A., R.P., G.B., and C.K. conceived and designed the experiments; C.A.A. and R.P. performed experiments; C.A.A. and R.P. analyzed data; C.A.A. and R.P. drafted the manuscript; C.A.A., R.P., G.B., and C.K. approved final version of manuscript.

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