NEUROPHYSIOLOGY

Hodgkin and Huxley model — still standing?

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Action potentials in cortical neurons show a variable threshold and a sudden rise in membrane potential at initiation. Naundorf *et al.*[1] fail to explain these features using single- or double-compartment Hodgkin–Huxley-style models, suggesting instead that they could arise from cooperative opening of Na^+ channels, although there is no direct biological evidence to support this. Here we show that these so-called unique features are to be expected from Hodgkin–Huxley models if the spatial geometry and spike initiation properties of cortical neurons are taken into account — it is therefore unnecessary to invoke exotic channel-gating properties as an explanation.

Cortical pyramidal cells initiate spikes in the axon initial segment (AIS) about 30–60 μm from their soma. These spikes then propagate antidromically through the soma and dendrites[2–4]. A well known feature of antidromic spikes is their sudden rise from baseline[5]. These critical properties were not considered by Naundorf *et al.*[1].

We made simultaneous whole-cell recordings from the AIS by patching the cut end of the axon (Fig. 1, legend) and the soma of layer-5 pyramidal neurons *in vitro*[6] during spontaneous spike generation (Fig. 1). Somatic spikes showed a rapid rise, or 'kink', at initiation (Fig. 1a, b) and the slope of the phase plot of spike dV/dt versus V at $dV/dt = 15\ mV\ ms^{-1}$ was $25 \pm 6.8\ ms^{-1}$ (mean ± s.d.; $n = 32$). The phase plots of dV/dt versus V typically revealed a biphasic rise, which was suggestive of two underlying components (Fig. 1b; $n = 30/32$), as observed in many cell types[7,8]. This biphasic component was not evident in the recordings of Naundorf *et al.*[1], although the low peak dV/dt of their recordings indicates that their spikes may not have been fully represented.

Intrasomatic injection of a noisy conductance that mimics the arrival of excitatory and inhibitory synaptic activity[9] resulted in significant variation in the apparent spike threshold ($n = 6$; Fig. 1c, green lines), as observed in the recordings of Naundorf *et al.*[1].

In contrast to somatic spikes, those recorded at the site of spike initiation, the AIS, showed a slower rise ($n = 10$; Fig. 1d, e). The slope of the phase plot of spike dV/dt versus V at $dV/dt = 15\ mV\ ms^{-1}$ was much lower for the AIS ($3.8 \pm 1.7\ ms^{-1}$; $n = 6$; $P<0.01$; Fig. 1d, e) than it was for the soma (Fig. 1a, b). The slow rise at spike initiation in the AIS is not an artefact of our method of axonal recording (Fig. 1, legend). On intrasomatic injection of a noisy conductance that mimics synaptic activity[9], the apparent spike threshold was less variable for the AIS ($n = 6$; Fig. 1f, green lines) than it was for the soma (Fig. 1c).

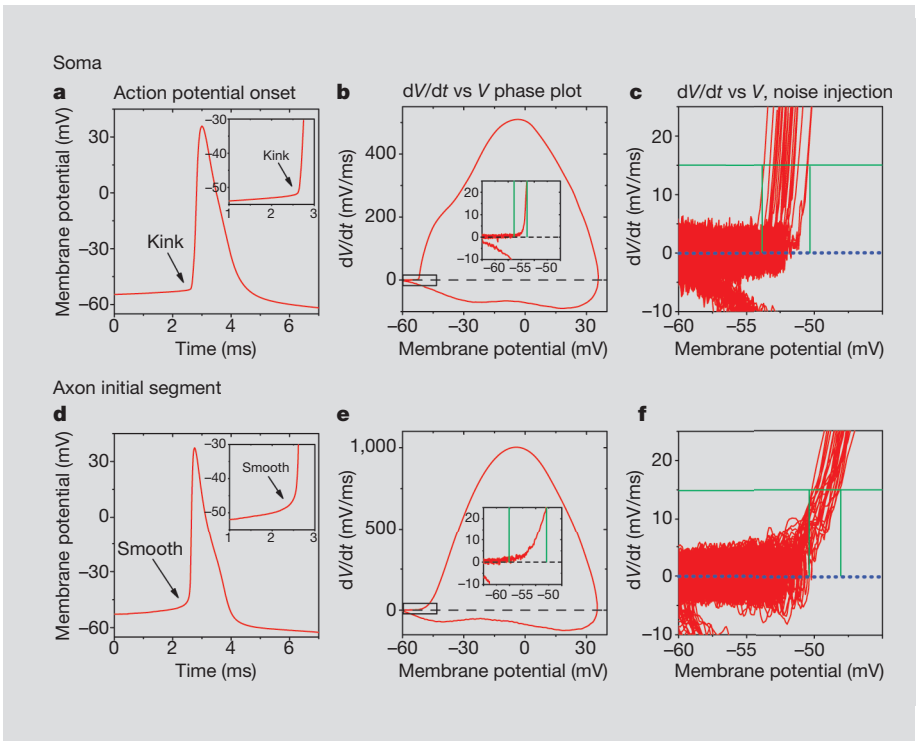

Figure 1 | Properties of spike initiation in the soma and axon of cortical pyramidal cells. a, Somatic spike exhibits a 'kink' at its onset. **b,** Phase plot (dV/dt versus V) and close-up of rapid initiation (inset) of the spike shown in **a**. **c,** Close-up of the phase plot of somatic spike initiation during noisy intrasomatic current injection[9], showing a broad distribution of thresholds (green lines). **d,** Whole-cell axonal recording (50 μm from the soma). **e,** Phase plot of the axonal spike. Note the smoothly rising dV/dt. **f,** Overlay of dV/dt versus V for the onset of axonal spikes, showing lower variability (compare with the soma) of spike threshold (green lines).
Methods. Simultaneous axonal and somatic whole-cell recordings were obtained with the multiclamp 700B amplifier from ferret prefrontal cortical layer-5 pyramidal cells in slices maintained *in vitro* at 36 °C (ref. 6). Spikes shown in **a**, **d**, as well as in **c**, **f**, were recorded simultaneously. Spikes occurred either during spontaneous synaptic activity[6] or in response to the intrasomatic injection of a noisy (10–15 mV) current injection[9]. Whole-cell axonal recordings obtained through patching the cut end of the axon (terminal bleb) do not result in abnormal smoothness of spikes because spikes recorded from distal (> 100 μm) axonal sites also show an onset kink owing to spike propagation (see also www.mccormicklab.org).

Spike initiation in the AIS is mediated by either a high Na^+-channel density in the AIS, as indicated by immunocytochemistry[10,11], or by a lesser density of Na^+ channels, which have a low threshold for activation[12]. Using a previous model of spike initiation in a layer-5 cortical pyramidal cell[13], we adjusted the axonal and somatic densities of Na^+ and K^+ channels until the spike waveform and its derivative were similar to those of our actual recordings (compare Figs 1 and 2).

Our Hodgkin–Huxley model initiated spikes in the AIS that then propagated antidromically through the soma and dendrites, as in real pyramidal cells. At the soma, these spikes showed a rapid rise at initiation (Fig. 2a, b), and the slope of the phase plot for spike initiation at $dV/dt = 15\ mV\ ms^{-1}$ was $21\ ms^{-1}$. Intrasomatic injection of artificial synaptic barrages[9] into the modelled neuron revealed a high variability of apparent spike threshold in the soma (Fig. 2c).

As in the whole-cell recordings, the rise in the model spike at initiation was smoother at the AIS (Fig. 2d, e) than at the soma (Fig. 2a, b). The slope of the phase plot for spike initiation at $dV/dt = 15\ mV\ ms^{-1}$ was considerably lower for the model AIS ($4\ ms^{-1}$) than for the soma, and both were in the range observed in normal cells. Intrasomatic injection of artificial synaptic barrages[9] showed a less variable threshold in

the AIS (Fig. 2f) than in the soma (Fig. 2c), as we found for real neurons (Fig. 1c, f).

We found that several other Hodgkin–Huxley models of cortical pyramidal cells, even one based on a relatively low density of Na^+ conductance in the axon, replicated the 'kink' and variability of somatic spikes (Fig. 2 legend). These features of spike initiation in the soma were dependent on the initiation of spikes in the AIS: increasing the somatic Na^+ conductance to a high level (7.5 nS μm^{-2}) and removing Na^+ conductance from the axon in the model presented here resulted in a loss of the kink at the foot of the spike (soma slope, 4.1 ms^{-1}) and a reduction in spike threshold variability in the soma (results not shown).

Our findings reveal that leading Hodgkin–Huxley models of cortical pyramidal cell spike initiation capture the so-called unique features observed by Naundorf *et al.*[1]. We attribute these features simply to recording from a site that is distant from the site of spike initiation and to the non-uniform distribution of spike properties over the somatic and axonal membrane. The initiation of spikes in the axon that then back-propagate into the soma can result in a rapid change in membrane potential (the kink) at the foot of the somatic spike. The large current supplied by the axonal spike precedes and overlaps with the current supplied by the local generation of the action potential in the soma during the rising phase of the spike. This results in a more rapid rise at the foot of the spike in the soma than would occur if there were no preceding spike in the axon. The apparent high threshold variability with intrasomatic recordings merely results from membrane potential differences between the soma and the actual site of spike initiation, the axon, at the time that spikes are generated. These membrane-potential differences arise from local electrophysiological differences, as well as spatial non-uniformity in synaptic activity. We conclude that the observations made by Naundorf *et al.*[1] are predictable by Hodgkin–Huxley theory and the known physiology of spike initiation[2–4], and that there is no need to invoke exotic interchannel cooperativity to explain their observations.

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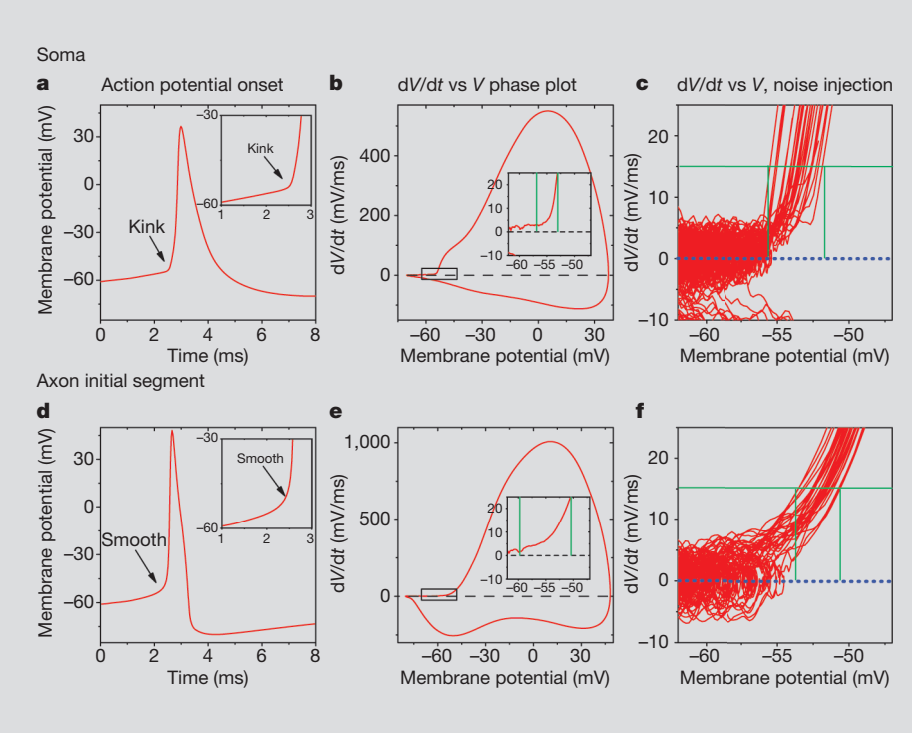

Figure 2 | Hodgkin–Huxley model of a layer-5 cortical pyramidal cell. a, Somatic spike shows a 'kink' at its onset, as in the real neuron. **b,** Phase plot (dV/dt versus V) and close-up of rapid initiation (inset) of the spike shown in **a**. **c,** Close-up of the phase plot of somatic spike during noisy intrasomatic current injection, showing a broad distribution of thresholds (green lines). **d,** Axonal spike (45 μm from the soma). **e,** Phase plot of the axonal spike. Note the smoothly rising dV/dt. **f,** Overlay of dV/dt versus V for the onset of axonal spikes, showing lower variability of spike threshold (green lines).
Methods. Results were obtained from a model layer-5 cortical pyramidal cell[13] with the intrasomatic injection of a 10–15 mV noisy conductance. The model contained the following conductances: soma (Na^+, 0.75 nS μm^{-2}; K^+, 0.15 nS μm^{-2}); axon hillock and initial segment (Na^+, 7.5 nS μm^{-2}; K^+, 1.5 nS μm^{-2}); dendrite (Na^+, 0.1 nS μm^{-2}; K^+, 0.002 nS μm^{-2}; M-current, 0.0003 nS μm^{-2}). Axonal length, 50 μm; soma size, 20 × 30 μm. These parameters were used to match the maximal dV/dt rates, durations and initiation site of spikes in our neurons (Fig. 1). Similar results are obtained from several Hodgkin–Huxley models of cortical pyramidal cells, including those using a high, medium or relatively low density of axonal Na^+ conductance[12–14], and the results from these simulations were well within the range of real cortical cells (see also www.mccormicklab.org).

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Naundorf et al. *reply*

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McCormick *et al.*[1] question whether the rapid onset and highly variable thresholds of action potentials[2] are genuine features of cortical action-potential generators — that is, whether they reflect the voltage-dependence of the underlying sodium currents. Instead, they consider these features to be epiphenomena, reflecting lateral currents from a remote initiation site, and, contrary to direct evidence[3], they assume that sodium currents show canonical kinetics.

Although the lateral current hypothesis of

McCormick *et al.* is superficially plausible, their recordings are inadequate for showing that the dynamics of axonal action-potential initiation conforms to the canonical model. Their so-called axonal recordings are actually obtained from 'blebs' — injury-induced swellings of cut axons on the slice surface. The injured axons, when forming blebs, reorganize their entire cytoskeleton, including the destruction of the sub-membrane spectrin network[4] that integrates sodium channels into the supramolecular machinery of the normal initial segment[5]. As the behaviour of axonal sodium channels is highly sensitive to their cellular environment[6], the smooth action-potential waveforms in the blebs, instead of revealing the true dynamics of action-potential initiation, are more likely to be caused by the disorganized state of the bleb membrane.

The model of McCormick *et al.*[1] does not conform with the known physiology of layer-5 pyramidal cells. Contradicting direct measurements[7,8], it assumes a high ratio of axonal-to-somatic sodium currents. Even with these physiologically unrealistic settings, their model still does not reproduce the experimental data. In their *in vitro* recordings, as in our *in vivo* recordings (Fig. 2 (panels a, c) in ref. 2), somatic action potentials rise almost vertically out of the cloud of subthreshold fluctuations. In their model, however, the range of action-potential onset potentials hardly overlaps with the range of subthreshold fluctuations, being shifted towards more depolarized potentials (Fig. 2 (panel c) in ref. 1). The model of McCormick *et al.* therefore in fact provides further evidence that canonical models are incapable of correctly describing the observed dynamics of action-potential initiation[2,3].

However, McCormick *et al.* highlight an important issue. How can the action-potential dynamics at a remote initiation site be critically probed, when action-potential waveforms recorded from thin processes, such as axons, are likely to be compromised by technical problems[9]? Our analysis identifies an essentially non-invasive approach for addressing this question (see supplementary information of ref. 2). It is based on quantifying the ability of a neuron to phase-lock its spikes to a weak test stimulus in the irregular firing regime[2,10,11].

Theoretical studies indicate that canonical generators of action potentials have a very limited ability to encode high-frequency inputs, showing cut-off frequencies of phase-locking (v_c) that are of the order of their mean firing rate[10,11]. By contrast, models with intrinsically high onset rapidness (r) can show arbitrarily high cut-off frequencies[2,10–12]. If the rapidness of the action-potential onset is genuinely increased by a factor of 10, then cut-off frequencies above 100 Hz are predicted by dimensional analysis ($v_c \propto r$), even for mean firing rates of around 10 Hz. Both *in vivo* and *in vitro* studies have revealed signatures of such fast responses in the neocortex[12,13], supporting genuinely rapid initiation of action potentials in cortical neurons (see also http://www.nld.ds.mpg.de/actionpotentials).

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