

The Elusive Action Potential

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Abstract

Recent evidence suggests that models using canonical kinetics of sodium currents are virtually incapable of quantitatively reproducing the dynamics of action potential initiation in cortical neurons (Naundorf et al., Nature 2006; Baranauskas & Martina, J. Neurosci. 2006). Here we analyze the dynamics of action potentials in canonical multi-compartmental models of layer 5 pyramidal neurons exhibiting axonal action potential initiation, including a model proposed by McCormick et al. (McCormick et al. Nature, 2007). We show that several canonical models constructed to match the spatial organization of axonal sodium currents measured in layer 5 pyramidal neurons fail to reproduce the rapid onset of somatic action potentials observed in these cells. The model proposed by McCormick et al. strongly deviates from the known spatial organization of axonal sodium currents, but is capable of partially mimicking the somatic action potential waveforms. All tested models failed to reproduce the range of AP onset potentials observed in both *in vivo* and *in vitro* intracellular recordings in the fluctuation driven firing regime (Naundorf et al., Nature 2006; McCormick et al. Nature, 2007). In addition, the models were incapable of explaining the transformation of somatic AP waveforms during bath application of low doses of the sodium channel blocker tetrodotoxin (Naundorf et al., Nature 2006). These results strongly suggest that axonal initiation of APs in layer 5 pyramidal neurons *per se* is not sufficient to explain the rapid initiation and broad range of AP onset potentials in these cells. The use of axonal bleb recordings to support this lateral current hypothesis is critically discussed.

We recently described that the dynamics of action potentials (APs) in all classes of cortical neurons exhibits a rapid onset of APs at variable onset potentials (Naundorf et al. 2006). This type of AP initiation dynamics can be robustly explained by the hypothesis of cooperative sodium channel activation, but is virtually impossible to reconcile with Hodgkin-Huxley type models (Naundorf et al. 2006). Because layer 5 pyramidal neurons are known to exhibit axonal action potential initiation in close proximity of the soma (Palmer, Stuart 2006), it is natural to ask how the spatial organization of the proximal axon underlying distal AP initiation can affect the dynamics of AP initiation and the nature of AP waveforms in these cells. Arguing in favour of a canonical model of AP initiation in layer 5 pyramidal neurons, McCormick et al. (2007) recently questioned whether rapid onsets and highly variable thresholds of APs are genuine features of cortical AP generators, i.e. reflect the voltage-dependence of the underlying sodium currents. Instead, they propose that these features are epiphenomena, reflecting lateral currents from the remote AP initiation-site. In a model implementing this *lateral current hypothesis*, they assumed sodium currents to exhibit canonical kinetics although substantial deviations from canonical behaviour have been demonstrated for the activation of sodium channels in cortical neurons by a recent biophysical study (Baranauskas, Martina 2006).

To clarify, how axonal AP initiation may affect the waveforms of somatic APs in canonical models, we analyzed the dynamics of action potential initiation in multi-compartmental models, including the model proposed by McCormick et al. (2007). In several models of layer 5 pyramidal neurons, we find that when the spatial organization of axonal sodium currents conforms with experimental observations these models fail to reproduce the waveforms of somatic action potentials recorded in these cells. The model proposed by McCormick et al. (2007) strongly deviates from the existing data on the spatial organization of axonal sodium currents by assuming an abrupt 10-fold increase of sodium current densities from the soma to the axon hillock. Due to this assumption it is capable of partially mimicking the somatic action potential waveforms. All tested models, however, fail to reproduce the range of AP onset potentials observed in both *in vivo* and *in vitro* intracellular recordings in the fluctuation driven firing regime (Naundorf et al., 2006; McCormick et al., 2007). In addition, the models are found incapable of explaining the transformation of somatic AP waveforms during bath application of low doses of tetrodotoxin (Naundorf et al., 2006). The use of axonal bleb recordings to support the lateral current hypothesis is critically discussed.

Axonal AP initiation and somatic AP waveforms in layer 5 pyramidal neurons

The model recently proposed by McCormick et al. (2007) achieves some resemblance of the onset dynamics between the simulated and recorded somatic APs by engineering in the model an initial segment that generates an unphysiologically large lateral current charging the somatic compartment. According to direct biophysical measurements, the organization of the proximal axon of layer 5 pyramidal neurons differs substantially from such an architecture (Figure 1). As described for pyramidal neurons in the hippocampus (Colbert, Johnston 1996) and for layer 5 pyramidal neurons in the neocortex (Colbert, Pan 2002, Ruben et al. 2003), sodium current properties and densities in the initial $30\mu m$ of the axon are largely indistinguishable from those of the somatic membrane. Only beyond this apparently homogenous initial region of the proximal axon, densities of sodium currents are mildly increased, indicating a somewhat increased density of sodium channels (Figure 1; see also Figure 2 in Pan, Colbert 2002 and Figure 2 in Colbert, Johnston 1996). Half-activation voltages of sodium channels in this region are reduced by about $7-8 mV$ (Figure 2 in Pan, Colbert 2002). When sodium current-densities and half-activation voltages in soma and axon in canonical multi-compartmental models are set according to these direct measurements, smooth APs are initiated in the distal axon, and remain perfectly smooth and rise gradually while invading soma and dendrites (Figure 2). The waveforms of these antidromically propagating APs in fact are virtually identical to those of the canonical single compartment models we analyzed previously (Naundorf et al., 2006; see also Figures 3b and ,4b in Colbert, Pan 2002). As pointed out before, the onset dynamics of these APs is thus qualitatively different from the rapid onset dynamics characteristic of somatic AP waveforms observed in all types of visual cortical neurons *in vivo* (Naundorf et al., 2006) and in layer 5 pyramidal neurons of the prefrontal cortex *in vitro* (McCormick et al., 2007) . These results indicate (1) that canonical models of axonal AP initiation, which conform with the existing experimental data on axonal organization are essentially incapable of reproducing the AP waveform of cortical neurons as observed at the soma and (2) that, in general, antidromically propagating APs in canonical multi-compartmental models do not exhibit an initial “kink”.

The initial kink exhibited by somatic APs in the model of McCormick et al. is not a signature of AP back propagation *per se*. Instead, it results from the contra-factual assumption of a pronounced drop in sodium current density from the most proximal part of the axon, the axon hillock, to the somatic membrane. In their model McCormick et al. (2007) are assuming that sodium current densities exhibit an abrupt, ten-fold increase at the transition from the somatic

membrane to the membrane of the axon hillock. Interestingly, even with this artificial axonal architecture, their model still fails to reproduce the recordings in several respects. Most importantly, in their *in vitro*, as well as in our *in vivo* recordings, APs in the soma rise virtually vertically out of the cloud of subthreshold fluctuations (Figure 1c in McCormick et al. 2007, and Figure 2 in Naundorf et al. 2006). In their model, however, the range of AP onset potentials barely overlaps with the range of subthreshold fluctuations. Instead, there is a clear threshold voltage: All membrane potential trajectories that reach the voltage range of AP onset potentials invariably lead to AP generation. Real neurons behave distinctly different: In neurons many “subthreshold” trajectories reach up to the highest AP onset potentials but return to more negative potentials without AP generation, leading to a cloud of subthreshold fluctuations overlapping the voltage range of AP onset potentials. This discrepancy between the model and the data of McCormick et al. stands out very clearly when Figures 2c and 1c of McCormick et al. 2007 are overlaid (Figure 3a). The disagreement between the behaviour of canonical multicompartmental models and the onset dynamics of cortical APs is also clearly evident when compared to *in vivo* recordings. Neither the model of McCormick et al. (2007), nor a canonical model of layer 5 pyramidal cells (Mainen, Sejnowski 1995), modified to match the experimentally observed axonal organisation, capture the rapid initiation and high variability of onset potentials displayed by APs of cortical neurons (Figure 3b,c). As previously shown (Naundorf et al. 2006), models based on cooperative sodium channel activation can perfectly reproduce both these features (Figure 3d).

Initiation of APs with reduced channel density: TTX experiments and simulation

Finally, the model proposed by McCormick et al. is also incapable of reproducing the transformation of somatic AP waveforms observed under bath application of TTX. Our *in vitro* experiments demonstrated that bath application of low doses of TTX reversibly transformed the somatic APs from having rapid AP onset to APs exhibiting a gradual onset (Figure 4a, see also Naundorf et al., 2006). This effect was predicted by and is consistent with the hypothesis of cooperative sodium channel activation. Because the model proposed by McCormick et al. can partially mimic the rapid onset of somatic APs, we tested whether this model is also capable of mimicking the transformation of AP waveforms, observed in the TTX experiments. Intriguingly, the model predicts an effect of partial sodium channel blockade which is very different from the experimentally observed behaviour (Figure 4b). With partial blockade of sodium channels, the onset dynamics of somatic APs does not become slower, as in the experiments. Instead, the bi-phasic shape of the initial AP slope

becomes more pronounced than under control conditions. In the McCormick et al. model, the dissociation of the axonal and somatic components of the AP progressively increases with increasing blockade of sodium currents. This is obviously due to the assumption of a much higher sodium channel density in the axon than in the soma. With partial blockade of sodium currents the somatic membrane in the model fails to support a locally generated AP but the tenfold higher conductance density in the axon is still capable of generating an axonal action potential and charging the soma completely externally.

In summary, (1) McCormick et al.'s assumption of a strong inhomogeneity of sodium currents in somatic and axon hillock membranes contradicts direct biophysical measurements, (2) their canonical axonal AP initiation model fails to reproduce the onset dynamics of somatic APs when driven by fluctuating input currents, and (3) it predicts changes of the shape of somatic APs in response to bath application of low doses of TTX that strongly disagree with the experimental observations. We therefore conclude that the lateral current hypothesis articulated by McCormick et al. is inconsistent with a wealth of experimental data including their own recordings and does not provide a satisfactory alternative explanation for our experimental observations.

Bleb recordings

McCormick et al. attempt to support the lateral current hypothesis and the assumption that cortical sodium currents exhibit canonical kinetics by a novel type of recording from axon initial segment membranes. These recordings, we believe, must be taken with a grain of salt. Their so-called axonal recordings are actually obtained from "blebs", injury induced swellings of cut axons on the slice surface. The same authors previously termed blebs "certainly abnormal ... structures" (Shu et al., 2006), but now consider the bleb-APs as "those recorded at the site of spike initiation, the AIS" (McCormick et al., 2007). The waveforms of APs recorded in blebs is substantially smoother and more gradual than those observed in normal somatic recordings (McCormick et al. 2007, Figure 1f). To our knowledge, such "bleb recordings" have never been used before in any published study of axonal AP initiation and probably for a good reason. The injured axons, when forming the blebs, undergo severe reorganization involving the dissolution and reformation of the entire cytoskeleton including the destruction of the sub-membrane spectrin network (Spira et al., 2002) that integrates sodium channels into the supramolecular machinery of the normal initial segment (Lacas-Gervais et al., 2004). Since the behaviour of axonal sodium channels is highly sensitive to

their cellular environment (e.g. Rush et al., 2005), the smooth AP waveforms in blebs instead of revealing the true dynamics of AP initiation more likely are caused by the disorganized state of the bleb membrane. The recordings of McCormick et al. are thus insufficient to demonstrate that the dynamics of axonal AP initiation conforms to the canonical model.

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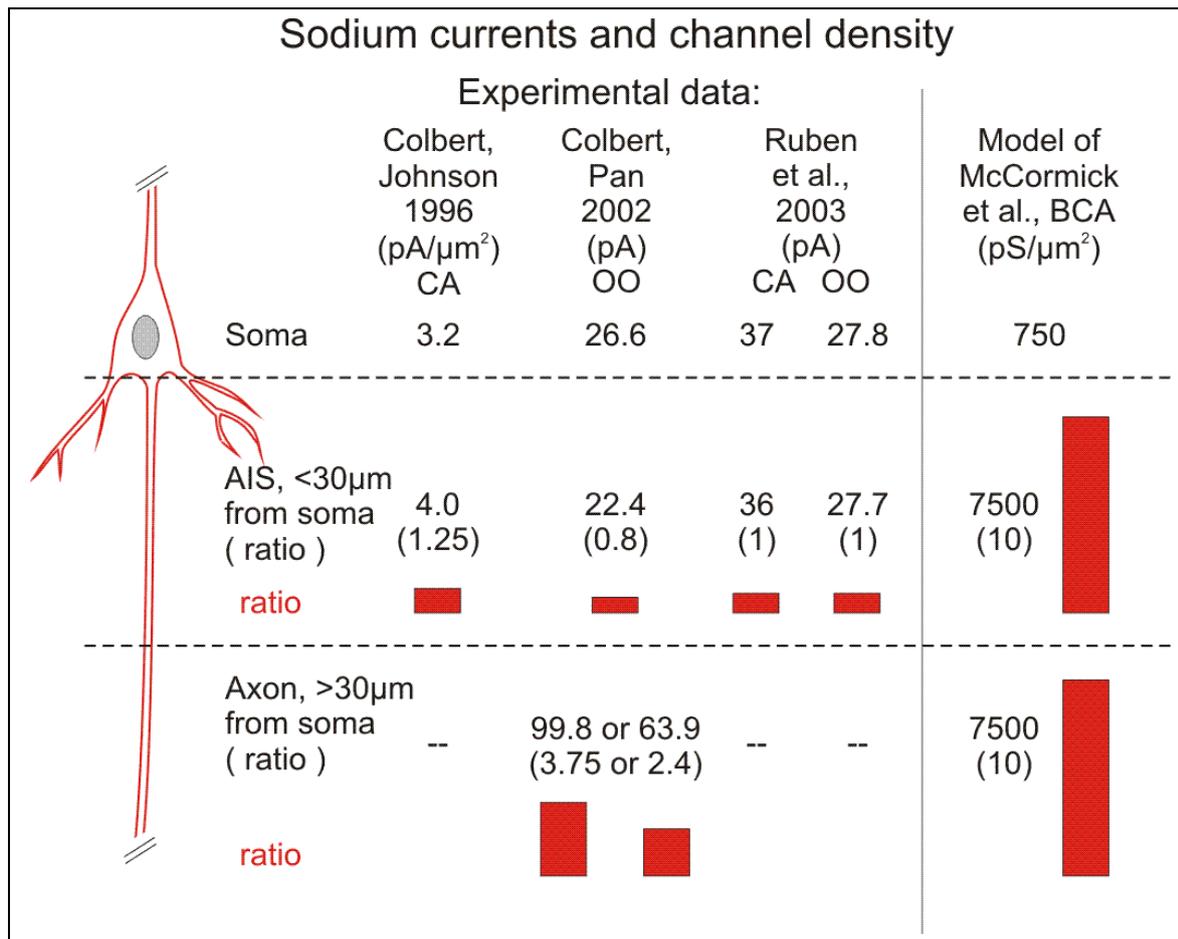


Figure 1. Sodium peak currents in the soma and axon of cortical pyramidal neurons compared to the model of McCormick et al.(2007, BCA).

Numbers show averaged peak sodium currents, recorded at the soma and at different distances along the axon. The ratio of axonal to somatic values is given in parentheses, and shown as bars. The rightmost column shows conductance values used in the model by McCormick et al. (2007).

Data for hippocampal pyramidal neurons (Colbert, Johnston 1996; cell-attached recordings, normalized by patch area), and neocortical layer 5 pyramidal neurons (Colbert, Pan 2002, outside-out patches; Ruben et al., 2003 cell-attached and outside-out patches). For distal axon, Colbert and Pan (2002) give two values, the second one excluding one outlier data point.

Note that the ratio of axonal to somatic conductances, used in the McCormick et al. model is substantially higher than any experimentally based estimate.

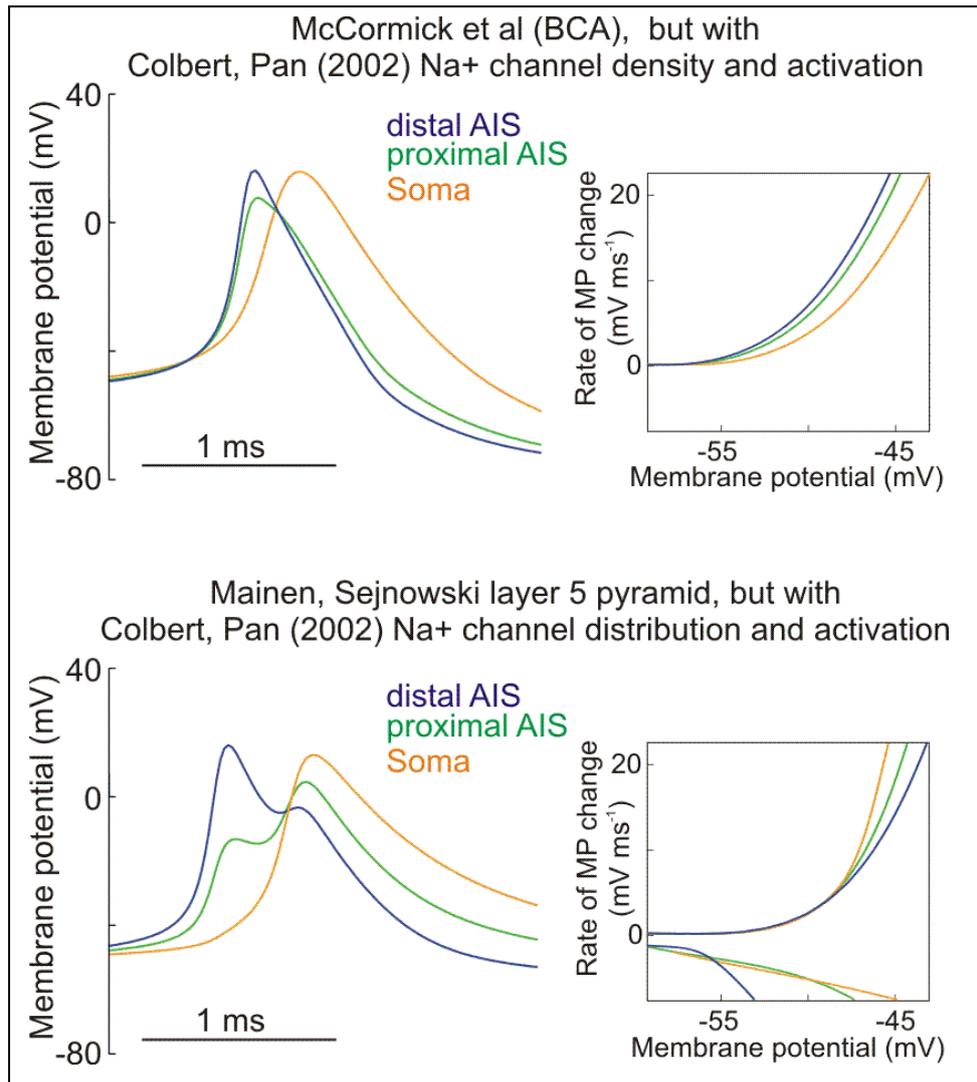


Figure 2. Canonical models of axonal AP initiation with biologically realistic sodium channels densities exhibit smooth AP waveforms in all compartments.

Super-imposed APs from different compartments, as indicated by colour code, and phase-plots of their initial portions.

Note that in both models, APs are initiated in the distal AIS and antidromically invade the soma. Nevertheless, the somatic AP has a smooth onset dynamics.

Top panels: McCormick et al. geometry and kinetics, Na⁺ conductances and half activation voltages adapted according to Colbert and Pan (2002). Soma, proximal AIS (20 μm) 336 pS/ μm^2 , Distal AIS (20 μm): 1008 pS/ μm^2 , activation $V_{1/2}$ shifted by 7 mV. K⁺ conductance in dendrites (g_{kv}) increased from 2 to 20 pS/ μm^2 to prevent dendritic AP initiation.

Bottom panels: Mainen, Sejnowski 1995 layer 5 pyramid, Na⁺ channel distribution and half activation voltages adapted according to Colbert and Pan (2002). Soma, proximal AIS (15 μm) 1000 pS/ μm^2 . Distal AIS (15 μm): 3000 pS/ μm^2 , activation $V_{1/2}$ shifted by 8 mV. Dendritic K⁺ conductance (g_{kv}) was increased from 0 to 20 pS/ μm^2 to prevent dendritic AP bursts.

Simulations were performed using the NEURON Simulator. Code files of the McCormick et al. model were generously provided by the authors. Code file of the Mainen & Sejnowski model was downloaded from the NEURON homepage.

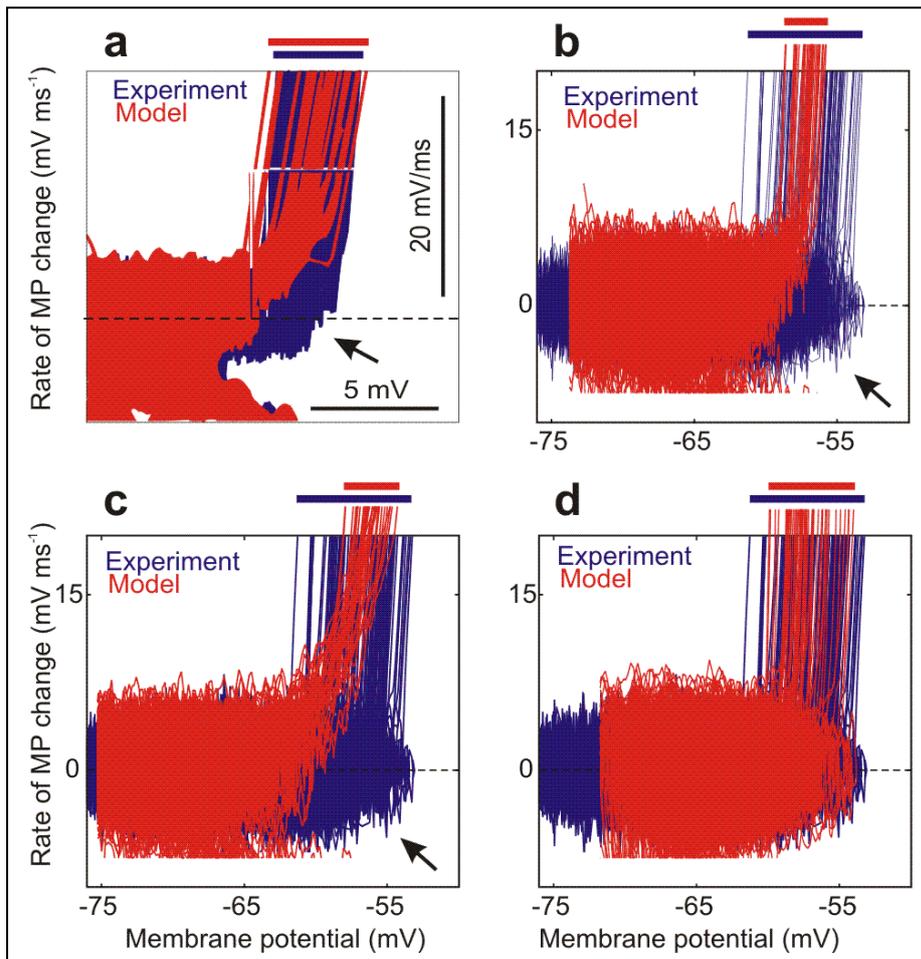


Figure 3. AP onset dynamics *in vivo* is not reproduced by multi-compartmental models based on canonical sodium channel activation, but can be explained by cooperative activation of sodium channels.

Each panel shows a close-up of a phase plot of the AP onset dynamics in cortical neurons (blue, A data from Figure 1c, McCormick et al. 2007, B-C data from Figure 2c, Naundorf et al. 2006). Arrows mark membrane potential trajectories reaching high depolarization levels and return to hyperpolarized potentials without AP initiation.

Superimposed are simulated APs during injection of fluctuating currents (red), shifted in voltage to facilitate comparison with the experimental data.

a: reproduced from Figure 2c of McCormick et al. (2007).

b: Model of McCormick et al.(2007).

c: Model of Mainen, Sejnowski (1995) layer 5 pyramid, Na⁺ channel distribution and activation adapted according to Colbert and Pan (2002) see Figure 2.

d: Model based on cooperative activation of sodium channels (Naundorf et al. 2006).

Note, that the model based on cooperative sodium channel activation reproduces all major features of the AP onset dynamics experimentally observed: (i) almost vertical take-off of the APs from the cloud of “subthreshold” fluctuations, and (ii) large variability of the AP onset potentials. Both canonical models do not reproduce these features.

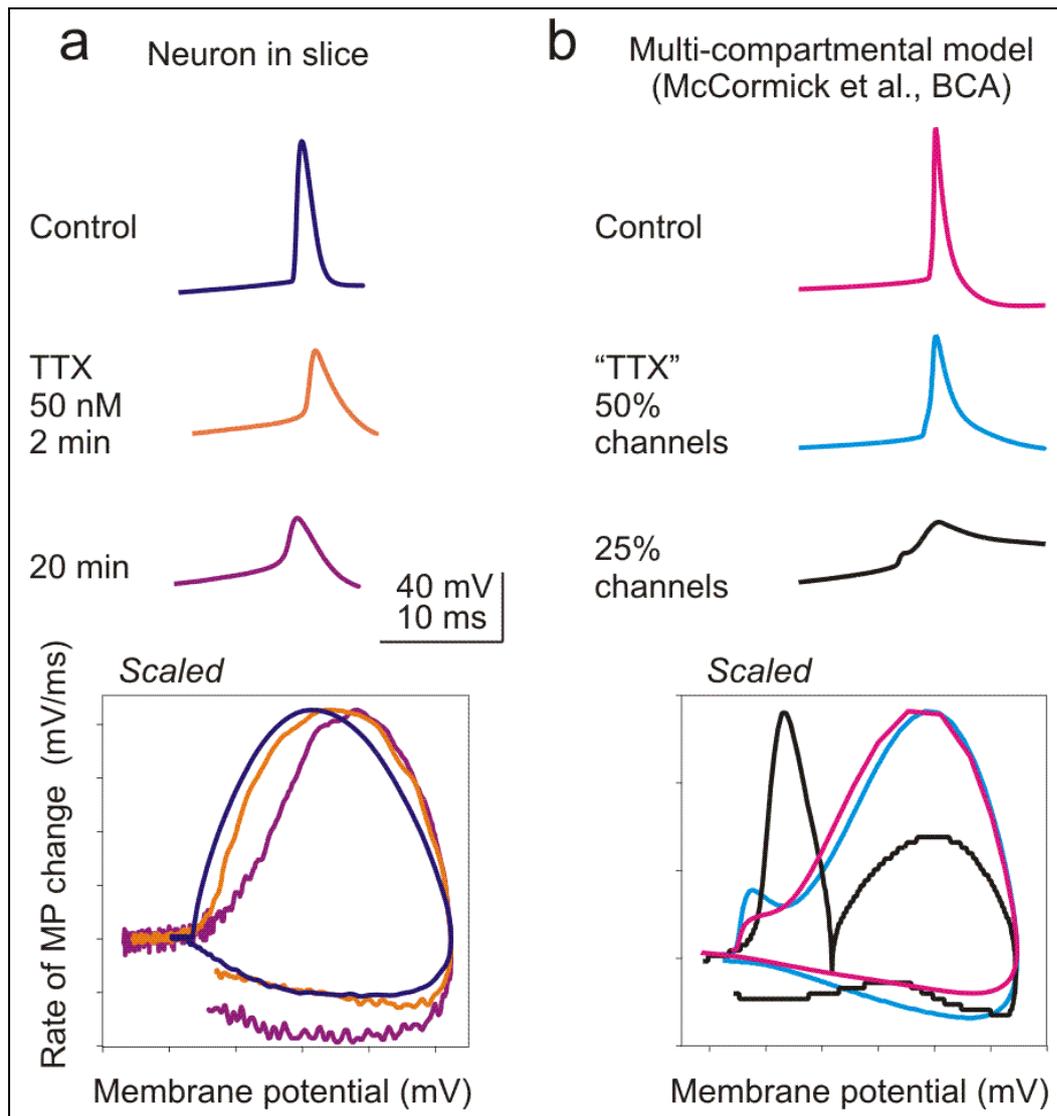


Figure 4. The model of McCormick et al. (2007) is inconsistent with the experimentally observed transformation of AP waveforms during bath application of low doses of TTX.

a, Action potentials recorded in a rat neocortical neuron in slice under control conditions and during bath application of low doses of TTX.

b, Predicted effect of bath application of TTX according to the model of McCormick et al. (2007). In the model, TTX application was implemented by a proportional reduction of sodium conductance density (to 50% and to 25%, as indicated) throughout the neuron.

The phase plots of APs were scaled by the maximal dV/dt and the AP amplitude to facilitate comparison of their onset dynamics.

Note: (i) Slow-down of the AP initiation dynamics during TTX application in the cortical neuron. (ii) Alteration of AP waveform, and dissociation of “axonal” and “somatic” components of the somatically recorded AP, predicted by the model of McCormick et al. (2007).