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Dendritic voltage-gated $K^+$ conductance gradient in pyramidal neurones of neocortical layer 5B.

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Abstract
Voltage-gated potassium channels effectively regulate dendritic excitability in neurones. In the distal apical dendrite of layer 5B (L5B) neocortical pyramidal neurones, potassium conductances are suggested to participate in active dendritic synaptic integration and to control regenerative dendritic potentials. The ionic mechanism for triggering these regenerative potentials has yet to be elucidated. Here we used two-electrode voltage-clamp (TEVC) to quantitatively record K⁺ conductance densities of a sustained K⁺ conductance in the soma and apical dendrite of L5B neurones of adult rats. We report that the somatic and proximal dendritic sustained voltage-gated K⁺ conductance density is more than 10-fold larger than previous estimates. The results obtained using TEVC were corroborated using current-clamp experiments in combination with compartmental modeling. Possible error sources, including inaccurate measurement of the passive membrane parameters and unknown axonal and basal dendritic conductance distributions, were shown not to distort the density estimation considerably. The sustained voltage-gated K⁺ conductance density was found to decrease steeply along the apical dendrite. The steep negative K⁺ conductance density gradient along the apical dendrite may help to define a distal, low threshold region for amplification of distal synaptic input in L5B pyramidal neurones.
Introduction

The apical dendrite of L5B pyramidal neurones has been shown to perform several non-linear transformations of synaptic input, most clearly exemplified by large, regenerative Ca\(^{2+}\) potentials that have been readily recorded from the apical dendrite of L5B neocortical pyramidal neurones (Amitai et al., 1993; Kim & Connors, 1993; Reuveni et al., 1993; Schiller et al., 1997; Larkum et al., 1999b; Zhu, 2000; Larkum et al., 2001). In these neurones, when a back-propagating action potential (AP) coincides with distal synaptic input, a regenerative dendritic Ca\(^{2+}\) potential is generated, triggering a burst of several APs at the soma (Larkum et al., 1999b) thus inducing a large change in the informational content of the neuronal output (Lisman, 1997). It has been hypothesized that the initiation of these regenerative Ca\(^{2+}\) potentials may require an increase in the density of voltage-gated Ca\(^{2+}\) conductance along the apical dendrite (Reuveni et al., 1993; Schaefer et al., 2003b). Alternatively, due to the intricate interaction of ionic currents in the generation of regenerative Ca\(^{2+}\) potentials (Larkum et al., 1999a; Larkum et al., 1999b, 2001) a substantial decrease in the relative density of voltage-gated K\(^{+}\) conductances in the distal dendrite might render the distal dendrite sufficiently excitable to allow the generation of regenerative dendritic Ca\(^{2+}\) potentials.

Such complex neuronal mechanisms should be explained by extracting the kinetics of the various voltage-gated conductances in these neurones and constructing a detailed compartmental model. This approach requires obtaining quantitative information on the density and kinetics of voltage-gated conductances.
conductances in various compartments of the neurone. Dendritic distributions of $K^+$ currents have been measured in dendrites from various type of neurones (Bischofberger & Jonas, 1997; Hoffman et al., 1997; Bekkers, 2000a; Korngreen & Sakmann, 2000; Martina et al., 2003). However, the experimenter is faced with technical problems inherent to the frequently used cell-attached recordings – the estimation of patch membrane area introduces a significant error to the determination of conductance densities. Moreover, the high variability in measurements due to the random sampling of the membrane with each patch containing only very few channels requires a large number of recordings.

We recently introduced a technique that enables the measurement of the density of voltage-gated $K^+$ conductances from the soma and apical dendrites of neurones by recording ionic currents using two-electrode voltage-clamp (TEVC) combined with measurement of passive membrane parameters and reconstruction of neuronal morphology (Schaefer et al., 2003a). Here we applied this technique to measure the density of voltage-gated $K^+$ conductances in the soma and along the apical dendrite of L5B neurones in the somatosensory cortex.
Methods

Slice preparation

Acute brain slices (sagittal, 300 µm thick) were prepared from the somatosensory cortex of 13-45 day old Wistar rats killed by rapid decapitation following shallow anesthesia with isoflurane or halothan, in accordance with the guidelines of the Max-Planck and Bar-Ilan University animal welfare committees, using previously described techniques (Stuart et al., 1993). Slices were superfused throughout the experiment with an oxygenated artificial cerebrospinal fluid (ACSF) containing: (mM) 125 NaCl, 15 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 25 Glucose (pH=7.4 with 5% CO₂, 310 mosmol kg⁻¹) at room temperature (20-22°C). Pyramidal neurones from L5B in the somatosensory cortex were visually identified using infrared differential interference contrast (IR-DIC) videomicroscopy (Stuart et al., 1993).

Solutions and Drugs

The standard pipette solution contained (mM): 125 K-gluconate, 20 KCl, 10 HEPES, 4 MgATP, 10 Na-phosphocreatin, 0.5 EGTA, 0.3 GTP and 0.2 % biocytin (pH=7.2 with KOH, 312 mosmol kg⁻¹). The bath solution for two-electrode voltage-clamp experiments contained (mM): 125 NaCl, 15 NaCO₃, 2.5 KCl, 1 MgCl₂, 2 CoCl₂, 25 glucose, 0.03 ZD7288, 100 nM TTX (pH=7.4 with 5% CO₂, 308 mosmol kg⁻¹). A liquid junction potential of 10 mV was corrected for a posteriori. For cell-attached recordings the pipette solution contained (mM): 135 NaCl, 5.4 KCl, 1 MgCl₂, 1.8 CaCl₂, 5 Hepes, 100 nM TTX (pH=7.2 with NaOH, 290 mosmol kg⁻¹). Tetrodotoxin (TTX, Tocris, Bristol, UK) was stored at -20°C as
stock solutions in doubly distilled water and added directly to the bath solution. ZD7288 (Tocris, Bristol, UK) was stored at +4 °C as stock solutions in doubly distilled water and added directly to the bath solution.

**Electrophysiology**

Two-electrode voltage-clamp (TEVC) recordings were made with two HS-2Ax0.1M head-stages and an Axoclamp-2B amplifier (Axon Instruments, Foster city, CA). Whole-cell recordings were obtained with two patch pipettes the tips of which were separated on average by 40 µm. Simulations have shown that the accuracy and stability of the conductance calculation were not affected by the inter-electrode distance (Supp. Fig. 3). No series resistance compensation was used. It is important to note that series resistance has a different manifestation in a TEVC circuit than in a single-electrode voltage-clamp. In the single-electrode voltage-clamp the same electrode is used for current injecting and voltage recording. Therefore, the part of the injected current falls on the electrode series resistance greatly impairing the accurate clamping of the membrane potential. The ideal TEVC circuit does not suffer from the electrode series resistance since one electrode is used for current injection and one for voltage recording. Since no current flows across the voltage recording electrode the electrode series resistance does not effect the accurate measurement of the membrane potential. In the real TEVC circuit the series resistance is mostly stray resistance from the bath and reference electrode. In the experiments reported in this manuscript the bath series resistance was very small (few kΩ) compared to the relevant membrane resistances and did not distort the recordings. Capacitive coupling
between the electrodes was minimized by placing a grounded shield that extended almost to the bath fluid level between the two electrodes. To increase clamp gain the feedback current injected via the current injecting electrode was phase shifted (phase-lag control of the Axoclamp-2B) with a time constant of 1 to 5 ms leading to filtering of the initial rise in the recorded current. The phase-shift had to be introduced in the TEVC circuit to allow for stability with maximal gain (Finkel & Gage, 1985). Achieving maximal gain is desirable as both the clamp fidelity and the actual bandwidth depend on the gain (Finkel & Gage, 1985). In this study we only report properties of the slow conductance activated 50 ms after the onset of the depolarizing voltage-clamp command. Hence, our results are unaffected by the limited bandwidth. Furthermore, we have shown in the previous article (Schaefer et al., 2003a), that systematic errors introduced by mis-estimation of channel kinetics become important only for very rapidly changing conductances (having current rise time smaller than 0.5 ms). Voltage and current were filtered at 10 kHz and sampled at 20 or 50 kHz using the program ‘Pulse’ (Version 8.1, Heka Electronic, Lambrecht, Germany), digitized by an ITC-16 interface (Instrutech, Greatneck, NY, USA), and stored on the hard disk of a computer. Capacitive and leak currents were subtracted off-line by scaling of pulses taken at hyperpolarized potentials. Patch pipettes (5-10 MΩ) were pulled from thick-walled borosilicate glass capillaries (2.0 mm outer diameter, 0.5 mm wall thickness, Hilgenberg, Malsfeld, Germany) and were coated with Sylgard 184 (Dow Corning) prior to the experiment. The distance of the dendritic recording from the soma and the distance between the tips of the current-
injecting and voltage-recording electrodes were measured from video pictures taken by a frame grabber. At the end of each experiment slices were fixed in cold 100 mM phosphate buffer (PBS, pH=7.4) containing 4 % paraformaldehyde. After fixation the slices were incubated for 2 hours in avidin-biotinilated horseradish peroxidase (ABC-Elite, Vector-Labs, Peterborough, UK) and the stain was developed using 0.015 % diaminobenzidine. The stained neurones were digitally traced using a Neurolucida system (MicroBrightField, Colchester, VT, USA).

Passive membrane parameters ($R_m$, $R_i$, $C_m$) were determined as previously described (Stuart & Spruston, 1998; Roth & Häusser, 2001). Briefly, prior to switching on the two-electrode voltage-clamp both electrodes were in bridge mode of the Axoclamp-2B. In this configuration a current pulse was injected via one of the electrodes and the voltage deflection was monitored by both electrodes. The passive membrane properties were determined simultaneously by fitting a passive membrane model using the reconstructed morphology of the soma and dendrites to the average of 30 such membrane potential traces measured in the same cell (Clements & Redman, 1989; Stuart & Spruston, 1998; Roth & Häusser, 2001). The fitting was carried out using NEURON routines kindly provided by A. Roth (University College London, UK).

TEVC currents were analyzed as described previously (Schaefer et al., 2003a). In brief, reconstructed morphology of the soma, axon and dendrites and measured passive membrane parameters were modeled in NEURON. TEVC experiments were simulated at the positions of the current injecting and voltage recording electrodes. Simulated clamp currents were fitted to the experimentally
recorded ones with the K⁺ conductance densities as the only free parameters yielding conductances densities for -90 to +30 mV in steps of 10 mV (Figs 1, 2). A one-gate Boltzmann curve was fitted to the median of the conductance densities from 45 to 55 ms for the voltages from -90 to +20 mV, where in all cases a stable voltage-clamp was achieved. To assess the influence of axonal and basal dendritic hotspots of voltage-gated K⁺ conductances, clamp currents were fitted with the additional constraint of high axonal or basal dendritic K⁺ conductance densities. The channel kinetics used for this purpose was derived from nucleated patch measurements (Korngreen & Sakmann, 2000) and can be obtained from the authors. All data analysis was performed using custom written routines in NEURON 5.4 (Hines & Carnevale, 1997) and Igor Pro 4.09 (Wavemetrics, Lake Oswego, OR).

Cell-attached recordings were carried out using an Axopatch-200B amplifier (Axon Instruments, Foster city, CA). All pipettes were coated with Sylgard and fire polished before use. In an effort to obtain patches with similar area a constant experimental procedure was used. Positive pressure (20-40 mbar) was applied to the pipette as it was advanced to the cell. The tip of the pipette was gently pressed against the membrane and a negative pressure that did not exceed 10 mbar was applied. The experiments on somata displayed in Fig. 2 were carried out with electrodes that had a resistance of 8-10 MΩ. To record K⁺ currents from the initial segment of the axon higher resistance electrodes were used (13-17 MΩ). To allow comparison, several recordings were carried out at the soma also with electrodes of identical properties. Linear leak and capacitance currents were
subtracted either on-line with a P/6 protocol from a hyperpolarized holding potential of -40 mV, or off-line from empty traces. The whole-cell recordings from the soma and apical dendrite in the current-clamp mode presented in Figs 3 and 4 were carried out using a Multiclamp-700B amplifier (Axon Instruments, Foster City, CA) with the same pipette and bath solutions used as in the voltage-clamp experiments.
Results

Somato-dendritic $K^+$ conductance densities measured with TEVC recordings

Figure 1B displays three TEVC experiments, one from the soma and two from the apical dendrite of three different L5B pyramidal neurones in acute brain slices prepared from the neocortex of 6-week (P42) old rats. The $K^+$ currents were recorded in response to voltage-clamp commands ranging from -90 to +20 mV shown below the current traces. Voltage-gated $Ca^{2+}$ currents were eliminated by complete substitution of bath $Ca^{2+}$ with $Co^{2+}$. Consequently, currents from $Ca^{2+}$ dependent $K^+$ conductances were attenuated. The nonselective cation current $I_h$ was blocked by bath application of 30 µM of ZD7288, and the voltage-gated $Na^+$ current was blocked by 100 nM TTX. Passive membrane properties were measured before the voltage-clamp protocol (see Methods). The neuronal morphology was reconstructed after biocytin filling (see Methods and Fig. 1A). Combined, these measurements provided for the quantitative calculation of $K^+$ conductance densities and kinetics (Schaefer et al., 2003a). The onset of the current recording was distorted due to the overshoot of the clamp-voltage during voltage commands produced by the phase lag imposed on the feedback signal of the TEVC (Fig. 1B). Therefore, we did not analyze the kinetics of the initial rise of the conductance, including the activation of the A-type $K^+$ conductance (Bekkers, 2000b; Korngreen & Sakmann, 2000). Activation curves were calculated from the corrected conductance density traces using the values recorded 50 ms after the start of the depolarizing voltage pulse. At this time point the A-type $K^+$ conductance has been reported to be fully inactivated (Bekkers, 2000b;
Korngreen & Sakmann, 2000). Thus, for the remainder of the paper the results and discussion relate only to the slowly inactivating K⁺ conductance.

Our initial observation was that the maximal K⁺ current decreased as a function of the distance along the apical dendrite (Fig. 1B top traces). The application of the space clamp correction algorithm to these current recordings revealed that the K⁺ conductance density decreased along the apical dendrite as a function of the distance from the soma from $172\pm41$ pS/µm² (mean±sem, n=4) at the soma to $9.6\pm1.4$ pS/µm² (n=4) beyond 300 µm along the dendrite (Figs 1B & 1C). The voltage of half activation ($V_{1/2}$) and the inverse slope (k) of the steady-state activation curve were not dependent on the distance along the dendrite ($R^2<0.1$, p>0.4, Fig. 1D) suggesting that the K⁺ conductance density gradient was unlikely due to changes in channel types along the dendrite.

**K⁺ conductance densities during development**

The somatic K⁺ conductance density was more than 10-fold larger than previous estimates from nucleated patches made from 2-week old (P14) animals (Bekkers, 2000b; Korngreen & Sakmann, 2000) but comparable to densities assumed in compartmental simulations of L5B pyramidal neurones (Mainen *et al.*, 1995; Rhodes & Llinás, 2001; Antic, 2003; Schaefer *et al.*, 2003b). To allow comparison of age groups and to assess whether the high somatic K⁺ conductance densities were also present in younger animals we made somatic TEVC experiments on L5B pyramidal neurones from animals that were 2, 4 and 6 week old (Fig. 2). K⁺ conductance densities displayed an apparent linear increase from $91\pm15$ pS/µm² (n=6) to $172\pm41$ pS/µm² (n=4) between 2 to 6
weeks of age that was highly correlated (Fig. 2A,B, r=0.6, p<0.03, ANOVA). The steady-state activation curve of the $K^+$ conductance (described by $V_{1/2, k}$) displayed only a slight developmental change (Fig. 2C). Voltage-clamp experiments in the cell-attached configuration, although failing to provide accurate density estimates, reproduced this relative developmental increase (Fig. 2A2). The average current in cell-attached patches increased significantly from 5.5±0.7 pA (n=40, P14) to 12.3±3.0 pA (n=9, P42, p<0.05, ANOVA).

Estimation of $K^+$-conductance densities from current-clamp recordings

The $K^+$-conductance densities reported in Figs 1 and 2 for the conductance densities were calculated using the previously published numerical algorithm for space-clamp correction (Schaefer et al., 2003a). To verify these results we measured membrane rectification in response to current stimuli at the soma and apical dendrite of L5B pyramidal neurones. As first reported by Cole & Curtis the response of the neuronal membrane potential is not linear when large depolarizing currents are injected into the cell (Cole & Curtis, 1941). This is caused by the activation of voltage-gated $K^+$ channels that draws the membrane potential towards the $K^+$ Nernst equilibrium potential. Here we used this nonlinearity to provide a rough estimate of the voltage-gated $K^+$ conductance density at the site of current injection. The kinetics of two voltage-gated $K^+$ conductances, an A-type fast inactivating voltage-gated $K^+$ conductance ($K_{fast}$) and a delayed rectifier slow inactivating voltage-gated $K^+$ conductance ($K_{slow}$), have been previously characterized in L5B pyramidal neurones (Bekkers, 2000b; Korngreen & Sakmann, 2000). Furthermore, Hodgkin-Huxley like models for
these conductances have been previously provided (Keren et al., 2005). These models were used to calculate the amount of membrane potential rectification at several levels of current injection and conductance densities (see Supp. Fig. 2 for examples of the effect of $K_{\text{slow}}$ and $K_{\text{fast}}$ on the simulated membrane potential).

Based on this rationale, we carried out current-clamp experiments using the whole-cell configuration of the patch-clamp technique at the soma and apical dendrite of L5B pyramidal neurones. Voltage-gated Ca$^{2+}$ currents were eliminated by complete substitution of bath Ca$^{2+}$ with Co$^{2+}$. Consequently, currents from Ca$^{2+}$ dependent K$^+$ conductances attenuated considerably; concurrently, the voltage-gated Na$^+$ current was blocked by 100 nM TTX. Depolarizing current pulses were injected via the whole-cell recording pipette after balancing of the bridge. The changes to the somatic membrane potential following the injection of current steps ranging from 0.05 to 3.95 nA with 0.15 nA increments are displayed in Fig. 3A1. After the experiment the neuronal morphology was reconstructed after biocytin filling and converted to a code readable by the simulation environment NEURON (Hines & Carnevale, 1997, 2000). The kinetic models of $K_{\text{fast}}$ and $K_{\text{slow}}$ were inserted with a homogenous conductance density throughout the somato-dendritic tree, a current-clamp electrode was simulated at the soma and current pulses, identical to those used in the experiment, were injected via this electrode. When the density of $K_{\text{fast}}$ and $K_{\text{slow}}$ was set to 5 pS/µm$^2$ each, similar to the values reported using nucleated patches, the simulated depolarization of the somatic membrane potential (Fig. 3A2) was substantially larger than the experimentally recorded depolarization.
(Fig. 3A1). Next we simulated the same experiment again only with a homogenous density of 170 pS/µm² that is similar to the density we obtained for the soma using TEVC recordings and the space-clamp correction algorithm. These simulations produced membrane potential depolarization (Fig. 3A3) of similar magnitude to those recorded experimentally (Fig. 3A1). In the second stage of the experiment presented in Fig. 3 a whole-cell pipette was attached to the apical dendrite 440 µm away from the soma (Fig. 3B). Injecting identical current pulses as those applied at the soma to the dendrite produced much larger changes to the membrane potential (Fig. 3B1) than those recorded at the soma (Fig. 3A1). As with the somatic experiment, the dendritic experiment was simulated using two values of the conductance density of \( K_{\text{fast}} \) and \( K_{\text{slow}} \), 5 pS/µm² (Fig. 3B2) and 170 pS/µm² (Fig. 3B3) respectively. Contrary to the simulations of the somatic membrane potential, the simulated changes of the dendritic membrane potential resembled the experimentally recorded traces when a conductance density of 5 pS/µm² was used more than when a conductance density of 170 pS/µm² was inserted into the model.

The experiment and simulations displayed in Fig 3 were performed on several neurones and the analysis of the results obtained is displayed in Fig. 4. Figure 4A plots the average current-voltage relationship recorded from 7 neurones in comparison with the average current-voltage relationships simulated in 4 reconstructed morphologies using conductance densities of 10 pS/µm² or 170 pS/µm² for \( K_{\text{fast}} \) and \( K_{\text{slow}} \). The simulated current-voltage relationship obtained using 170 pS/µm² was in close agreement with the experimentally
recorded relationship whereas that simulated with a conductance density of 10 pS/µm² deviated considerably from the experimental one (Fig. 4A). The average simulated depolarization obtained following 3.95 nA current injection via the somatic electrode is plotted in Fig. 4B as a function of several conductance densities. The average experimental depolarization obtained following injection of the same amount of current was also plotted as a horizontal line on the same graph and the conductance density value calculated from the voltage-clamp experiments with the space-clamp correction algorithm values a vertical line with their respective error ranges indicated as gray bands. The intercept of these two experimental results was in good agreement with the simulated values (Fig. 4B). Finally, the current-voltage relationship was measured for two dendritic experiments at 440 µm (Fig. 4C) and 154 µm (Fig. 4D) along the apical dendrite of two different L5B pyramidal neurones. The simulated current-voltage relationships, assuming different conductance density values were shown as dashed lines. In the experiment carried out with the whole-cell electrode connected at 440 µm along the apical dendrite the simulated curve that overlapped the experimental curve was calculated using a 3 pS/µm² homogenous conductance density of both K\text{slow} and K\text{fast} (Fig. 4C). In the experiment carried out with the whole-cell electrode connected at 154 µm distance along the apical dendrite the simulated curve that overlapped the experimental curve was calculated using a 15 pS/µm² homogenous conductance density of both K\text{slow} and K\text{fast} (Fig. 4D). Taken together, the somatic and dendritic recordings and simulations described in Figs 3 and 4 were consistent with the
results obtained using voltage-clamp experiments and the space-clamp correction algorithm (Figs 1 and 2).

**Impact of passive parameter measurements**

Measurement of $K^+$ conductance densities using TEVC recordings and morphological reconstruction requires estimation of the passive membrane parameters for each experiment (Schaefer et al., 2003a). Could possible errors in the estimation of these parameters account for the difference in the densities measured by the TEVC technique compared to the densities estimated from nucleated and cell-attached membrane patch measurements? As detailed in the methods, for each neurone the passive membrane parameters in the vicinity of the recording site were estimated by injecting a current pulse prior to the voltage-clamp experiment. The reconstructed soma and dendrite geometry was used in combination with this voltage recording to calculate the passive membrane parameters (Fig. 5A1). The passive membrane parameters obtained from 14 somatic recordings are displayed in Fig. 5A2. The scatter in the parameters decreased as a function of postnatal age. This is clearest for the membrane capacitance ($C_m$) and resistance ($R_m$, Fig. 5A2). While the scatter in the values of the axial resistance ($R_i$) also decreased as a function of postnatal age it still displayed variation even at P42 and is generally least constrained by local current injection protocols (Major et al., 1994; Stuart & Spruston, 1998). We then applied Monte Carlo simulations to investigate whether the scatter in $R_i$ could cause large errors in the estimation of the conductance density. For each of the 14 somatic recordings $R_i$ was randomly changed within the range given by the
age group distribution (Fig. 5B1), the two additional passive membrane parameters ($R_m$ and $C_m$) were then re-estimated by fitting the original voltage traces (Fig. 5B2) and the new set of passive membrane parameters (Fig. 5B3) was used in the calculation of the conductance densities (Fig. 5B4). This procedure was repeated 10 times and the error caused by the scatter of the passive membrane parameters was estimated from the standard deviation of the conductance densities (Fig. 5C1). The measured variability in the values of $R_i$ (Fig. 5A2) could not account for more than a 10% error in the measurement of the conductance density (Fig. 5C2, Supp. Fig. 1, and Supp. Table 1). It has to be emphasized that this procedure is likely to strongly overestimate the influence of erroneous estimates of $R_i$ as it assumes that all variability between recordings is due to measurement errors rather than reflecting true cell-to-cell heterogeneity.

As detailed in our previous paper (Schaefer et al., 2003a) the spatial resolution of the space-clamp correction algorithm is on the order of a few tens of micrometers. Thus, even if the passive parameters are different distal to the recording electrode it is predicted to have little impact on the correction of the conductance density. To further explore this point, we also repeated the determination of passive parameters assuming a dendritic gradient of $R_m$ (defined by 4 parameters: $C_m$, $R_i$ and two parameters specifying a linear somato-dendritic gradient in $R_m$). Using these inhomogeneous passive parameters as a basis for the analysis of conductance densities, however, resulted in only a small deviation of 1.6% in the conductance density estimate.
To further assess the contribution of inhomogeneous passive parameters we analyzed the passive data in a way similar to that presented in Fig. 5: We introduced a somato-dendritic gradient of $R_i$ (somatic value=0.5 times the original, homogeneous, $R_i$ value linearly increasing with distance to twice its original value at 1000 µm), refitted the passive traces and re-analyzed the conductance measurements. This was repeated for a 2-0.5 fold gradient of $R_i$, a 0.5-2 as well as a 2-0.5 fold gradient for $R_m$ and $C_m$. Although in virtually all cases the resulting passive model was clearly a poor description of the cell, conductance measurements were robust with respect to these distortions displaying on average only 14% deviation (Supp. Fig. 1). In summary and together with the results of Fig. 5 and of our previous paper (Schaefer et al., 2003a), we conclude that the passive model only marginally influences the conductance density estimation by the space clamp correction algorithm. This is likely due to two reasons. Firstly, the fitting procedure to obtain the passive model ensures that critical parameters such as input resistance and time constants are comparatively accurately described by the passive parameters. Secondly, as soon as voltage-gated conductances are activated, the membrane properties (space constant and time constant) are dominated by the voltage-gated conductances, marginalizing the influence of misestimating in $R_i$, $R_m$ and $C_m$.

**Impact of K⁺ conductances in the axon and basal dendrites on somatic K⁺ conductance density measurements**

It has been suggested that the density of voltage-gated K⁺ channels in the axon hillock is several fold higher than at the soma (Mainen et al., 1995). Such a K⁺ conductance "hot-spot" may be activated by the somatic voltage-clamp...
command and contribute current to the somatic recording. Although this is unlikely to affect distal dendritic recordings it might cause an overestimation of the somatic K$^+$ conductance density. To assess the error in the conductance density measurement we simulated experiments with an axonal hot spot of voltage-gated K$^+$ conductances. A high conductance density ($10^4$ pS/µm²) of a delayed rectifier K$^+$ conductance model ($K_{\text{slow}}$, as in Figs. 3, 4 and Supp. Fig. 2) was placed in the axonal initial segment 10 µm away from the soma, while the density of K$^+$ conductances was homogeneous and low in the remainder of the cell (Fig. 6A), and a voltage-clamp experiment was simulated. Following a voltage-clamp step from -110 to 0 mV the recorded current was larger than the current recorded when all the neurone was equipped with a low and homogenous conductance density (Fig. 6A). When the hot spot was moved to a position 20 µm away from the soma only a small fraction of the current recorded at the soma was due to activation of K$^+$ conductances in the hot spot (Fig. 6A2 and - for 40 µm – Fig. 6A3). The fraction of the somatic current due to activation of conductances in the hot spot was dependent on the distance of the hot spot (Fig. 6B1) and the density of the K$^+$ conductance at the site of the hot spot (Fig. 6B2) but only weakly on its spatial extent (Fig. 6B1). Currently, there are no direct recordings of the conductance density of voltage-gate K$^+$ from the initial segment of the axon of L5B neurones. However, it has been reported that the density of the voltage-gated Na$^+$ conductance in the initial 30 µm of the axon in L5B pyramidal neurones was similar to that recorded at the soma (Colbert & Pan, 2002). Assuming that the density of the voltage-gated Na$^+$ conductances is
counterbalanced by similar densities of voltage-gated K⁺ conductances it is possible to hypothesize that the initial segment of the axon of L5B neurones does not contain high densities of voltage-gated K⁺ conductances. To verify this hypothesis we made cell-attached recordings from the axon’s initial segment of L5B pyramidal neurones. The mean maximal voltage-gated K⁺ current recorded following depolarization of the patches from -20 mV relative to the resting membrane potential to +140 mV relative to the resting membrane potential was 7.4 ± 3.8 pA (n=10) for somatic patches and 3.8 ± 1.8 pA (n=9) for patches made at distances of 6-21 µm along the initial segment (data not shown). These results support the hypothesis that in the first 20 µm of the axon initial segment the voltage-gated K⁺ conductance density is not higher than the corresponding somatic density.

To obtain an upper limit of the error which a hot spot of K⁺ conductance more distal than 20 µm along the axon would produce on the K⁺ conductance density measurements, we re-corrected the 14 somatic experiments using the space-clamp correction algorithm assuming a drastically increased K⁺ conductance density of 10⁴ pS/µm² stretching from 20 µm from the soma along the entire axon. This assumption indeed led to a smaller estimate of the somatic conductance density (Fig. 6C). However, the error introduced by an axonal hot spot was on average 7 % (P28, P42) or 14% (P14) and thus did not increase significantly the estimate of the somatic conductance density (Fig. 6C). As predicted by cable theory, dendritic measurements were essentially unaffected (Supp. Fig. 1 and Supp. Table 1). Errors introduced by incomplete knowledge of
passive membrane parameters and by potential axonal $K^+$ conductance hotspots are summarized in Supplementary Figure 1 and Supplementary Table 1.

Another possible source of error for the somatic $K^+$ conductance density measurements are $K^+$ conductance densities in the basal dendrites that deviate substantially from the somatic ones. It has been recently reported (Antic, 2003), based on voltage-sensitive dye recordings from basal dendrites of L5 neurones, that the conductance density in these dendrites should be ~200 pS/$\mu$m$^2$. This complies with our estimates of the somatic conductance density and should not contribute additional current to the soma. Nevertheless we repeated our conductance density analysis assuming basal dendritic channel densities of between 0.3 and 3-fold of the somatic value. As expected, only small deviations in the conductance measurements were observed for the dendritic measurements (-3.6 to 3.1 %); however, somatic deviations were larger (-20.1 to 14.3 %). This error analysis was incorporated in the summary Supplementary Figure 1.
**Discussion**

Using a newly developed numerical technique (Schaefer *et al.*, 2003a), we corrected space-clamp distorted voltage-gated K⁺ currents recorded using TEVC from the soma and apical dendrite of L5B pyramidal neurones. We show that in 6-week old rats the decrease in the density of voltage-gated K⁺ conductances along the apical dendrite is considerably steeper than the gradient we previously reported on using cell-attached recordings from 2-week old rats (Korngreen & Sakmann, 2000). Furthermore, we show that the somatic conductance density increased approximately 2-fold from P14 to P42 animals. In addition we used an alternative approach, extracting an estimate of the conductance density from membrane rectification to corroborate the results obtained using TEVC and the space-clamp correction algorithm. Finally, the somatic density we measured was substantially larger than estimates previously obtained from nucleated patches (Bekkers, 2000b; Korngreen & Sakmann, 2000) and similar to the values postulated in several simulation studies (Mainen *et al.*, 1995; Rhodes & Llinás, 2001; Antic, 2003; Schaefer *et al.*, 2003b). This discrepancy is likely not due to variability in passive parameters or axonal hot-spots of voltage-gated K⁺ conductance.

**Estimating voltage-gated K⁺ conductance density in non-spherical cells.**

First consider the simplest case of a cylindrical neuron with a delayed rectifier-like voltage-gated K⁺ conductance and assume that the leak reversal potential is -60 mV and that the K⁺ reversal potential is -90 mV. At rest the voltage-gated K⁺ conductance is low. Therefore, the leak conductance will draw
the resting membrane potential towards the leak reversal potential (-60 mV). Depolarizing the membrane potential of the cylinder with a small voltage-clamp command will cause a local depolarization that decays along the cylinder according to passive cable theory. Now deflect the membrane potential using a larger voltage-command that will activate the voltage-gated $K^+$ conductance. Once activated the $K^+$ current will draw the membrane potential on both sides of the voltage-clamp electrode (which are not clamped by the voltage-clamp circuit as the non-spherical morphology of the cylindrical neuron renders it non-isopotential) towards the $K^+$ reversal potential (-90 mV in this case). In other words, voltage-gated $K^+$ channel activation increases membrane conductance which effectively decreases the passive space constant. Thus, distal to the recording pipette the membrane potential will be lower than the threshold for $K^+$ channel activation. Consequently, the spatial activation of the $K^+$ conductance is limited to a relatively small region around the voltage-clamp electrode as shown graphically in Fig. 1 of a review by Häusser (Häusser, 2003). This local activation allows to use a relatively simple, stepwise linear approximation for the construction of the kinetics of the $K^+$ conductance (Schaefer et al., 2003a). More importantly, since the relevant membrane area is close to the voltage-clamp electrode the detailed morphology and passive properties of the dendritic tree distal to the voltage-clamp electrode are not important for the accuracy of the correction.

Consider the same cylindrical neuron with a current-clamp electrode inserted at its center instead of the voltage-clamp electrode. The only difference
is that the voltage-clamp circuit uses the recorded potential as a feedback signal to increase the injected current until the recorded membrane potential matches the command potential. Assuming, in addition, that the kinetics of the K⁺ conductance are known the membrane potential recorded in the current-clamp experiment can be used to calculate the conductance density of the K⁺ conductance around the recording electrode. The advantages of this approach are that the current-clamp experiment is considerably simpler than the voltage-clamp experiment and that the analysis does not require the use of time consuming space-clamp correction algorithm.

Since we have previously characterized the kinetics of the voltage-gated K⁺ conductances in L5B pyramidal neurones (Korngreen & Sakmann 2000), we were able to analyze current-clamp experiments of these neurones. We did not perform actual curve fitting of the data but rather plotted the results in comparison to several possible conductance density values. The results illustrated in Figs 3 & 4 were similar to the results we obtained using the space-clamp correction algorithm predicting a higher density of K⁺ conductance at the soma and lower density along the apical dendrite. This corroborates the numerical validity of the space-clamp correction algorithm and the adequate use of the TEVC recording mode.

**Relation to previous work.**

It has been reported that in order to perform realistic simulations, the effective somatic conductance density of voltage-gated K⁺ channels had to be several fold higher than that measured from nucleated patches (Mainen et al.,
Rhodes & Llinás, 2001; Antic, 2003; Schaefer et al., 2003b). Therefore, it is possible that while recording from nucleated outside-out patches provides an adequate measurement of channel kinetics it underestimates the value of conductance density. This could be due to altered function of channels in excised membranes (Fenwick et al., 1982) or due to an overestimation of the apparent size of the patch that might be a consequence of applied suction. Furthermore, channels recorded from outside-out patches are sensitive to the selection of the pipette solution. Distinct differences were observed between voltage-gated K$^+$ currents recorded in nucleated patches from L5B neurones that were made with a gluconate based pipette solution (Korngreen & Sakmann, 2000) to those recorded when the pipette solution was based on methyl-sulphonate (Bekkers, 2000b). Thus, it is possible that “rundown” of the K$^+$ current in nucleated patches may be responsible for the large difference between somatic conductance densities obtained by nucleated patches and by TEVC recording (Fig. 2).

The combination of whole-cell voltage-clamp recordings from somata and dendrites in brain slices with a measurement of morphology and passive parameters merges the advantages of the whole-cell recording technique with the visual identification of neuronal sub-types. It allows not only estimation of channel kinetics but in particular a quantitative measurement of the conductance density (Schaefer et al., 2003a).

In this work we investigated two major sources of possible error to the conductance density estimate provided by the space-clamp correction method. First, Monte-Carlo simulations were used to investigate whether the inaccuracy in
the estimation of \( R_i \) may cause over estimation of the conductance density (Fig. 5). This procedure revealed that the observed scatter in the values of \( R_i \) (Fig. 5A2) could not account for more than a 10% error in the measurement of the conductance density. Second, simulating a hypothetical increase in the voltage-gated \( K^+ \) conductance density in the axon revealed that the error introduced by an axonal hot spot was on average 7% (P28, P42) or 14% (P14) and as such did not significantly increase the estimate of the somatic conductance density (Fig. 6C). Hence, error sources such as incomplete knowledge about passive membrane parameters or current contributions from unknown sources did not bring about a significant uncertainty in the conductance density measurements. Statistical errors, on the other hand, are very small compared to cell-attached recordings as the whole-cell currents measured are large and consist of contributions from many channels distributed in the membrane around the recording pipette. Application of specific channel antagonists could aid the space-clamp correction method. In this study, our approach was to investigate the compound \( K^+ \) conductance density, and in particular focus on the slowly inactivating component as the transient phase is less readily reconstructed and so obtain a map of the somato-dendritic \( K^+ \) conductance distribution.

**Possible functional significance.**

We have not attempted to present a complete mechanism for the electrophysiology of the apical dendrite of L5 neurones. This would require at the very least a detailed characterization of dendritic \( Ca^{2+} \) and \( Na^+ \) channels in adult animals combined with a construction of a detailed compartmental model.
However, it is tempting to speculate what might be the function of the K$^+$ conductance gradient in the physiology of L5B neurones. The steep decrease of the K$^+$ conductance density along the apical dendrite of L5B pyramidal neurones of the neocortex differentiates them from other neuronal cell type studied so far. In Purkinje neurones of the cerebellum the density of the sustained voltage-gated K$^+$ conductance was observed to gradually decrease as a function of distance from the soma (Martina et al., 2003). In these neurones, contrary to L5B neurones, dendritic Ca$^{2+}$ potentials do not propagate to the soma (Llináš & Sugimori, 1979, 1980) and axonally generated APs do not back-propagate into the dendritic tree (Stuart & Häusser, 1994) – in part due to the high degree of branching and low Na$^+$ channel densities (Vetter et al., 2001). It has, however, been observed that in proximal dendrites, voltage-gated K$^+$ conductances are activated by passive membrane responses and help to further attenuate the amplitude of the back-propagating AP (Martina et al., 2003). In more distal dendrites, voltage-gated K$^+$ conductances have been observed to inactivate only at rather depolarized levels (Martina et al., 2003). Thus, due to their large conductance, effectively shaping dendritic Ca$^{2+}$ spikes without completely abolishing them.

In CA1 pyramidal neurones of the hippocampus the density of an A-type voltage-gated K$^+$ conductance along the apical dendrite has been observed to increase steeply with the distance from the soma (Hoffman et al., 1997). This K$^+$ conductance gradient has been proposed to act as a “dendritic shock absorber” in modulating the amplitude of the back-propagating AP (Hoffman et al., 1997).
As a result, the dendritic tree in CA1 pyramidal neurones receives highly variable feedback information from the soma based on the level of inactivation of the A-type voltage-gated K$^+$ conductance that controls the amplitude of the back-propagating AP (Hoffman et al., 1997).

It is clear that the kinetic properties and distribution of the voltage-gated K$^+$ conductances in L5B are different from those observed for dendritic voltage-gated K$^+$ conductances in Purkinje and CA1 pyramidal neurones and may reflect differences in their function. In L5B pyramidal neurones the AP readily back-propagates into the dendritic tree (Amitai et al., 1993; Kim & Connors, 1993; Reuveni et al., 1993; Schiller et al., 1997; Larkum et al., 1999b; Zhu, 2000; Larkum et al., 2001). In these neurones, when a back-propagating AP coincides with distal synaptic input, a regenerative dendritic Ca$^{2+}$ potential is generated, triggering a burst of several APs at the soma (Larkum et al., 1999b). This coincidence mechanism has been shown to be regulated by membrane potential changes in the proximal apical dendrite (Larkum et al., 2001). Thus, similarly to Purkinje neurones the steep ~13-fold decrease of the voltage-gated K$^+$ conductance density from the soma along the apical dendrite of L5B pyramidal neurones may be responsible for segmenting the apical dendrite into several functional regions including a distal low threshold region for the initiation of dendritic regenerative Ca$^{2+}$ potentials and a proximal region that may modulate the propagation of dendritic potentials to the soma. Nevertheless, it is important to note that, due to the restrictions in the temporal resolution of the TEVC we could not resolve the gradient of the A-type voltage-gated K$^+$ conductance along
the apical dendrite. It may well be that the gradient of the A-type K⁺ conductance differs from that of the delayed K⁺ conductance and may confer additional function to the apical dendrite of L5B neurones. Keeping this constraint in mind, it may be that the dendritic "shock absorbers" are loosened in the distal apical dendrite of L5B pyramidal neurones. This would allow the synaptic input to the apical dendrite to generate dendritic regenerative Ca²⁺ potentials, to boost distal synaptic input, and readily modulate the neurones AP output.
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Figure legends

Figure 1: Somato-dendritic distribution of voltage-gated $K^+$ conductance densities in L5B pyramidal neurones.

A, reconstruction of a L5B pyramidal neurone from a P42 rat used for the recording of dendritic currents 340-380 $\mu$m distal to the soma. Schematic illustration of the dendritic TEVC recording configuration has been added and the command potential has been indicated ($V_c$).

B, TEVC recordings of voltage-gated $K^+$ currents from the soma (left traces) and apical dendrites of three L5B pyramidal neurones as indicated. The voltage-clamp command potential is shown below the current records. The $K^+$ conductance density determined using reconstructed morphology and passive membrane parameters is displayed below the voltage traces.

C, peak $K^+$ conductance density calculated by fitting a one gate Boltzmann function to the measured $K^+$ conductance at the 50 ms time point. The vertical error bars display the error estimated by the curve fit routine. The horizontal error bars indicate the positions of the voltage recording and current injection electrodes of the TEVC circuit (Methods). The continuous line is a shifted exponential fit $G=8+164*\exp(-x/80)$ where $G$ is the $K^+$ conductance density in pS/$\mu$m$^2$ and $x$ is the distance from the soma along the apical dendrite in micrometers.

D, the voltage of half activation ($V_{1/2}$) and inverse slope ($k$) of the activation curve, obtained by fitting a one gate Boltzmann function to the conductance 50
ms after the onset of depolarization, are independent of the distance from the soma. Vertical error bars in C and D reflect the error of the Boltzmann fit.
Figure 2: Somatic $K^+$ conductance densities increase throughout development.

**A1**, reconstructions of neurones, from left to right, were from P14, P28 and P42 animals. Below, voltage-clamp recordings of voltage-gated $K^+$ currents from the soma of the three L5B pyramidal neurones are shown above the voltage traces. Note distortions to the onset of the clamp potential because of incomplete voltage control. The voltage-clamp potential and the conductance density for each record are shown below the current recordings.

**A2**, recordings of voltage-gated $K^+$ currents recorded using the cell-attached configuration from the soma of three different neurones (from P14, P28 and P42 animals). Responses were evoked by a voltage step to +140 mV relative to the resting membrane potential.

**B**, summary of the developmental changes in the average maximal current from cell-attached recordings (gray bars, P14 n=40, P28 n=9, P42 n=9) and the average maximal conductance density measured by the TEVC technique (black bars, P14 n=6, P28 n=4, P42 n=4). Error bars are SEM.

**C**, summary of the developmental changes to the voltage of half activation ($V_{1/2}$) and inverse slope (k) of the activation curve, obtained by fitting a one gate Boltzmann function to the conductance 50 ms after the onset of depolarization. Error bars are SEM between cells within each age group.
Figure 3: Recorded and simulated membrane rectification due to the activation of voltage-gated $K^+$ conductances.

A1, membrane potential traces recorded in the current-clamp mode following injection of a series of increasing current steps (0.05 to 3.95 nA in steps of 0.15 nA) via a whole-cell electrode at the soma of a L5B neocortical pyramidal neurone from a P42 animal. The neurone was filled with biocytin, stained, reconstructed using a Neurolucida system, and converted to a computer code readable by the simulation environment NEURON.

A2, membrane potential traces simulated using the same morphology of the neurone from which the experimental traces displayed in A1 were recorded. In this simulation a conductance density of 5 pS/$\mu$m$^2$ for $K_{fast}$ and 5 pS/$\mu$m$^2$ for $K_{slow}$ was homogenously inserted into the somato-dendritic tree. The passive parameters were set to $R_i=150$ $\Omega$cm, $R_m=15000$ $\Omega$cm$^2$ and $C_m=0.75$ $\mu$F/cm$^2$ for all the simulations displayed in this figure.

A3, membrane potential traces simulated using the same morphology of the neurone from which the experimental traces displayed in A1 were recorded. In this simulation a conductance density of 170 pS/$\mu$m$^2$ for $K_{fast}$ and 170 pS/$\mu$m$^2$ for $K_{slow}$ was homogenously inserted into the somato-dendritic tree.

B1, membrane potential traces recorded in the current-clamp mode following injection of a series of increasing current steps (0.05 to 3.95 nA in steps of 0.15 nA) via a whole-cell electrode at the apical dendrite of a L5B neocortical pyramidal neurone 440 $\mu$m away from the soma. The neurone was filled with
biocytin, stained, reconstructed using a Neurolucida system, and converted to a computer code readable by the simulation environment NEURON.

**B2**, membrane potential traces simulated using the same morphology of the neurone from which the experimental traces displayed in B1 were recorded. In this simulation a conductance density of 5 pS/µm² for $K_{fast}$ and 5 pS/µm² for $K_{slow}$ was homogenously inserted into the somato-dendritic tree.

**B3**, membrane potential traces simulated using the same morphology of the neurone from which the experimental traces displayed in B1 were recorded. In this simulation a conductance density of 170 pS/µm² for $K_{fast}$ and 170 pS/µm² for $K_{slow}$ was homogenously inserted into the somato-dendritic tree.
Figure 4: Analysis of experimental and simulated current clamp experiments.

A, current-voltage relationship of the somatic experiments displayed in Fig. 3A. The average deflection of the membrane potential measured 50 ms following the onset of the depolarization is displayed as a function of the injected current (●, n=7). The same IVs were also calculated for simulations using the morphologies of the same cells with a homogenous conductance density of $K_{\text{slow}}$ and $K_{\text{fast}}$ set to 10 pS/µm² (○, n=4) and 170 pS/µm² (□, n=4). Error bars are SEM. The passive parameters were set to $R_i=150$ Ωcm, $R_m=15000$ Ωcm² and $C_m=0.75$ µF/cm² for all the simulations displayed in this figure.

B, the average simulated deflection of the membrane potential 50 ms after the onset of the depolarization due to the injection of 3.95 nA into the models that contained homogenous distributions of $K_{\text{slow}}$ and $K_{\text{fast}}$ at several values (○, n=5). The conductance density we report in Figure 1 (172±42 pS/µm²) is displayed as a vertical line and the recorded membrane potential deflection after 3.95 nA current injection from A as a horizontal line. The SEM for both of these values are displayed as gray bands.

C, an IV calculated from the dendritic recording displayed in figure 3B1 from 440 µm along the dendrite (●). The continuous lines are simulated IVs of 1 pS/µm² (dashed) 3 pS/µm² (solid) and 100 pS/µm² (dotted) lines.

D, an IV calculated from another dendritic recording from 150 microns along the dendrite of another neurone (●). The continuous lines are simulated IVs (using
the same morphology) of 1 pS/µm² (dashed) 15 pS/µm² (solid) and 100 pS/µm² (dotted) lines.
Figure 5: The effect of erroneous estimates of passive membrane parameters on the calculation of the K⁺ conductance density.

A, illustration of the procedure used to estimate the passive membrane parameters in each somatic and dendritic experiment. A brief current (0.6 nA, 1 ms) was injected via the ME2 electrode of the AxoClamp-2B in bridge mode. The voltage deflection was filtered at 10 kHz and measured with a 50 kHz sampling rate with both ME1 and ME2 (A1). To reduce experimental noise 30 sweeps were averaged. The current pulse was always injected via ME2 since this electrode has larger intrinsic voltage recording noise in the AxoClamp-2B amplifier. Following reconstruction of the cells using Neurolucida the passive membrane parameters were estimated by custom written routines in NEURON. The result of this fit is shown as red lines. A2, The passive membrane parameters (R_m, R_i, and C_m) are displayed versus the experiment identifier of each somatic experiment that was analyzed (six experiments from P14 rats, and 4 experiments from P28 and P42 rats each). The solid line in each group indicates the mean for the age group and the dashed lines indicate ±SD from this mean.

B, Monte Carlo simulation used to investigate the sensitivity of the fit to R_i measurement. For each of 10 runs of the passive membrane parameters’ estimation fit (shown in A1) the value of R_i was fixed to a random number drawn from a normal distribution based on the originally determined R_i as a mean and the age population standard deviation displayed in A2 as SD (B1). The fit was repeated 10 times with the 10 fixed R_i values from B1 (one example shown in B2,
data - black lines, fit – red lines, scale bar 10 ms, 1 mV) and the resulting new values of $R_m$ and $C_m$ (B3) were used to calculate $K^+$ conductance densities in the same neurone (one example shown in B4, scale bar 20 ms, 40 pS/µm²).

C, $K^+$ conductance density calculated using the originally measured passive membrane parameters (A) and using those obtained from the Monte Carlo simulation (B). The results of 10 repetitions of the conductance measurement for the different passive membrane parameters were averaged and the standard deviation was calculated for each data point. The mean value of each data point obtained by this Monte Carlo simulation (C1 black lines) did not differ considerably from the conductance density calculation using the original set of passive membrane parameters (C2 gray lines). The standard deviation determined by the Monte Carlo simulation is shown only for some of the points to avoid visual cluttering. Each mean conductance density (the result of 10 repetitions) was analyzed by fitting a Boltzmann function to the 50 ms time point. The results of this curve fitting are shown in C2 for each of the 10 repetitions. The value obtained from the original parameters is indicated by a thick line. The mean from the Monte Carlo simulation is shown as a thin line; dashed lines indicate ±SD.
Figure 6: Axonal conductance hot spot does not result in substantial overestimation of the K⁺ conductance.

A, in this simulation a high density (10⁴ pS/µm²) of the slow delayed rectifier K⁺ conductance \( K_{\text{slow}} \) was inserted, at 10 µm (A1), 20 µm (A2) and at 40 µm (A3) away from the soma for 20 µm along the axon (shown in red in the picture of the reconstructed cell). The soma was subjected to a voltage step from -110 to 0 mV. The current traces simulated without an axonal hot spot (black lines) and with axonal hot spot (red lines) are shown below each neurone after leak subtraction (scale bars are 20 ms, 20 nA and 50 mV).

B, the dependence of the deviation (expressed in percentage and displayed in color coding) of the current simulated at the soma in the presence of an axonal hot spot from the current simulated at the soma when no hot spot was incorporated into the axon. B1, the dependence of the deviation on the size and distance of the hot spot from the soma. B2, the dependence of the deviation on the magnitude and distance of the hot spot from the soma. The X marks the values used for the simulations shown in C.

C, the deviation in the maximal conductance density for each of the somatic simulations when a hot spot of 10⁴ pS/µm² was positioned starting at 20 µm along the entire axon of each neurone. The conductance values in the absence of a hot spot are shown in blue circles while those in the presence of an axonal hot spot are marked in black triangles. Simulations in A and B are from neurone B1110.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Dendritic voltage-gated K+ conductance gradient in pyramidal neurones of neocortical layer 5B
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