Altered dendritic complexity affects firing properties of cortical layer 2/3 pyramidal neurons in mice lacking the 5-HT$_{3A}$ receptor

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van der Velden L, van Hooft JA, Chameau P. Altered dendritic complexity affects firing properties of cortical layer 2/3 pyramidal neurons in mice lacking the 5-HT$_{3A}$ receptor. J Neurophysiol 108: 1521–1528, 2012. First published June 13, 2012; doi:10.1152/jn.00829.2011.—We have previously shown that the serotonergic input on Cajal-Retzius cells, mediated by 5-HT$_3$ receptors, plays an important role in the early postnatal maturation of the apical dendritic trees of layer 2/3 pyramidal neurons. We reported that knockout mice lacking the 5-HT$_{3A}$ receptor showed exuberant apical dendrites of these cortical pyramidal neurons. Because model studies have shown the role of dendritic morphology on neuronal firing pattern, we used the 5-HT$_{3A}$ knockout mouse to explore the impact of dendritic hypercomplexity on the electrophysiological properties of this specific class of neurons. Our experimental results show that hypercomplexity of the apical dendritic tuft of layer 2/3 pyramidal neurons affects neuronal excitability by reducing the amount of spike frequency adaptation. This difference in firing pattern, related to a higher dendritic complexity, was accompanied by an altered development of the afterhyperpolarization slope with successive action potentials. Our abstract and realistic neuronal models, which allowed manipulation of the dendritic complexity, showed similar effects on neuronal excitability and confirmed the impact of apical dendritic complexity. Alterations of dendritic complexity, as observed in several pathological conditions such as neurodegenerative diseases or neurodevelopmental disorders, may thus not only affect the input to layer 2/3 pyramidal neurons but also shape their firing pattern and consequently alter the information processing in the cortex.

cortex; dendrites; morphology; excitability

cortical layer 2/3 pyramidal neurons have apical dendrites extending toward the pia that branch and develop into a tuft within cortical layer 1. These apical dendritic tufts represent an important part of the neuronal surface area, because they receive the majority of synaptic input arising from other cortical areas (Bannister 2005; Spruston 2008). The extent of branching of dendrites is not a static feature of neurons. In fact, we have previously shown that serotonin, via action on 5-HT$_3$ receptors located on Cajal-Retzius cells in layer 1 of the cortex, regulates the dendritic complexity of cortical layer 2/3 pyramidal neurons during early postnatal development (Chameau et al. 2009). We found that the dendritic complexity of cortical layer 2/3 pyramidal neurons in mice lacking the obligatory 5-HT$_{3A}$ subunit of the 5-HT$_3$ receptor is increased compared with wild-type mice (Chameau et al. 2009). This prompted us to investigate what consequences such a difference in dendritic morphology has on the functional properties of layer 2/3 pyramidal neurons.

Besides their role in receiving and integrating synaptic input, there is evidence that dendrites also influence the neuronal firing pattern. Several experimental studies have reported a correlation between the apical dendritic morphology and the action potential firing pattern, allowing for the classification of pyramidal neurons on the basis of either their dendritic morphology or their action potential firing pattern (Bannister 2005; McCormick et al. 1985). For instance, work focusing on layer 5 pyramidal neurons showed two subclasses of pyramidal neurons, regular spiking neurons with thin and slender apical dendrites and bursting neurons with thick and tufted apical dendrites (Kasper et al. 1994; Mason and Larkman 1990). These differences in firing pattern between classes of neurons could depend on the types and distributions of the ion channels over the neuronal membrane (Migliore and Shepherd 2005), but there is growing evidence that the dendritic morphology itself influences neuronal firing. Using a two-compartment model of CA3 pyramidal neurons, Pinsky and Rinzel (1994) showed a correlation between burst occurrence and the degree of electrical coupling between soma and dendrites. Other modeling studies that used reconstructed neurons from various cortical layers, given identical ion channel distributions and densities, showed that the various firing patterns found experimentally (regular to burst firing) can solely be attributed to differences in dendritic morphology (Mainen and Sejnowski 1996; van Ooyen et al. 2002). In addition, van Ooyen et al. (2002) found that the topology (branching pattern) of abstract dendritic trees influences the firing frequency of a model neuron. Building on these results, van Elburg and van Ooyen (2010) showed that the burst firing regime depends on both the size and topology of the dendrite in a reconstructed layer 5 pyramidal neuron and simplified neuron model.

Because model studies have emphasized the role of dendritic morphology in determining the neuronal firing pattern, we investigate the consequences of changes in apical dendritic complexity of cortical layer 2/3 pyramidal neurons on their functional properties. Passive and active electrophysiological properties of neurons in wild-type mice were compared with those observed in 5-HT$_{3A}$ knockout mice, which have more complex dendritic trees. In addition, we used model neurons with schematized dendritic trees of various complexity with otherwise identical properties and reconstructed dendrites of wild-type and 5-HT$_{3A}$ knockout mice to establish the sensitivity of the relation between morphological and functional properties.

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**METHODS**

*Ethical approval.* Wild-type and 5-HT$_{3A}$ receptor knockout mice (Zeitz et al. 2002) between the age of postnatal day 14 and postnatal day 21, both males and females, were used for this study. In total, 26 animals were used. All experiments were approved by the committee on animal experiments of the University of Amsterdam.

*Slice preparation.* Mice were anesthetized with isoflurane and killed by decapitation. Coronal slices (300 μm thick) of the somatosensory cortex were cut with a vibroslicer (Leica VT1000S) in ice-cold slicing solution containing (in mM) 120 choline chloride, 3.5 KCl, 0.5 CaCl$_2$, 6 MgSO$_4$, 1.25 NaH$_2$PO$_4$, 25 NaHCO$_3$, and 25 glucose, continuously bubbled with 95% O$_2$-5% CO$_2$ (pH 7.4). Slices were incubated at 32°C for 1 h in artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 3.5 KCl, 2.5 CaCl$_2$, 1.3 MgSO$_4$, 1.25 NaH$_2$PO$_4$, 25 NaHCO$_3$, and 25 glucose, continuously bubbled with 95% O$_2$-5% CO$_2$ (pH 7.4).

*Electrophysiology.* During the recordings, slices were kept submerged at 30°C and were continuously superfused with ACSF. Patch pipettes were pulled from borosilicate glass and had a resistance between 2 and 3 MΩ when filled with internal solution containing (in mM) 130 K-gluconate, 10 KCl, 0.5 EGTA, 10 HEPES, 4 Mg-ATP, and 0.4 Na-GTP, plus 4 mg/ml biocytin (pH 7.3 with KOH). Whole cell current-clamp recordings from layer 2/3 pyramidal neurons were made using an EPC9 patch-clamp amplifier controlled by PULSE software (HEKA Electronic, Lambrecht, Germany) and custom software. Signals were filtered at 5–10 kHz and sampled at 20–50 kHz. Series resistance ranged from 5 to 10 MΩ and was not compensated for.

*Morphology.* During the recordings, neurons were filled with biocytin (4 mg/ml; Sigma Chemical, St. Louis, MO) for identification of the cell morphology. Slices were fixed overnight in 0.1 M phosphate-buffered saline (PBS; pH 7.4) containing 4% paraformaldehyde. After 30-min permeabilization in 0.3% Triton X-100-PBS, slices were incubated for 60 min in avidin-biotin-peroxidase complex (Vectastain ABC Elite kit; Vector Laboratories; Burlingame, CA). Biocytin was visualized as a dark brown substrate using 3,3’-diaminobenzidine-4 HCl reaction (Sigma Chemical). Biocytin-filled layer 2/3 neurons were scanned with a confocal microscope (Zeiss LSM 510) equipped with a dry Plan Neofluor ×200/0.75 objective. The morphological analysis was performed using ImageJ (National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/image/) in combination with the Neuron Morpho plugin for reconstruction and LMeasure software to define the parameters necessary to determine the dendritic complexity index (DCI). The DCI was calculated as previously described (Lom and Cohen-Cory 1999) according to DCI = [Σ(branch tip order + no. of branch tips) × total arbor length]/no. of primary dendrites.

The Sholl analysis was performed as described by Sholl (1953), with a distance of 10 μm between concentric rings. The numbers of neurons used in this study are 21 wild-type (WT) and 24 knockout (KO) neurons. Morphological parameter values are means ± SE and compared using the one-way ANOVA test. Effects are considered significant when *P < 0.05 and **P < 0.01.

*Analysis.* The analysis of the electrophysiological properties was performed using scripts written in MATLAB (The MathWorks, Natick, MA). The passive properties were estimated from voltage responses (20 consecutive sweeps were averaged) evoked by 400-ms-duration hyperpolarizing current injections (20 pA). The membrane time constant ($\tau_m$) and the input resistance ($R_{in}$) were determined by fitting the voltage response with a monoexponential function: $V(t) = I_{app} \times R_{in} \times e^{-t/\tau_m}$, where $I_{app}$ is the applied current. The capacitance of the neuron ($C_{m}$) was calculated according to $\tau_m = R_{in} \times C_{m}$, where $R_{in}$ estimates $R_{in}$. Single action potential (AP) properties [threshold, amplitude measured from threshold to the peak of the spike, duration at half amplitude (half-width)] were determined from voltage responses (50-kHz sampling frequency) to 200-ms-duration depolarizing current injections ranging from 0 to 400 pA in 10-pA increments. Repetitive AP firing properties were determined from voltage responses (20-kHz sampling) to long-duration (1 s) depolarizing current injections, and statistics were done in the range from 200 to 450 pA in 10-pA steps. AP firing properties were characterized by the rheobase (defined as the minimal current amplitude that initiated an AP), the mean spike frequency, and the instantaneous frequency (inverse of the interspike interval). Afterhyperpolarization (AHP) amplitude was measured from the AP threshold to the peak of the AHP, and AHP slope was estimated from the linear regression of the voltage signal between the peak of the AHP and the threshold of the following AP (see Fig. 3A). To characterize the dynamics of the spike frequency, the instantaneous spike frequency in response to current injections ranging from 200 to 450 pA were superimposed and fitted with a monoexponential function (see Fig. 2C). From this fit, the time constant of adaptation ($\tau$), the initial frequency ($a + c$), the steady-state frequency ($c$), and the depth of adaptation ($a/(a + c)$) were determined. Electrophysiological parameter values are means ± SE and were compared using the one-way ANOVA test. Effects are considered significant when *P < 0.05 and **P < 0.01.

*Computational modeling.* All simulations were performed using NEURON (Hines and Carnevale 1997) with the segmentation length kept under 30 μm for all models to prevent errors due to spatial discretization. The output was analyzed with the same MATLAB scripts used for analysis of whole cell recordings.

The binary trees used in this study are commonly used as abstract models of dendrites (van Elburg et al. 2010; van Ooyen et al. 2002). In contrast to van Ooyen et al. (2002), who studied topological effects by manipulating dendritic symmetry, our trees are kept symmetric. This allows us to manipulate the dendritic complexity by increasing the number of branch lengths largely independently of dendritic topology, which is defined by all connectivity patterns one can make with a fixed set of dendritic compartments.

Specific morphological properties of these binary trees were based on our reconstructed natural neurons. For instance, the mean path length from soma to dendritic tip was kept constant at 250 μm, under manipulation of the number of branch levels (1 to 6 in Fig. 4A) (“abstract model”), based on the mean distance from soma to pia measured from biocytin-filled neurons. To this end, a dendritic branch was replaced by one mother and two daughter branches with a 0.5:1 length ratio between the new and old branches. Under these conditions, each branch level also contributed equally to the total arbor length, and the DCI values of the dendritic geometries covered the range of the experimental data [geometries 1–6 (DCI): 1.500, 6,000, 20,000, 60,000, 168,000, 448,000]. The diameter of the primary dendrite was kept constant at 2.5 μm according to measurements performed on biocytin-filled neurons. For subsequent branch levels, the Rall diameter rule was applied to produce trees with identical passive properties. The surface-to-volume ratio at each branch level was calculated by adding the surface area of all compartments of that branch order and dividing it by the sum of their volumes. This means that the surface-to-volume ratio is increasing with each branch level. It also prevents more complex dendrites from becoming unmanageably leaky. A cylindrical soma (10-μm length and 15-μm diameter) was attached to these trees, and a small axon hillock (2-μm length and 2-μm diameter) was connected to the basal part of this soma.

To model more realistic neurons, apical dendrites from reconstructed WT and KO neurons were converted to the NEURON format using CA software. Besides sampling dendrites around the mean DICI of, respectively, the WT and KO populations, we also selected the dendrites on the basis of symmetry to avoid assigning unrealistically small dendritic diameters with the Rall diameter rule. The oblique branches were set to one-third the diameter of the primary dendrite, which was fixed at 2.5 μm. The same soma and axon hillock as in the abstract model were connected to the reconstructed dendrites.

For both “abstract” and “realistic” models, the maximal conductances (g) of the active currents were as follows. In the axon hillock,
DENDRITIC MORPHOLOGY AFFECTS FIRING IN 5-HT$_{3A}$ KNOCKOUT MICE

Fig. 1. Increased dendritic complexity of layer 2/3 pyramidal neurons in 5-HT$_{3A}$ receptor knockout (KO) mice correlates with cellular capacitance. A: typical examples of reconstructed apical dendrites from wild-type (WT; left) and 5-HT$_{3A}$ receptor KO (right) layer 2/3 pyramidal neurons. Apical KO dendrite shows exuberant branching. B: dendritic complexity index (DCI) was significantly higher in 5-HT$_{3A}$ receptor KO mice (n = 24) compared with WT mice (n = 21). This increased DCI originated from the dendritic tufts, not the proximal part of the dendrite. **P < 0.01. C: Sholl analysis of the dendritic tufts of WT (n = 21) and 5-HT$_{3A}$ receptor KO neurons (n = 24), expressed as the number (nr.) of intersections of the dendritic tree as a function of distance from the start of the tuft. The amount of branching in the tuft of the 5-HT$_{3A}$ receptor KO neurons was higher. D: cellular capacitance increased with the complexity of the dendrite. Plot shows the correlation between cellular capacitance and DCI.

The model neurons were activated by long somatic depolarizing stimulations. AP firing properties, recorded from the soma, were analyzed according to the same definitions used for the experimental data, and the effect sizes were calculated between the most simple and most complex dendritic geometries (geometries 1 and 6 in Fig. 4A).

RESULTS

Passive and active electrophysiological properties. The dendritic complexity of all the recorded and filled neurons was estimated using the DCI (see METHODS). DCI values of the whole apical dendrite (total tree, Fig. 1B) for KO neurons were almost two times higher than those of WT neurons (KO: 205.385 ± 21,454, WT: 116.141 ± 12.642, P < 0.01). The effect was consistent for all the constituent parameters of the DCI (Table 1). These observations confirmed the previously reported dendritic hypercomplexity of layer 2/3 pyramidal neurons from 5-HT$_{3A}$ KO mice (Chameau et al. 2009). The comparison of DCI values calculated for the proximal apical dendrites (minus tuft, Fig. 1B) suggest that the main differences between WT and KO neurons originate from the distal apical tuft. To refine our findings, we performed a Sholl analysis on the dendritic tufts, which confirmed that differences in dendritic complexity between WT and KO neurons reside in the apical dendritic tuft (Fig. 1C). Comparison of passive properties between WT and KO neurons showed that both populations had similar input resistances but differed in their cellular capacitance and membrane time constant: KO neurons had a higher cellular capacitance and a longer membrane time constant (Table 1). The cellular capacitance correlated with the dendritic complexity (β > 0, t = 3.14, P < 0.01, 2-tailed, R$^2$ = 0.19, Fig. 1D); thus neurons with more complex apical dendrites had a higher cellular capacitance.

Next, we determined single AP properties from APs evoked by brief (200 ms) depolarizing current injections and recorded at a high sampling rate (50 Hz). The mean AP threshold was significantly lower in KO compared with WT neurons (Table 1), relating to their lower resting membrane potential (Table 1). We also observed that APs evoked in KO neurons had significantly higher amplitudes and shorter durations at half-height.

Table 1. Morphological and electrophysiological parameter comparisons between the 5-HT$_{3A}$ KO and WT neurons

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WT</th>
<th>KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dendritic length, μm</td>
<td>1,095.3 ± 63.6</td>
<td>1,476.0 ± 69.2 $^*$</td>
</tr>
<tr>
<td>No. of endpoints</td>
<td>15.6 ± 0.91</td>
<td>20.6 ± 0.99†</td>
</tr>
<tr>
<td>Sum of branch order</td>
<td>83.8 ± 6.8</td>
<td>112.2 ± 8.2*</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>128.5 ± 12</td>
<td>118.4 ± 11.0</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>14.9 ± 1.2</td>
<td>18.9 ± 1.1*</td>
</tr>
<tr>
<td>Membrane resistance, pF</td>
<td>120 ± 0.6</td>
<td>180 ± 1.2†</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>−58.2 ± 1.9</td>
<td>−66.9 ± 2.5†</td>
</tr>
<tr>
<td>First AP amplitude, mV</td>
<td>82.4 ± 3.4</td>
<td>92.6 ± 1.6†</td>
</tr>
<tr>
<td>Sequential AP amplitude, mV</td>
<td>71 ± 2.8</td>
<td>80.5 ± 1.7†</td>
</tr>
<tr>
<td>First AP half-width, ms</td>
<td>1.15 ± 0.064</td>
<td>0.92 ± 0.027†</td>
</tr>
<tr>
<td>Sequential AP half-width, ms</td>
<td>1.88 ± 0.16</td>
<td>1.38 ± 0.067†</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>−30.87 ± 0.91</td>
<td>−37.38 ± 0.80‡</td>
</tr>
<tr>
<td>Rheobase, pA</td>
<td>170.95 ± 16.85</td>
<td>146.67 ± 11.56</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>10.3 ± 0.48</td>
<td>10.1 ± 0.53</td>
</tr>
<tr>
<td>AHP slope, mV/ms</td>
<td>0.19 ± 0.01</td>
<td>0.26 ± 0.11‡</td>
</tr>
<tr>
<td>Normalized AHP slope</td>
<td>0.79 ± 0.024</td>
<td>0.87 ± 0.013†</td>
</tr>
<tr>
<td>Normalized AHP amplitude</td>
<td>0.975 ± 0.017</td>
<td>0.959 ± 0.012</td>
</tr>
</tbody>
</table>

Values are means ± SE for wild-type (WT; n = 21) and 5-HT$_{3A}$ receptor knockout (KO; n = 24) neurons. AP, action potential; AHP, afterhyperpolarization. *P < 0.05 and †P < 0.01.
state level with a similar time constant of adaptation (WT: decrease of their instantaneous spike frequency to a steady-state with time (Fig. 2B)). All recorded neurons showed spike frequency adaptation with time (Fig. 2B). Both WT and KO neurons had a fast decrease of their instantaneous spike frequency to a steady-state level with a similar time constant of adaptation (WT: 14.8 ± 1.0 Hz, KO: 18.6 ± 1.4 Hz, P < 0.05, Fig. 2A). All recorded neurons showed spike frequency adaptation with time (Fig. 2B). Both WT and KO neurons had a fast decrease of their instantaneous spike frequency to a steady-state level with a similar time constant of adaptation (WT: 0.178 ± 0.024 s, KO: 0.132 ± 0.027 s). However, WT and KO neurons differed in their magnitude of spike frequency adaptation. KO neurons had a significantly higher steady-state spike frequency level than WT neurons (KO: 17.8 ± 1.5 Hz, WT: 11.9 ± 0.9 Hz, P < 0.01, Fig. 2C), whereas their initial firing frequency was similar (KO: 37.7 ± 4.1 Hz, WT: 28.9 ± 2.3 Hz). This translated into a significantly smaller depth of adaptation, as defined by the ratio between the peak and the steady-state frequency (KO: 0.50 ± 0.023, WT: 0.58 ± 0.025, P < 0.05, Fig. 2D); thus KO neurons exhibited less spike frequency adaptation than WT neurons (Fig. 2E). This reduced spike frequency adaptation correlated with the apical dendritic complexity, because a higher DCI related to a lower AP frequency adaptation (black line, Fig. 2F: WT and KO, β < 0, t = −4.6, P < 0.001, 2-tailed, \( R^2 = 0.34 \)). This correlation also held for the WT and KO populations separately (gray lines, Fig. 2F: WT, β < 0, t = −2.72, P < 0.05, 2-tailed, \( R^2 = 0.28 \); KO: β < 0, t = −2.45, P < 0.05, 2-tailed, \( R^2 = 0.21 \)), indicating that the effect is independent of other differences between the WT and KO neurons. These results suggest that the complexity of the apical dendrite influences the neuronal excitability by reducing the amount of spike frequency adaptation.

Repetitive firing properties. To assess the influence of apical dendritic complexity on the AP firing pattern, we examined the repetitive AP firing properties of layer 2/3 pyramidal neurons evoked by 1-s-duration depolarizing current injections ranging from 100 to 450 pA. KO neurons fired APs at a higher mean frequency than WT neurons at all depolarizing somatic current injections (WT: 14.8 ± 1.0 Hz, KO: 18.6 ± 1.4 Hz, P < 0.05, Fig. 2A). All recorded neurons showed spike frequency adaptation with time (Fig. 2B). Both WT and KO neurons had a fast decrease of their instantaneous spike frequency to a steady-state level with a similar time constant of adaptation (WT: 0.178 ± 0.024 s, KO: 0.132 ± 0.027 s). However, WT and KO neurons differed in their magnitude of spike frequency adaptation. KO neurons had a significantly higher steady-state spike frequency level than WT neurons (KO: 17.8 ± 1.5 Hz, WT: 11.9 ± 0.9 Hz, P < 0.01, Fig. 2C), whereas their initial firing frequency was similar (KO: 37.7 ± 4.1 Hz, WT: 28.9 ± 2.3 Hz). This translated into a significantly smaller depth of adaptation, as defined by the ratio between the peak and the steady-state frequency (KO: 0.50 ± 0.023, WT: 0.58 ± 0.025, P < 0.05, Fig. 2D); thus KO neurons exhibited less spike frequency adaptation than WT neurons (Fig. 2E). This reduced spike frequency adaptation correlated with the apical dendritic complexity, because a higher DCI related to a lower AP frequency adaptation (black line, Fig. 2F: WT and KO, β < 0, t = −4.6, P < 0.001, 2-tailed, \( R^2 = 0.34 \)). This correlation also held for the WT and KO populations separately (gray lines, Fig. 2F: WT, β < 0, t = −2.72, P < 0.05, 2-tailed, \( R^2 = 0.28 \); KO: β < 0, t = −2.45, P < 0.05, 2-tailed, \( R^2 = 0.21 \)), indicating that the effect is independent of other differences between the WT and KO neurons. These results suggest that the complexity of the apical dendrite influences the neuronal excitability by reducing the amount of spike frequency adaptation.

AHP dynamics. Spike frequency adaptation depends on the development of AHPs with successive APs in the spike train (Madison and Nicoll 1984). To get more insight into the effects found on the spike frequency adaptation, we examined the dynamics of the AHP and, in particular, how its amplitude and slope developed with successive APs in the spike train. The AHP amplitude was similar in size and development for both neuronal populations (Table 1), yet in the KO neurons the AHP slope remained higher during repetitive AP firing (Table 1 and Fig. 3B). This was clearly seen when the successive AHP slopes were normalized to the slopes of the first two AHPs.

Fig. 2. Differences in firing pattern correlate with changes in dendritic complexity. A: mean firing frequency as a function of depolarizing current injections for WT (n = 21) and 5-HT1A receptor KO neurons (n = 24). The mean action potential (AP) firing frequency was consistently higher for the 5-HT1A receptor KO neurons for all current intensities. B: example data for a neuron, showing instantaneous (Inst.) spike frequency expressed as a function of time. After an initial adaptation, the firing frequency achieved a steady-state level. C: steady-state frequency after adaptation was higher in KO compared with WT neurons. **P < 0.01. D: frequency adaptation depth [ratio between initial frequency (peak) and steady-state frequency] was significantly lower in KO neurons (less adaptation). *P < 0.05. E: example of AP firing of WT and KO neurons in response to suprathreshold depolarizing current injection. From the same initial frequency, the KO neuron decayed to a higher steady-state frequency (dotted lines indicate the timing of WT APs). F: frequency adaptation depth correlates with the dendritic complexity (DCI) within (gray lines) and between (black line) the KO and WT populations.
The normalized AHP slope of the KO population decayed to a higher steady state during AP firing than did that of the WT population (Fig. 3C). The correlation between the normalized AHP slope and the adaptation depth ($\beta = -0.79$, $t = -5.54$, $P < 0.001$, 2-tailed, $R^2 = 0.42$, Fig. 3D) suggests that a differential development of the AHP slope during AP firing is tightly coupled to the altered spike frequency adaptation depth. In addition, the normalized AHP slope also correlated directly with the DCI ($\beta > 0$, $t = 2.45$, $P < 0.05$, 2-tailed, $R^2 = 0.14$, Fig. 3E). This suggests that the dendritic complexity influences the spike frequency adaptation by modulating the AHP slope during repetitive firing.

**Neuronal modeling.** An abstract neuronal model was developed (see METHODS and Fig. 4A) in which the apical dendritic complexity could be manipulated through increased branching in the dendritic tuft, capturing the profile shown in the Sholl analysis of our recorded neurons (Fig. 1D). To investigate the impact of apical dendritic complexity on the firing pattern, all other neuronal properties were held constant, such as dendritic ion channel expression, density, and distribution. The passive dendritic model (without dendritic ion channels) reproduced the finding that the mean firing frequency decreases with longer total dendritic length (van Ooyen et al. 2002; Fig. 4B), showing that the dendritic calcium channels and calcium-dependent potassium channels are crucial for spike frequency adaptation to occur. We observed that for a given dendritic complexity, the depth of adaptation varied smoothly with the dendritic density of both channel types (Fig. 4C). Increasing the apical dendritic complexity in the active dendritic model reduced the adaptation depth by 30% and increased the normalized AHP slope by 20%, while the normalized AHP amplitude was not affected (Fig. 4B). In addition, more realistic models, based on reconstructed dendrites, were made (see METHODS). Eleven reconstructed dendrites of WT and 11 dendrites of KO neurons were stimulated and their firing patterns analyzed (similar to Fig. 1A). The reconstructed KO dendrites generated less spike frequency adaptation than the WT dendrites ($P < 0.05$; Fig. 4D). Altogether, the abstract model reproduced all the essential effects related to the dendritic complexity in our electrophysiological data, and the realistic models closed the gap between our abstract model and the realistic morphologies found in the brain.
To understand the underlying mechanisms linking the dendritic complexity to spike frequency adaptation, we hypothesized that the dendritic complexity influences the dendritic calcium dynamics that regulate the currents underlying the AHPs. We reasoned that increasing the dendritic complexity would result in an increase of the surface-to-volume ratio of the dendrite and therefore alter the speed of the calcium dynamics.

To test our hypothesis, we measured the calcium dynamics in our dendritic model, where higher order branches of a given complex dendrite had higher surface-to-volume ratios due to the Rall diameter rule (tapering) (Fig. 5A). We observed that the calcium dynamics evoked by a single AP were faster in distal branches (higher surface-to-volume ratio) compared with the proximal dendrite (lower surface-to-volume ratio) (Fig. 5B). The decay time constant of the dendritic calcium concentration was reduced for higher branch orders (Fig. 5C). This confirms that the speed of the calcium dynamics, induced by a single AP, is faster in branches that have a higher surface-to-volume ratio.

We next compared the calcium dynamics evoked by repetitive AP firing in dendritic branches of distinct surface-to-volume ratios (branches with branch orders 0 and 4). The time courses of the calcium concentration within these branches show that distal branches (branch order 4) have faster calcium concentration dynamics and lower calcium concentration.
tion buildup compared with the proximal branch (branch order 0) (Fig. 5D). Thus more complex dendrites, having increased surface-to-volume ratios, exhibit faster decay of the intracellular calcium concentration that would reduce the AHP slope and consequently affect the spike frequency adaptation.

**DISCUSSION**

In this study, we provide evidence that changes in dendritic complexity within a single class of neurons (layer 2/3 pyramidal neurons in the somatosensory cortex) affect the electrophysiological properties of these neurons. In addition to an increase in their cellular capacitance and membrane time constant, we also observed that neurons with a more complex dendritic tuft had a reduced spike frequency adaptation. These differences in firing pattern were related to an altered development of the AHP slope with successive APs. Our model study corroborated our experimental findings by showing that an increase in apical dendritic complexity per se can result in a faster AHP slope and a reduction of spike frequency adaptation.

Spike frequency adaptation is most likely mediated by calcium-dependent potassium channels located in dendrites (Bekkers 2000; Power et al. 2011; Sah and Bekkers 1996), which are activated by increases in cytosolic calcium concentration (Lancaster and Adams 1986; Madison and Nicoll 1984). The dynamics of the internal calcium concentration are known to depend on the surface-to-volume ratio of the cellular membrane versus the cytosol such that the kinetics of the influx and efflux of calcium are faster in dendritic compartments with a higher surface-to-volume ratio (Hille 1978; Regehr and Tank 1994). Using our neuronal model, we observed that complex dendrites with an increased number of thinner branches have a higher surface-to-volume ratio. This is due to the tapering of the branch diameters along the dendrite as has been shown for cortical pyramidal neurons (Kabaso et al. 2009; Rothnie et al. 2005). Consequently, the calcium concentration dynamics measured in our model were faster (faster decay rate) for dendritic branches having a higher surface-to-volume ratio. Because the kinetics of the calcium transients determine the activation kinetics of the calcium-dependent potassium channels responsible for spike frequency adaptation (Lancaster and Zucker 1994; Wang 1998), we suggest that the link between the dendritic complexity and spike frequency adaptation is mediated by altered calcium dynamics. This can alter the development of the AHP slope during firing and, consequently, the amount of frequency adaptation.

Most experimental studies on the relation between dendritic morphology and firing properties have compared distinct classes of neurons that originated from different cortical layers or were of clearly disparate phenotypes (Kasper et al. 1994; Mason and Larkman 1990; McCormick et al. 1985). Only a few studies, combining experimental and modeling approaches, have described a relationship between dendritic morphology and electrophysiological properties within a single class of neurons (Schaefer et al. 2003; Vetter et al. 2001). Vetter et al. (2001) showed that the pattern of backpropagating AP into the dendritic tree depends on the dendritic morphology and is insensitive to changes in channel densities. Schaefer et al. (2003) emphasized that variations in the apical dendritic branching pattern of layer 5 pyramidal neurons affect the degree of electrical coupling between the soma and the apical dendrite. However, both studies focused on the influence of dendritic arborization on the backpropagation of AP into the dendritic tree that modulates coincidence detection of synaptic input arising at the dendrites. In the present study, we focused on the correlation between dendritic complexity and AP firing pattern within one class of neurons, namely, the layer 2/3 pyramidal neurons, which would determine the output of these neurons. This class of neurons is interesting because it takes in a pivotal...
position in the cortical network. Layer 2/3 pyramidal neurons project locally onto other layer 2/3 and layer 5 pyramidal neurons and provide long distance cortico-cortical feedback connections (Bannister 2005). Because frequency adaptation is believed to be critical for the proper cortical information processing (Benda et al. 2005; Wang 1998), changes in dendritic complexity of layer 2/3 pyramidal neurons can have a great impact through these local and long-range cortical projections.

There are many studies that link alterations in dendritic morphology to pathological conditions such as neurodegenerative diseases or neurodevelopmental disorders (Kaufmann and Moser 2000; Luebke et al. 2010). Similarly, disturbances in serotonergic signaling have been linked to neurodevelopmental disorders (Daubert and Condron 2010; Gaspar et al. 2003). In this context, it is of interest to note that 5-HT3A receptor knockout mice display abnormalities in social behavior, reminiscent of an autistic phenotype (Smit-Rigter et al. 2010). Although a direct link between the morphological and behavioral abnormalities has yet to be established, the present results may help us to understand the relation between morphology and function during (patho)physiological development and maturation of neuronal circuits.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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