Regulation of AMPA and NMDA receptor-mediated EPSPs in dendritic trees of thalamocortical cells

Francis Lajeunesse\textsuperscript{1,2}, Helmut Kröger\textsuperscript{2} and Igor Timofeev\textsuperscript{1,3}\textdagger

\textsuperscript{1}Centre de recherche de l'Institut universitaire en santé mentale de Québec (CRIUSMQ), Laval University, Québec, G1J 2G3, Canada

\textsuperscript{2}Department of Physics, Laval University, Québec G1V 0A6 Canada

\textsuperscript{3}Department of Psychiatry and Neuroscience, Laval University, Québec G1V 0A6 Canada

Abbreviated title: Synaptic transfer in thalamocortical cells

\textdagger To whom correspondence should be addressed

Igor Timofeev

Centre de recherche de l'Institut universitaire en santé mentale de Québec (CRIUSMQ), Laval University, Québec, G1J 2G3, Canada

e-mail: Igor.Timofeev@phs.ulaval.ca

Phone: 418-663-5747 ext. 6396

Fax: 418-663-8756
Abstract

Two main excitatory synapses are formed at the dendritic arbor of first order nuclei thalamocortical neurons. Ascending sensory axons primarily establish contacts at large proximal dendrites, while descending corticothalamic fibers form synapses on thin distal dendrites. Using a multicompartent computational model based on fully reconstructed thalamocortical neurons from the ventroposterolateral nucleus of the cat, we compared local responses at the site of stimulation as well as somatic responses induced by both AMPA and NMDA receptor-mediated currents. We found that AMPA receptor-mediated responses, when synapses were located at proximal dendrites, induced a larger depolarization at the level of soma, while NMDA receptor-mediated responses were more efficient for synapses located at distal dendrites. The voltage transfer and transfer impedance were higher for NMDA than for AMPARs activation at any location. For both types of synaptic current and for both input locations at the dendritic arbor, somatic responses were characterized by a low variability despite the large variability found in local responses in dendrites. The large neurons had overall smaller somatic responses than small neurons, but this relation was not found in local dendritic responses. We conclude that in thalamocortical cells the dendritic location of small synaptic inputs does not play a major role in the amplitude of somatic response, but the size of the neuron does. The variability of response amplitude between cells was much larger than the variability within cells. This suggests possible functional segregation of thalamocortical neurons of different size.
Keywords: Thalamus, synaptic integration, neuronal morphology, signal propagation
Introduction

Early investigations have shown that local synaptic responses dramatically depend on the amount of neurotransmitters released and on the location of the neurotransmitter release sites at the dendritic arbor of the postsynaptic cell (Katz 1969; Komendantov and Ascoli 2009; Magee and Cook 2000; Redman 1990; Rinzel and Rall 1974). Despite some variability in the amplitude of somatic responses, this variability is rather small compared to the variability of local responses in dendrites except for neurons lacking an electrotonically compact structure (i.e. apical dendrite of pyramidal cells) (Chitwood et al. 1999; Jaffe and Carnevale 1999).

Thalamocortical (TC) cells are characterized by a complex morphology with dendrites organizing themselves in a way that each TC neuron occupies a rather spherical region in the dorsal thalamus (Crunelli et al. 1987; Friedlander et al. 1981; Turner et al. 1997; Zomorrodi et al. 2010). TC neurons of the ventroposterior complex of the thalamus receive excitatory inputs from somatosensory afferents at thick proximal dendrites (Liu et al. 1995; Williams et al. 1994), but excitatory synapses are also established at thin distal dendrites by corticothalamic (CT) fibers (Hoogland et al. 1991; Liu et al. 1995). At lemniscal synapses (LM), the main contribution to the postsynaptic responses comes from AMPA receptors (AMPARs) activation, while at CT synapses NMDA receptors (NMDARs) activation contribute more than AMPARs to the postsynaptic responses (Golshani et al. 1998; Miyata and Imoto 2006). A higher contribution of NMDARs to the postsynaptic response is found at early stages of life for both LM and CT synapses (Arsenault and Zhang 2006; Golshani et al. 1998). Previous studies demonstrated that the activation of CT synapses on TC neurons elicits low-amplitude, long-lasting responses,
but the activation of ascending sensory fibers induces large-amplitude, short-lasting
responses (Timofeev et al. 1996; Turner and Salt 1998). This difference can be explained
by a large number of release sites formed at each LM synaptic bouton and a single release
site formed at each CT terminal on ventroposterolateral (VPL) nucleus neurons (Liu et al.
1995). The question that we asked was whether identical synaptic stimulation (AMPA or
NMDA) applied to proximal vs. distal dendrites of TC neurons from the VPL nucleus
would result in comparable amplitude of somatic responses.

To answer this question we used a multicompartmment computational model based
on eight reconstructed TC neurons from the VPL nucleus of the cat’s thalamus. Major
morphological features of those cells have been analyzed and reported in a previous study
(Zomorrodi et al. 2010) and the exact morphological data can be downloaded from our
website (http://www.cru1rg.ulaval.ca/pages_perso_chercheurs/timofeev_i/shared.html).
Out of these eight neurons, seven were retrogradely stained by Fluoro Ruby injection into
the somatosensory cortex and one neuron was stained intracellularly with neurobiotin.
The objective was to investigate how the morphological features of VPL neurons affect
signal propagation in these neurons.

We investigated the variability of responses depending on cell size, type of
receptors (AMPA vs. NMDA) and specific location of synaptic inputs. We found that
despite major differences in the local amplitude of EPSPs elicited in various parts of the
dendritic tree their somatic amplitude was similar.
Materials and Methods

Multicompartment model

To compare the somatic responses to excitatory synapses innervating different dendrites or different branches of the same dendritic tree we used a multicompartment model based on three-dimensional reconstructions of eight real TC neurons from the VPL nucleus of the cat that were processed with minimal shrinkage. The detailed morphological description of these cells was given previously (Zomorrodi et al. 2010). Simulations were carried out on the NEURON simulation environment (Hines and Carnevale 1997). The minimal number of compartments assigned to each cell was initially evaluated using the $d$-lambda rule (Hines and Carnevale 2001), but could be increased in some sections (see below). Somatic EPSPs were not altered by this local increase in the number of compartments.

The model was conductance based and each compartment could be described by the Hodgkin-Huxley equations (Hodgkin and Huxley 1952). Passive properties were assumed constant over all compartments and cells. The membrane capacitance ($C_m = 0.878 \, \mu F/cm^2$), the leak conductance ($g_{\text{leak}} = 0.0379 \, mS/cm^2$), its associated reversal potential ($E_{\text{leak}} = -69.85 \, mV$) and the axial resistivity ($R_a = 173 \, \Omega \cdot cm$) were assigned according to Destexhe and colleagues, who fitted the model outcome to experimental data using a simplex algorithm (Destexhe et al. 1998). These values are very similar to those obtained in other studies of TC neurons (Briska et al. 2003; Perreault and Raastad 2006). In all performed simulations, Na$^+$ and K$^+$ channels were included in the somatic compartment, with the kinetics of fast action potentials taken from a model of
hippocampal pyramidal cells (Traub and Miles 1991). The corresponding peak conductance and reversal potential for both Na\(^+\) and K\(^+\) currents were respectively set to \(g_{\text{Na}} = 200 \text{ mS/cm}^2, E_{\text{Na}} = 50 \text{ mV}, \quad g_{\text{K}} = 200 \text{ mS/cm}^2 \text{ and } E_{\text{K}} = -100 \text{ mV.}\)

Active conductance for the low-threshold Ca\(^2+\) current \((I_T)\) was included in the model. This typical current of the TC neuron was modeled via Hodgkin-Huxley-like equations based on (Huguenard and McCormick 1992). However, the kinetics of \(I_T\) were taken from a more recent study (Destexhe et al. 1998). T-channels mediating \(I_T\) were inserted in all sections. However, based on previous experimental and computational studies (Destexhe and Sejnowski 2002; Williams and Stuart 2000; Zhou et al. 1997; Zomorrodi et al. 2008), a higher density of T-channels was assigned to the proximal part of dendrites through the adjustment of the maximal permeability \((\bar{P}_{\text{Ca}})\):

\[
\bar{P}_{\text{Ca}} = \begin{cases} 
20.6 \times 10^{-5} \text{ cm/s} & \text{in soma} \\
41.2 \times 10^{-5} \text{ cm/s} & \text{if } x < 40 \mu\text{m} \\
5 \times 10^{-5} \text{ cm/s} & \text{otherwise,} 
\end{cases}
\]

where \(x\) is the distance to the cell body. Ca\(^2+\) extrusion and buffering within a shell of 0.1 \(\mu\text{m}\) of thickness beneath the membrane was modeled with a first-order differential equation (McCormick and Huguenard 1992). The intracellular Ca\(^2+\) concentration at equilibrium was set to 240 nM and for fast Ca\(^2+\) handling a time constant \(\tau_{\text{Ca}} = 5 \text{ ms}\) was used (Destexhe et al. 1998).

Given that model the resting membrane potential was around -67 mV for all cells. In all simulations a small hyperpolarizing current was injected at the cell body of the neuron to set the membrane potential to -70 mV prior to stimulation, unless otherwise is indicated. All simulations were carried out assuming a temperature of 34 °C. To control
for a larger range of parameters we also investigated effects of $I_T$ at a membrane potential of -75 mV. In these experiments the maximal permeability was set to 0, 1 or $1.5 \times P_{Ca}$.

**AMPAR- and NMDAR-mediated synaptic currents**

For all reconstructed TC cells, we stimulated with an excitatory synaptic current $I_{\text{syn}}$ all possible dendritic branches at distances of 60 μm (47 - 78 branches in different cells) and 150 μm (59 - 131 branches in different cells) to the cell body in order to compare somatic responses to proximal and distal inputs. To increase the spatial resolution at proposed locations of synapses, we divided stimulated dendritic sections to form compartments of about 1 μm length. A realistic fast excitatory and voltage-independent conductance ($g_{\text{AMPA}}$) and a slower voltage-dependent conductance ($g_{\text{NMDA}}$) were modeled to respectively represent AMPAR- and NMDAR-mediated currents.

Standard equations (Baker et al. 2010; Jahr and Stevens 1990) described the synaptic conductance:

$$g_{\text{AMPA}} = a \left[ \exp \left( -\frac{t}{\tau_{1,\text{AMPA}}} \right) - \exp \left( -\frac{t}{\tau_{2,\text{AMPA}}} \right) \right]$$  \hspace{1cm} (2)

$$g_{\text{NMDA}} = b \frac{\exp \left( -\frac{t}{\tau_{1,\text{NMDA}}} \right) - \exp \left( -\frac{t}{\tau_{2,\text{NMDA}}} \right)}{1 + \frac{[\text{Mg}^{2+}]_{\text{ext}}}{3.57} \exp \left[ -0.062 V_m(t) \right]}$$  \hspace{1cm} (3)

where $t$ refers to the time after the onset of the EPSC, $\tau_{1,\text{AMPA}} = 0.1$ ms, $\tau_{2,\text{AMPA}} = 1.4$ ms, $\tau_{1,\text{NMDA}} = 0.5$ ms and $\tau_{2,\text{NMDA}} = 13$ ms are time constants, $a = 2.5$ nS and $b = 6.1$ nS are coefficients used to set the maximal conductance at the synapse and $[\text{Mg}^{2+}]_{\text{ext}} = 1$ mM is the extracellular concentration of Mg$^{2+}$. The intensity of the synaptic current is given by Ohm’s law: $I_{\text{syn}} = g_{\text{syn}}(V_m - E_{\text{syn}})$. The reversal potential of the synaptic current $E_{\text{syn}}$ was set to 0 mV for both AMPA and NMDA synapses. These parameters of synaptic currents
yielded peak conductances of 1.9 nS for AMPAR- and 0.23 nS (at -70 mV) for NMDAR-mediated currents. The somatic responses obtained from those stimuli were of the order of 0.4 mV, which roughly correspond to the amplitude of somatic EPSPs induced by the activation of a single vesicle release site (CT synapse) at TC neurons (Golshani et al. 2001). Unless indicated, the parameters for the synaptic currents were as indicated above. To explore a larger range of parameters we also used peak AMPAR- or NMDAR-mediated conductances of 0.128 nS and 0.5 nS. Finally, in one experiment we simultaneously activated both AMPARs and NMDARs. Here the target amplitude of somatic EPSP was also 0.4 mV. To achieve that for proximal (LM) stimuli, we simply changed parameters $a$ and $b$ of equations (2) and (3) to 1.9 nS and 2.6 nS, respectively. For distal (CT) stimuli, we set $a = 0.46$ nS and $b = 5$ nS. Based on (Miyata and Imoto 2006) the ratios of the NMDAR-mediated component to the non-NMDAR-mediated component were in the mean 60% and 192% for LM and CT, respectively.

Mean dendritic diameter

To estimate how the diameter of dendrites changes with the distance to soma, we measured the dendritic diameter $D$ as a function of the distance $x$ to the cell body for every possible path from the soma to a dendritic tip. Dendritic sections were compartmentalized in bins of 1 μm. The mean diameter at a point $x$ was given by the following equation:

$$\langle D(x) \rangle = \frac{1}{N(x)} \sum_{k=1}^{N(x)} D(x)$$

(4)
where $N(x)$ is the number of paths reaching the point $x$. Therefore, the dendrites with multiple branches had a higher weight in the calculation as opposed to dendritic trees with a few branches.

The mean dendritic diameter is given for each distance $x$ to the soma. Later in the study we will refer to the average path diameter. We define this quantity as the averaged diameter of the path joining the soma and a site of stimulation.

*Input impedance, voltage transfer and transfer impedance*

To describe the excitability of cells or of dendritic sites, we calculated the input impedance $R_{in}$ by applying in the model (passive membrane) a 20 pA hyperpolarizing current pulse of 200 ms duration and by measuring the amplitude of the voltage deflection induced. To quantify the degree of attenuation of EPSPs propagating from the site of stimulation to the soma, we calculated the voltage transfer $V_T$, defined as the ratio of the amplitude of the somatic response to the amplitude of the local response. $V_T$ has values between 0 and 1. Low values indicate a strong attenuation of the EPSP, while values close to 1 indicate a small attenuation. To additionally quantify the efficiency of the transfer of synaptic signals we calculated the transfer impedance $Z_T$ (Jaffe and Carnevale 1999; Norenberg et al. 2010), defined as the ratio of the amplitude of the voltage somatic response to the amplitude of the synaptic current at the site of stimulation. Large values of $Z_T$ indicate an efficient transfer of the synaptic signal, while small values indicate a weak signal transfer.

*Analysis*
Analysis was done on the MATLAB environment (The MathWorks, Natick, MA).

The normality of the data obtained for each cell was estimated with a one-sample Kolmogorov-Smirnov test. To compare responses obtained for proximal vs. distal inputs for each cell (n \geq 47), we used a two-sample \( t \)-test (two-tailed, unequal variances). To compare responses to the activation of different receptors (AMPA vs. NMDA), we used a paired \( t \)-test. Small samples (n = 8) were compared with a Mann-Whitney \( U \)-test. To estimate the dependence between two variables, Spearman’s rank correlation coefficient was used when linear correlation could not be assumed (nonlinear dependence and/or small sample [n < 15]). Otherwise, Pearson’s correlation coefficient was computed. Correlation coefficients are denoted \( r \). For plots that display fittings, the coefficient of determination \( R^2 \) that describes the goodness of fit is calculated when needed. To measure the dispersion of the data, the coefficient of variation was calculated as the standard deviation of the sample divided by its mean. To obtain standardized samples (see Fig. 6), the data were processed by applying the \textit{zscore} function of MATLAB (statistics toolbox), where the mean was subtracted from each sample, and the result was divided by the standard deviation of the sample. Statistical significance was assumed when \( p < 0.05 \). On all occasions * means \( p < 0.05 \), ** - \( p < 0.01 \), *** - \( p < 0.0005 \), NS - non-significant.
Results

Variability of synaptic signals in modeled TC neurons

In the first experiment, we stimulated independently two dendritic trees from two different cells, one cell of median size (cell 2, Fig. 2C from (Zomorrodi et al. 2010)) and the cell with the largest total membrane area from our sample (cell 6, Fig. 2C from (Zomorrodi et al. 2010)). In each cell the stimuli were applied to one proximal branch (15 μm from the soma, Fig. 1A and B) and to one distal branch (150 μm from the soma, Fig. 1A and B). Responses of the neuron to a single excitatory stimulus were recorded at different locations on the path of propagation, as indicated in figure 1C.

For both cells, the activation of AMPARs at a thin distal branch (150 μm) induced a large and fast depolarization of the membrane at the site of stimulation (Fig. 1C1, right). The application of the same conductance at a thick proximal branch (15 μm) generated a much smaller EPSP (Fig. 1C1, left). The EPSPs evoked at the distal part of dendritic arbors were dramatically attenuated during their propagation toward the soma (Fig. 1C1, right), while the EPSPs generated near the soma were weakly attenuated (Fig. 1C1, left). As a result, the somatic responses to proximal stimuli had higher amplitude (Fig. 1C1, left, soma) than responses to distal stimuli (Fig. 1C1, right, soma).

The activation of NMDARs also induced EPSPs of larger amplitude at distal dendrites (150 μm, Fig. 1C2, right) than at proximal dendrites (15 μm, Fig. 1C2, left). However, the attenuation for NMDAR-mediated responses propagating toward the soma was smaller than for AMPAR-mediated responses. As a consequence, somatic responses to the activation of distally located NMDARs were higher in amplitude than somatic
responses to the activation of the same receptors located proximally. Despite the small amplitude of NMDAR-mediated EPSPs evoked at proximal dendrites, their amplitude attenuated only to a minor extent in their propagation toward the soma.

As expected, responses evoked by stimulation with NMDAR-mediated current (Fig. 1C2) had a slower time course compared to responses to stimulation with AMPAR-mediated current. This slow time course is likely the explanation for a lower attenuation of NMDAR-mediated EPSPs (Magee 2000). Thus, only a low intensity NMDAR-mediated synaptic current (see Materials and Methods) was sufficient to generate somatic responses of the same order of magnitude as the ones obtained for AMPARs (Fig. 1, compare C1 and C2, soma).

The somatic responses evoked in the largest cell had a faster time course than those obtained in the smaller cell. The fast responses of the largest cell to the activation of proximal AMPARs (Fig. 1C, soma, see arrow) exhibited a shape similar to a fast pre-potential (FPP), a voltage deflection observed at the soma and usually associated to attenuated dendritic spikes (Crochet et al. 2004; Maekawa and Purpura 1967; Spencer and Kandel 1961; Timofeev and Steriade 1997).

**Geometrical and electrotonic properties of reconstructed neurons**

Primary dendrites of VPL neurons are thick, frequently end in tufts of multiple secondary branches and these second order dendritic collaterals are fine in caliber (Sawyer et al. 1994; Spreafico et al. 1983; Turner et al. 1997; Yamamoto et al. 1991; Yamamoto et al. 1985; Yen et al. 1985; Zomorrodi et al. 2010). Using equation 4, we quantified the mean dendritic diameter as a function of the distance to the soma for 4
cells: the smallest (cell 4), the largest (cell 6) and the 2 cells of median size (cells 1 and 2; Fig. 2A). We found a steep initial drop in the mean diameter at the perisomatic region of dendrites (up to $\approx 60 \, \mu m$), but a moderate decrease in diameter at remote dendritic branches. The overall change in the mean diameter of dendrites could be appropriately fitted by a decaying exponential function (Fig. 2A, black curve). We defined initial 60 $\mu m$ of dendrites as the proximal part of the dendritic arbor and the remaining part as the distal part.

Despite similar profile of dendritic diameters the cells had different size and therefore different input impedance that showed highly significant ($p < 0.0001$) relationship (Fig. 2B). By fitting our data with an equation derived from Rall’s work (Rall 1977); see figure 2B), we estimated the electrotonic length $L$ of investigated TC neurons, which was $L = 1.15$, a value similar to that of the dorsal lateral geniculate nucleus X and Y neurons (Bloomfield et al. 1987). The small value of electrotonic length suggests that distal dendritic inputs provide important contribution to somatic responses. The estimated membrane time constant was $\tau_m = 20 \, ms$.

We also calculated the local input impedance at dendritic sites stimulated with the synaptic currents. The amplitude of local responses to the activation of both AMPAR- (Fig. 2C1) and NMDAR-mediated conductances (Fig. 2C2) depended on the local input impedance for both proximal (60 $\mu m$) and distal inputs (150 $\mu m$).

According to the cable theory the input impedance depends on both the diameter of the innervated cable and on its extent in space (see appendix). Here we show that indeed the local input impedance was highly correlated to the local diameter of dendritic
branches stimulated in TC neurons for both proximal and distal sites \( r = -0.68, p = 6 \times 10^{-12} \) for proximal inputs; \( r = -0.60, p = 4 \times 10^{-11} \) for distal inputs; Fig. 2D). This relationship was well fitted by a power law \( \propto D^{-3/2} \) such as found for cables (Fig. 2D; see also the appendix). The number of dendritic tips and the dendritic path length in forward and backward directions also correlated with the local input impedance, but to a lesser extent (not shown). Therefore the local diameter is the main morphological parameter determining the input impedance at dendritic sites.

Dendritic diameters modulate local and propagating EPSPs

We investigated the impact of both the local diameter and the average path diameter on local dendritic and somatic responses, respectively. The stimuli were applied to proximal (60 μm from soma) and distal (150 μm from soma) branches in four investigated cells. Because the dendritic diameter determines the local input impedance and the local input impedance determines the amplitude of local response (Fig. 2C and D), we found a clear relation between the local amplitude of responses and the local dendritic diameter (Fig. 3). The local amplitude of EPSPs was smaller at thick branches than at thin ones for both AMPARs and NMDARs (Fig. 3; \( r < -0.55; p < 1 \times 10^{-4} \)). The results obtained with the activation of distally located AMPARs could be fitted properly by the theoretical function obtained by Softky (Softky 1994) for fast synaptic currents (Fig. 3A1-D1, see appendix). In contrast, the activation of proximally located AMPARs generally evoked weaker local responses than predicted by the theoretical function (Fig. 3A1-D1), due to their proximity to the somatic expanse that reduces the local input
impedance in the perisomatic region (Fig. 2D, compare circles with diamonds). Local responses obtained with the activation of NMDARs were also generally smaller for proximal inputs (Fig. 3A2-D2) than for distal inputs. The local amplitude of responses steeply decreased with an increase in the dendritic diameter. This relation could not be approximated by Softky’s expression, but remained highly significant in all cases \((r < -0.55; p < 1 \times 10^{-4})\). Due to the voltage dependence of NMDAR-mediated conductance, the intensity of the synaptic current also varied with the local diameter. For large diameter branches, local NMDAR-mediated EPSPs were very small in amplitude, because the weak depolarization at the point of stimulation was not efficient in removing Mg\(^{2+}\) block. To control for a larger range of synaptic conductances we also used peak AMPAR-mediated conductances of 0.128 nS, 0.5 nS and 1.9 nS and peak NMDAR-mediated conductances of 0.128 nS, 0.23 nS and 0.5 nS. Using smaller or higher conductances did not alter the dependence of local responses on the dendritic diameter, but as expected, it changed the magnitude of responses (not shown).

Next, we estimated in details the propagation of AMPAR- and NMDAR-induced EPSPs in a single dendritic branch (Fig. 4A-B; cell 1, 150 \(\mu\)m from the soma). In control conditions, at the site of stimulation the AMPAR-mediated EPSP was higher in amplitude than the NMDAR-mediated EPSP, but it was lower at the level of soma (Fig. 4B). The steeper decrease in the depolarization from the site of stimulation to the soma for the AMPAR-mediated EPSP reveals a stronger attenuation than for the NMDAR-mediated EPSP, as confirmed by voltage transfer values (Fig. 4B, inset, see numerical values \([V_T]\)). The larger attenuation rate of EPSPs induced by AMPAR activation could be the result of higher local amplitude. Therefore, we reduced the synaptic conductance
to obtain the same local amplitude as the one obtained for NMDARs (Fig. 4B, AMPA, reduced conductance). In this case too, the somatic response induced by AMPAR activation was lower than NMDAR-mediated response, indicating a larger attenuation of AMPAR-mediated EPSPs than NMDAR-mediated EPSPs. NMDAR-mediated responses have a slower time course and they are enhanced by membrane depolarization. To ensure that indeed the time course of NMDAR-mediated EPSPs was responsible for its better transmission to the soma, we removed the voltage-dependence of NMDARs by fixing $V_m$ to -70 mV in equation 3. The NMDAR-mediated EPSP reached the soma with higher amplitude than the EPSP mediated by AMPARs with matched amplitude at the site of stimulation, but lower than for control AMPARs and NMDARs. The voltage transfer associated to NMDARs with a fixed conductance remained higher than for AMPARs (control and reduced conductance; Fig. 4B, inset). The voltage-dependence of the NMDAR-mediated conductance was therefore not necessary to transmit efficiently the dendritic EPSPs to the soma. The calculation of voltage transfers (Fig. 4C) and transfer impedances (Fig. 4D) for all possible proximal and distal positions on cell 1 confirmed that NMDAR-mediated responses ensure a better transfer of synaptic signals than AMPAR-mediated responses. Note that the strongest attenuation occurs before distal EPSPs reach the proximal part of dendrites (Fig. 4B).

To probe attenuation rates of EPSPs propagating toward the soma we systematically investigated the voltage transfer as a function of the average path diameter of dendrites for all dendritic branches at studied locations in four neurons (Fig. 5). We found that the voltage transfer was positively correlated ($r \geq 0.3947; p \leq 0.002$) to the average path diameter for both proximal and distal synapses and for both AMPARs and
NMDARs, indicating that in larger dendritic path the EPSPs propagate with lower attenuation.

Accordingly, following the activation of AMPARs, the somatic responses were generally higher in amplitude for dendrites with a thick dendritic path than for dendrites with a thin dendritic path (Fig. 6A1-D1). For the four cells investigated and for both proximal and distal AMPARs activation the somatic amplitude positively correlated to the average path diameter ($r > 0.45; p \leq 3 \times 10^{-4}$). In thin dendrites the local responses were of high amplitude, but they attenuated dramatically in their propagation toward the soma; in thick dendrites the local responses were small, but the attenuation rate was low and overall the somatic responses were higher in amplitude than for small dendritic averaged path diameters. In contrast, the activation of NMDARs induced a larger depolarization at the cell body when the stimulus was applied to thin dendrites, compared to the depolarization induced at thick dendrites (Fig. 6A2-D2). In all cases but one (proximal stimuli at cell 6), the somatic amplitude was negatively and significantly correlated to the average path diameter ($r < 0.42; p \leq 2 \times 10^{-6}$). Due to the voltage dependence of NMDAR-mediated conductance and to high local input impedance (Fig. 2C2), the local responses induced by the activation of NMDARs on thin (mainly distal) dendrites were of higher amplitude (Fig. 3A2-D2). Owing to a much slower time course, the amplitude of NMDAR-induced propagating EPSPs was attenuated less by low-pass filtering properties of dendrites than AMPARs-mediated EPSPs and resulted in higher amplitude somatic responses when thin or distal dendrites were stimulated despite low voltage transfer values (Fig. 5A2-D2).
The use of peak AMPAR-mediated conductances of 0.5 nS yielded to similar patterns of the amplitude of somatic responses vs. dendritic diameter, but of different magnitude (not shown). When the AMPA conductance was decreased to 0.128 nS the somatic responses were at the noise level (0.03-0.0335 mV, not shown). The variation in peak NMDAR-mediated conductances (see methods) did not alter the dependence of somatic amplitudes on the dendritic diameter, but it was scaled in relation to the stimulation strength (not shown).

In all eight investigated TC neurons, the mean local amplitude induced by the stimulation of distal dendrites was significantly higher than that produced by the stimulation of proximal dendrites and it was true for both AMPAR- and NMDAR-mediated EPSPs (Fig. 7A). The absolute difference in the somatic amplitude of EPSPs induced by proximal vs. distal stimuli was smaller than for the local amplitude. However, the somatic amplitude was significantly higher when an AMPAR-mediated current was applied at proximal dendrites (Fig. 7B1). This relation was opposite for NMDAR-dependent responses. The activation of NMDAR-dependent currents in distal dendrites resulted in significantly larger somatic responses, as compared to the activation of the same receptors located on proximal dendrites (Fig. 7B2).

In the same data set, we investigated the time to peak of somatic responses evoked by an AMPAR-mediated current. It was significantly longer for distal inputs than for proximal inputs (Fig. 7C1). However, when an NMDAR-mediated current was applied, the time to peak was smaller for distal inputs than for proximal inputs in most cells (Fig. 7C2).
Above we demonstrated that despite the fact that we applied stimuli to fixed distances from the soma (60 μm or 150 μm), both local and somatic responses were of variable amplitude due to different diameters of dendrites. At this point we hypothesized that the complexity of dendritic trees may affect the amplitude of responses. We observed that bushy dendritic subtrees tend to emerge from large dendritic sections, while only a few branches are typically formed at thin dendrites. The analysis of the dendritic complexity of cell 1 (Fig. 8A) shows that when the first order dendritic sections (primary branches) were relatively thin (up to 1.6 μm) the total number of subsections formed by this primary branch was smaller or equal to 8 (Fig. 8A1). In contrast, when the primary branches were thicker, the number of formed subsections was higher (Fig. 8A1). Such a significant correlation was observed for the shown neuron (cell 1, filled circles and full line) and for the whole analyzed population of 8 neurons (dotted line). That stands in agreement with results published for X and Y cells from the dorsal lateral geniculate nucleus (Bloomfield et al. 1987). The same principle remained true for the second, third and fourth orders of dendritic branches as demonstrated by a high and significant coefficient of correlation (Fig. 8A2-4).

Earlier, we showed that dendritic diameters modulate local and somatic EPSPs, and here we show that dendritic diameters vary with the complexity of dendritic trees. Therefore, we analyzed the relation between the amplitude of local (Fig. 8B1-B2) and somatic (Fig. 8C1-C2) responses and the complexity of dendritic trees. We found that the local responses evoked both at proximal and distal locations and induced by the activation of both AMPARs and NMDARs were negatively correlated with the
complexity of dendritic trees (Fig. 8B1-2). The picture was different for AMPAR-
mediated somatic responses. The activation of proximally located AMPARs induced
responses that fail to show a significant correlation with the total number of sections of
the stimulated tree. However, the smallest somatic responses were observed when simple
dendritic trees (small number of sections) were stimulated (Fig. 8C1). On the other hand,
the activation of the same receptors at distal dendritic branches induced somatic
responses that were significantly correlated with the total number of sections. Complex
dendritic trees gave higher somatic responses than simple dendritic trees (Fig. 8C1).

When NMDARs were activated, the stimuli applied to simple dendritic trees induced
larger somatic responses than the stimuli applied to complex dendritic trees,
independently of the distance to soma (proximal or distal input, Fig. 8C2).

Between cells / within cell variability of synaptic responses

Results shown in figures 3, 6 and 7 suggest that the mean amplitudes of responses
differ across neurons. Because the metric properties of analyzed TC neurons were
different (Zomorrodi et al. 2010), we investigated the relation between the amplitude of
synaptic responses and the size of stimulated cells, as quantified by the total membrane
area (Fig. 9A-B). We found that in general, the amplitude of somatic voltage responses
was higher for cells with a small total membrane area than for cells with larger membrane
area. Cells with a similar membrane area had similar amplitudes of somatic responses
(Fig. 9B). The amplitude of somatic responses showed a clear monotonic relation to the
total membrane area in all tested conditions (proximal and distal inputs, AMPARs and
NMDARs; Fig. 9B, \( p < 0.0005 \)). However, this dependence of the amplitude of voltage
responses to the size of the cell was less pronounced for local responses (Fig. 9A).
Generally, the relation was not monotonic and for responses induced by the activation of NMDARs at proximal dendrites the relation was not significant.

The results shown in the figure 9A and B indicate that the variability in the amplitude of local responses was much higher than the variability in the amplitude of somatic responses. We computed the coefficient of variation of all responses (proximal and distal inputs, AMPARs and NMDARs, all cells). We found that indeed the variability of local responses was 3-10 times higher than the variability of somatic responses (Fig. 9C), suggesting that the morphological properties of dendritic trees are tuned to normalize the impact on the soma of synaptic inputs coming to different dendrites.

In one middle size neuron (cell 1) we investigated whether $I_T$ was responsible for the normalization of somatic responses. Removal of $I_T$ slightly reduced the mean amplitude of local and somatic responses for all tested stimuli (proximal and distal stimuli) by $<0.02$ mV when AMPARs were activated. When NMDARs were activated, the mean amplitude of somatic responses was decreased by $<0.1$ mV, but the mean amplitude of local responses in distal dendrites was decreased by $0.21$ mV ($2\%$ of amplitude). We used the coefficient of variation to describe the variability of responses. For all AMPAR-mediated EPSPs (local and somatic EPSPs, proximal and distal stimuli), the coefficient of variation remained rather stable (Fig. 9 D1). The highest reduction in the coefficient of variation ($2\%$) was observed for local responses to the activation of proximal NMDARs (Fig. 9D2). We also tested the effects of $I_T$ on local and somatic responses when the neuron was hyperpolarized to -75 mV and for various values of maximal $Ca^{2+}$ permeability ($0$, $1$ and $1.5 \times P_{Ca}$). Changes in the $Ca^{2+}$ permeability in this range of parameters did not influence the amplitude of local responses and the
dependence of somatic responses on the dendritic diameter. However, the somatic responses without Ca\(^{2+}\) permeability (0 × \(P_{\text{Ca}}\)) were on average 11% (AMPARs) or 35% (NMDARs) smaller than the responses obtained with a peak Ca\(^{2+}\) permeability of 1.5 × \(P_{\text{Ca}}\) (not shown). These results indicate that \(I_T\) does play a role in controlling the amplitude of somatic responses, but does not play major role in the normalization of somatic responses.

Validation of the computational model

The results shown in the figure 1C1 indicate that the time course of voltage responses may differ for identical stimuli applied to different locations on dendrites or to distinct cells. For example the response to the application of an AMPAR-mediated current at proximal dendrites of cell 6 often showed a sharp FPP-like shape (fig. 1C1, see arrow). This type of response was not observed when distal synapses were activated or when other neurons from our sample were stimulated. As mentioned in the introduction, one of the neurons investigated in this study (cell 6) was recorded intracellularly. To validate our model, we compared the somatic responses of that modeled neuron to the activation of AMPARs at proximal dendrites with electrophysiological responses of the same neuron recorded \textit{in vivo} to medial lemniscus stimulation. The model’s response was much smaller in amplitude than the responses to medial lemniscus stimulation (Fig. 10A-C). It is known that LM synaptic terminals establish several release sites on proximal dendrites and that a single LM axon forms several synaptic knobs in proximity (Jones 2007). Therefore, we simulated the activation of AMPARs on three different branches to mimic the synaptic contacts formed by three LM terminals and at each terminal we
modeled the release of eight vesicles through eight release sites. The membrane potential prior to stimulation of both the modeled neurons and the neuron recorded in vivo was \(-65\) mV. In these conditions, the response generated by the model was identical to the response recorded in vivo (Fig. 10 B and D).

To investigate the nature of FPPs observed in the largest cell from our sample (cell 6; Fig. 10A), we first removed the channels underlying the different intrinsic currents that were present in the model. Blocking Na\(^+\) and K\(^+\) channels inserted in the soma had no effect on the shape of EPSPs (Fig. 10E). Removing the T-channels from our model affected the latter part of the repolarizing phase of EPSPs (Fig. 10E). However, the absence of \(I_T\) had no effect on the initial part of the repolarizing phase of EPSPs, suggesting that \(I_T\) does not mediate the FPPs. Finally, simulations made on a purely passive cell showed the same EPSPs shape as observed without \(I_T\) but with Na\(^+\) and K\(^+\) channels present in the model (Fig. 10E).

Because the FPPs were only observed in the largest cell of our sample, we hypothesized that the size of the cell modulate the time course of EPSPs and is responsible for the generation of FPPs by providing current sinks toward extensive dendrites. To investigate this issue, we modified in a progressive fashion the total surface area of cell 6 by cutting dendrites to a maximal path length of 250, 200 and 150 \(\mu m\). As the majority of branching points in this cell were located within the first 120 \(\mu m\) from soma (Zomorrodi et al. 2010) the topological structure of the neuron was preserved. A slight decrease in the total surface area (maximal path length of 250 \(\mu m\)) did not affect the shape of EPSPs (Fig. 10F). Reducing any further the total surface area progressively removed the FPPs and smoothened the peak of EPSPs (Fig. 10F, compare the last traces...
These results indicate that the large dendritic expense was sufficient to generate somatic FPPs.

Finally, we investigated the effects of a simultaneous activation of AMPA and NMDARs as found in LM and CT synapses. For the LM synapse, the response to the co-activation of AMPA and NMDARs was indistinguishable from the arithmetic sum of their individual responses (Fig. 11A, left). For the CT synapse, the co-activation of AMPA and NMDARs induced a response that was slightly larger than the arithmetic sum of individual responses (Fig. 11A, right). To examine the role of the NMDAR-mediated component in the transfer of EPSPs from dendrites to soma, we performed simulations on cell 1 for both LM (all possible locations at 60 μm from soma) and CT synaptic currents (all possible locations at 150 μm from soma) and calculated the voltage transfer and the transfer impedance. For both LM and CT synaptic currents, the voltage transfer and the transfer impedance were significantly reduced when the NMDAR-mediated component was blocked (Fig. 11 B). This reduction was much larger for CT synapses than for LM synapses (62% [voltage transfer] and 72% [transfer impedance] reductions for CT synapses compared to 18% [voltage transfer] and 20% [transfer impedance] reductions for LM synapses). These results show that the use of synaptic stimuli with previously estimated ratio of NMDAR-mediated currents to AMPAR-mediated currents as found at LM and CT synapses (Miyata and Imoto 2006) was sufficient to enhance the transfer of synaptic signals.
Discussion

In this modeling study conducted on morphologically reconstructed TC neurons we show that: (a) the activation of either AMPARs or NMDARs induced large depolarizing responses in distal dendrites that were dramatically reduced in amplitude at the level of soma; (b) despite the large variability of responses in dendrites that depends on the dendritic diameter and as follow on the complexity of the dendritic tree, their variability at the level of soma was small; (c) in contrast, the overall cell size played a major role in the amplitude of somatic EPSPs, the smallest neurons generated large EPSPs and the largest neurons generated small EPSPs; (d) the activation of AMPARs at proximal dendrites had a slightly larger but highly significant impact on the soma than the activation of those receptors at distal dendrites; while the somatic impact of the activation of NMDARs was significantly larger when these receptors were located on distal dendrites.

Amplitude of local responses in dendrites. The stimulation of dendrites with excitatory conductances of 1.9 nS (for AMPARs) and 0.23 nS (for NMDARs) evoked somatic postsynaptic voltage responses similar to the one expected from the release of a single vesicle at the CT synapse (Golshani et al. 2001), but it induced dendritic responses spanning from several up to fifty millivolts. The largest responses were elicited at thin dendrites and the smallest responses were found at thick (usually more proximal) dendrites. This result was expected from Ohm’s law and it was previously demonstrated for other types of neurons (Komendantov and Ascoli 2009; Magee and Cook 2000; Rinzel and Rall 1974). However, there are possibly some details specific to TC neurons. We found that only the relation between the local amplitude of distal AMPAR-mediated
EPSPs and the diameter follows the mathematical expression obtained by Softky for fast synaptic currents (Softky 1994). The amplitude of AMPAR-mediated EPSPs at proximal dendrites was smaller than what was predicted from that expression. It likely occurred because the proximity to the large somatic compartment reduced the impedance at the perisomatic region (Fig. 2D, compare circles with diamonds).

Regulation of somatic responses. There are three important aspects to mention. For a given neuron, we found that despite the high variability in the amplitude of local responses in dendrites stimulated at the same distance from the soma, the amplitude of somatic responses for the same neuron was much less variable (Fig. 9C). These results are consistent with other studies of fast synaptic events in TC neurons (Briska et al. 2003; Perreault and Raastad 2006), but we demonstrated that this normalization also stands for NMDAR-mediated EPSPs. A similar phenomenon of normalization was found in other neurons that lack extended apical dendrites (Chitwood et al. 1999; Jaffe and Carnevale 1999; Schmidt-Hieber et al. 2007). Second, we found that stimulation of proximal vs. distal dendrites of the same neuron induced somatic responses comparable in amplitude (Figs. 6, 7B). Small amplitude local responses elicited at proximal dendrites attenuated to a minor extent in their propagation to soma, while large amplitude responses elicited at distal dendrites attenuated to a major extent, mainly before reaching the proximal part of dendrites. This is consistent with the pronounced tapering of proximal dendrites and with higher geometrical ratios (see appendix) at the perisomatic region of VPL neurons (Zomorrodi et al., 2010). Such attenuation ensures that large dendritic distal EPSPs do not dramatically interfere with small dendritic proximal EPSPs. The previously described mechanisms for the so-called dendritic democracy were limited to
amplification of distal inputs by dendritic intrinsic current, scaling of synaptic strength and increased sensitivity of postsynaptic sites (Häusser 2001). Our current study on TC neurons combined with previous computational investigations (Chitwood et al. 1999; Jaffé and Carnevale 1999) demonstrates that morphological properties of neurons by themselves can ensure the regulation of dendritic signals evoked at the different parts of dendritic trees by a progressive increase of input impedance with distance to soma. Therefore, our model predicts that in any electrotonically compact neuron the somatic responses to a small synaptic current will depend in a minor extent to the location where this current is applied. This mechanism might ensure the linear integration of inputs to TC neurons at the level of soma. Third, we found that due to input impedance differences a synaptic input to small neurons induced larger responses at the soma than the same input to large neurons. We found this variability across cells to be more pronounced than the variability within each cell. This implies a functional segregation of TC neurons. Large neurons possess more membrane surface and are likely innervated by more synapses as in the case of cortical pyramidal neurons (DeFelipe and Farinas 1992). They will respond (generate spikes) to multiple inputs compared to small neurons in which a few simultaneously activated synapses will elicit an action potential. We found that the clear monotonic relation of somatic responses to the cell size could not be validated for dendritic sites. This suggests that dendritic branches are rather independent processing units and constitute a first stage of integration, as supported by previous studies (Branco et al. 2010; Losonczy and Magee 2006; Poirazi et al. 2003; Williams 2004).

Difference between AMPAR and NMDAR-mediated responses. We found that despite a dendritic normalization of EPSPs, the activation of AMPARs had a stronger impact on
the soma when the receptors were found at proximal dendrites rather than at distal
dendrites (Fig. 5B1). In contrast, the activation of NMDARs had a stronger impact on
soma when distal dendrites were stimulated (Fig. 5B2). There are at least two factors that
explain this difference. It is well known that NMDARs are voltage dependent (Mayer et
al. 1984; Nowak et al. 1984). Then, due to a high input resistance at distal dendrites, a
relatively small current will depolarize them to the voltages at which NMDAR-mediated
conductances progressively reinforce the voltage response. The second aspect is a lower
attenuation rate for propagating NMDAR-mediated EPSPs than for AMPAR-mediated
responses. The reduced attenuation rate depends on the slow time course of NMDAR-
mediated EPSPs. Our model predicts that large somatic effects induced by the activation
of NMDARs at distal (CT) synapses (Golshani et al. 1998) could be mediated by a
relatively small number of NMDARs. In contrast, the effects of NMDARs activation
would be smaller if they would be localized on proximal dendrites. This might have an
important role for TC neuron physiology because NMDARs are present in higher
proportions at distal CT synapses than at proximal LM synapses. A similar distribution of
NMDARs with distance to soma has also been found for pyramidal cells (Branco and
Häusser 2011; Otmakhova et al. 2002). Our results suggest that co-activation of AMPA
and NMDARs on distal dendrites ensures a reliable transfer of CT excitation to the soma
of VPL TC neurons.

One of the objectives of our study was to investigate why experimentally recorded
CT EPSPs had a longer time course than LM EPSPs responses (see introduction). Here
we show that the activation of AMPARs on distal dendrites induced slower responses at
soma compared to their activation at proximal dendrites (Fig. 7C1). In the majority of
investigated neurons the activation of NMDARs on distal dendrites induced faster somatic responses compared to proximal activation of the same receptors (Fig. 7C2). These results predict that distally located synapses with AMPARs could be a factor of the slower time course of somatic responses induced by the stimulation of CT fibers. However, we found in somatic recordings that the time to peak of NMDAR-mediated EPSPs was approximately 4 times slower than for AMPAR-mediated EPSPs. Our data suggest that the activation of NMDAR-dependent currents in distal dendrites could be primarily responsible for the longer time course of somatic EPSPs induced by the activation of CT synapses.

Generation of FPPs in TC neurons. Multiple studies reported the presence of fast pre-potentials in TC (Deschênes et al. 1984; Maekawa and Purpura 1967; Timofeev and Steriade 1997), hippocampal (Spencer and Kandel 1961) and neocortical (Crochet et al. 2004) neurons. FPPs are characterized by fast rising and fast decaying initial components of the depolarizing response. Because of their voltage dependence in pyramidal neurons (Crochet et al. 2004; Spencer and Kandel 1961), it was proposed that FPPs are the result of the activation of dendritic active conductances (dendritic spikes). In TC neurons FPPs were recorded in a large range of voltages (Timofeev and Steriade 1997). Here we show that without the implication of any active conductance the shapes that resemble FPPs could be recorded in large neurons when proximal AMPARs were activated (Fig. 1C1, Fig. 10). We did not observe this type of response neither in smaller neurons nor when distal synapses or NMDARs were activated. Fast rising and decaying phases of EPSPs are explained purely by the morphological properties of dendrites; larger cells promote faster voltage responses due to the current outflow in other parts of the neuron.
Limitations and validation of our study. Here we did not consider multiple active conductances that are present in TC neurons. They can have a significant impact on the expression of both local and somatic responses. The major difficulty of implementing active conductances is that for many of them the density of their channel distribution on different neuronal compartments is unknown. We have recently shown that a change in the distribution of T-channels can lower the threshold of the low-threshold spike generation by up to 64% (Zomorrodi et al. 2008). Adding other conductances to dendritic locations might affect neuronal output as it was demonstrated for pyramidal neurons (Cook et al. 2007; Hu et al. 2009; Ulrich 2002). However, the low-threshold spike response appears to be identical in TC neurons with or without H current when the stimuli were applied at similar levels of the membrane potential (Meuth et al. 2006). This is also true for pyramidal neurons that when electrotonically more compact (basal dendrites) were investigated, the H current blockage affected only slightly the attenuation of synaptic responses (Golding et al. 2005). Another limitation is that we investigated the effects of a very small (presumably single vesicle-dependent) synaptic conductance. However, it is known that LM synapses in VPL TC neurons contain multiple release sites (Liu et al. 1995). The activation of multiple release sites in dendrites may lead to nonlinear summation of EPSPs, which would affect the neuronal output.

The choice of parameters describing passive cell properties ($C_m$, $R_a$ and $g_{\text{leak}}$) could influence the obtained results. We did not investigate the dependence of EPSPs to $C_m$. It has been shown that $C_m$ is rather constant across cell types and do not depend on proteins embedded in the membrane (Gentet et al. 2000). We tested effects of modulation of $R_a$ and $g_{\text{leak}}$ on neuronal responsiveness. An increase or a decrease of $R_a$ by 25%
affected local, but not somatic responses. An increase or a decrease of $g_{\text{leak}}$ by 25% slightly affected the maximal amplitude of somatic, but not local responses. This was true for both AMPAR- and NMDAR-mediated responses (not shown). Our main conclusions remain unchanged: AMPARs were more efficient in depolarizing the soma when they were located at proximal dendrites, while the opposite was true for NMDARs, and somatic responses remained relatively constant in amplitude.

Despite described above limitations, we believe that our modeling study provides reliable physiological information because the activation of several synapses on proximal dendrites in the model perfectly reproduced the shape of the EPSP induced by the medial lemniscus stimulation as recorded in vivo (Fig. 10B and D).
Appendix

*Dendritic morphology determines the attenuation of synaptic signals*

In their propagation toward the soma, EPSPs are subject to attenuation, which is mainly governed by two physical processes. First, the thickness of the path determines axial and membrane resistances and therefore the electrotonic attenuation in dendritic cables defining that path. In uniform semi-infinite cables, this is properly described by the length constant, generally denoted $\lambda$ (Rall 1959):

$$\lambda = \left[ \frac{D R_m}{4 R_a} \right]^{1/2}$$

$D$, $R_m$ and $R_a$ are respectively the diameter of the cable (in units of length), the passive membrane resistance (in units of resistance times units of surface) and the axial resistivity (in units of resistance times units of length). A steady-state voltage deflection initiated by constant current injection at the extremity of a semi-infinite cable will be attenuated to $1/e \approx 37 \%$ of its amplitude at a distance $\lambda$ from the extremity. The thinner is the path the smaller is $\lambda$ and the stronger is the electrotonic voltage attenuation.

Second, the particular geometrical and topological features of dendritic trees impose specific input impedances at branching points. For branches of several $\lambda$ in length connected together, the behavior of signals approaching the branching point can be properly described by Rall’s geometrical ratio (Goldstein and Rall 1974):

$$GR = \frac{\sum_j b_j^{3/2}}{b_p}$$
where $D_p$ is the diameter of the section along which the signal propagates to the branching point, and $D_j$ is the diameter of each section $j$ emerging from that branching point. This relation also stands when only two segments of different diameters are connected together. For a thin segment receiving a synaptic input and connecting to a much thicker one, which is generally the case in forward propagation of dendritic signals toward the soma, there is low input impedance at the branching point, causing the voltage transient to attenuate when spreading from the thin segment to the thick one, despite a high current flow toward the thick segment. Therefore, the EPSPs evoked at dendrites are largely attenuated during their propagation toward the cell body. The relatively thin dendritic trunks connecting the large somatic expanse can account for a considerable amount of that attenuation.

Local responses depend on the dendritic diameter

For a semi-infinite cable, the input impedance at the origin is given by (Rall 1959; Rall 1977)

$$R_{\infty} = (R_m R_a)^{1/2} \frac{2}{(\pi D)^{3/2}}$$

When the membrane resistance and the axial resistivity remain fixed all along the cable, the input impedance depends only on the diameter of the cable. For a finite cable with a sealed end at a distance $L$ from the origin, the input impedance at the origin is given by

$$R_{in} = R_{\infty} \coth L$$

If the end of the cable at a distance $L$ from the origin is rather an open end, $R_{in}$ is described as
\[ R_{\text{in}} = R_{\infty} \tanh L \]

Those equations reveal that the input impedance is function of both the thickness of the cable and its extent in space.

Based on the cable theory, Softky previously obtained an expression that allows the estimation of the amplitude \( V_{\text{peak}} \) of an EPSP evoked by a fast current input (Softky 1994):

\[
V_{\text{peak}} = 1.5 \frac{R_{\infty}^{1/2} I_{\text{peak}}}{(\pi D)^{3/2} C_m^{1/2} \sqrt{t_{\text{peak}}}}
\]

where \( I_{\text{peak}} \) is the peak value of the current applied and \( t_{\text{peak}} \) is the time taken by the current to reach this peak, starting from its onset. \( C_m \) is the membrane capacitance, in units of capacitance per units of area. This expression predicts that the amplitude of an EPSP increases considerably as the diameter of the targeted branch decreases. Note that here \( V_{\text{peak}} \) does not depend on \( R_m \). This equation is only an approximation, because it does not take into account the reversal potential of the excitatory synaptic current, which is around 0 mV for glutamatergic receptors, and may not be valid for slower synaptic currents.
Grants:
This study was supported by National Science and Engineering Research Council of Canada (grant 298475), Canadian Institutes of Health Research (MOP-37862, MOP-67175), and National Institute of Neurological Disorders and Stroke (1R01NS060870). I.T. is Fonds de la Recherche en Santé du Québec Research Scholar.

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

Author Contributions: F.L. performed modeling experiments, contributed to design of study, wrote paper; H.K. contributed to modeling experiments; I.T. design study, contributed to modeling experiments performed in vivo experiment and wrote paper.
Figure legends

Figure 1. Variability of synaptic responses. A and B, Reconstructed dendritic trees from cell 2 (A) and from cell 6 (B). A synaptic current was applied at the proximal or the distal part of dendritic trees as indicated (pipette 1, 15 μm from soma and pipette 2, 150 μm from soma). C, Attenuation of voltage signals in their propagation toward the soma for both AMPAR- (C1) and NMDAR-mediated (C2) currents. Voltage responses were induced by the synaptic current application at proximal (Left columns) and distal (Right columns) locations depicted in A and B. Voltage responses are shown at different locations: 15, 10, 5 μm and at the soma for the proximal stimulus (1) and at 150, 100, 50 μm and at soma for the distal stimulus (2). Colour traces in C correspond to depicted dendrites in A and B.

Figure 2. Geometrical and electrotonic properties of reconstructed neurons. A. Mean dendritic diameter vs. distance to the cell body in 4 thalamocortical cells. The investigated neurons revealed a similar profile in their dendritic diameters as a function of the distance to soma. The diameter decreased in a steep fashion with distance to soma at the perisomatic region, but this decrease in diameter was moderate at distal dendrites. The mean curve was properly fitted by a decaying exponential function. The vertical dashed line defines the respective domains of the proximal (≤ 60 μm) and the distal (> 60 μm) parts. B. Relationship of neuronal input impedance to total membrane area in 8 reconstructed TC neurons. C. Dependence of the local amplitude of responses in one neuron (cell 1) to the local input impedance (C1 for AMPARs, C2 for NMDARs). D. The rate of increase in local input impedance with the dendritic diameter depends on the location in dendrites.
Figure 3. Dependence of local responses on the local dendritic diameter. A, Amplitude of local responses to a proximal (60 μm, circles, n = 74) or a distal (150 μm, diamonds, n = 110) stimulus vs. the diameter at the site of stimulation in cell 1 for both AMPAR- (A1) and NMDAR-mediated currents (A2). Curves correspond to a theoretical function obtained by Softky that fits to distal AMPAR-mediated EPSPs only (see Appendix). B, C and D, Same as A, but for cell 2 (B, proximal n = 70; distal n = 120), cell 4 (C, proximal n = 47; distal, n = 59,) and cell 6 (D, proximal n = 56; distal n = 131), respectively.

Figure 4. Attenuation of EPSPs, voltage transfer and transfer impedance. A. Dendritic tree of a reconstructed TC neuron indicating the position of a stimulating electrode (pipette, 150 μm from soma). B. Peak amplitude of EPSP in a dendrite at different distances from soma. Black curves – AMPAR-mediated responses; grey curves – NMDAR-mediated responses. To fit the local amplitude of the NMDAR-mediated EPSP at the site of stimulation, the AMPAR-mediated conductance was reduced (dashed black curve). To probe voltage dependency of NMDAR-mediate EPSPs the Mg²⁺ block mechanism was inactivated (dashed grey curve). Inset shows peak amplitude of responses at the perisomatic region. Voltage transfer values (Vₜ) are indicated for each case. C. Mean voltage transfer values for cell 1, D. Mean transfer impedance values for cell 1.

Figure 5. Voltage transfer values vs. the average path diameter for each dendritic branch at each investigated location (proximal [60 μm, circles] vs. distal dendrites [150 μm, diamonds]) for 4 studied neurons (A-D). AMPAR-mediated stimuli – left column, NMDAR-mediated stimuli – right column. Voltage transfer values increase with the thickness of the path and are larger for proximal stimuli than for distal stimuli.
Figure 6. Dependence of somatic responses on the average path diameter. A, Amplitude of somatic responses to a proximal (60 μm, circles, n = 74) or a distal (150 μm, diamonds, n = 110) stimulus vs. the average path diameter measured from the site of stimulation to the soma in cell 1 for both AMPA (A1) and NMDA (A2) receptor-mediated currents. B, C and D, Same as A, but for cells 2, 4 and 6.

Figure 7. Amplitude and time to peak of synaptic responses elicited by proximal and distal stimuli. A, Amplitude of AMPA (A1) and NMDA (A2) receptor-mediated local responses for proximal (60 μm, white bar) and distal (150 μm, grey bar) stimuli. B, Same as A, but for somatic responses. C, Time to peak of AMPA (A1) and NMDA (A2) receptor-mediated somatic responses for proximal (60 μm, white bar) and distal (150 μm, gray bar) stimuli.

Figure 8. Correlation between dendritic diameter and amplitude of voltage responses to the complexity of dendritic arbors. A, Number of dendritic subsections emerging from a given dendritic section vs. the mean diameter of that section. Data are shown and linearly fitted (dots and black line) for the first four orders of branching of cell 1 (A1 to A4, respectively). The mean fit obtained for all investigated cells is also shown (dashed lines). B, Z-scored (see Materials and Methods) mean local amplitude of responses to proximal (60 μm, circles) and to distal (150 μm, diamonds) stimuli obtained for each tree of all investigated cells vs. the total number of dendritic sections in the stimulated dendritic tree. Data are shown for both AMPA (B1) and NMDA (B2) receptors. Mean
amplitudes were z-scored to pool the results obtained in all cells together. C, Same as B, but for the somatic amplitudes.

**Figure 9.** Dependence of the amplitude of voltage responses to the cell size. A, Amplitude of local responses to the activation of proximal (60 μm, open symbols) or distal (150 μm, filled symbols) AMPA (A1) and NMDA (A2) receptors vs. the total surface area of stimulated cells. B, Same as A, but for somatic responses. C, Mean coefficient of variation (n=8) of the amplitude of both local (gray bar) and somatic (white bar) responses to the activation of AMPARs (C1) and NMDARs (C2). D, Coefficient of variation of somatic responses in cell 1 was only slightly affected by the presence of $I_T$ in TC neurons. In each column (proximal or distal input), the left symbols stand for passive dendrites, and right symbols stand for dendrites containing $I_T$.

**Figure 10.** Validation of the computational results. A, Morphology of reconstructed cell 6. The same neuron was used to investigate effects of T-channel distribution in thalamocoritcal neurons (Zomorrodi et al. 2008). B, Intracellular responses of that cell to the stimulation of the medial lemniscus in *in vivo* experiment. C, Response of modeled neuron to the activation of AMPARs at an individual site located on proximal dendrite. D, Response of modeled neuron to the activation of AMPAR at 24 sites located on proximal dendrite. Note the similarity between the response of modeled neuron to the stimulation of 24 release sites and the response recorded *in vivo* to the stimulation of the medial lemniscus. E, Active conductances do not affect peak amplitude of EPSP and its early part of the decaying phase that gives rise to FPP-like shape. F, Cutting dendritic branches to the indicated maximal path lengths reduced the total membrane area ($A_m$) and progressively abolished the FPP-like shape.
Figure 11. Effects of co-activation of AMPA and NMDARs in lemniscal and cortico-thalamic synapses. A. Amplitude of EPSCs and EPSPs induced by activation of AMPARs, NMDARs, their arithmetic sum and their co-activation in lemniscal and cortico-thalamic synapses. Voltage transfer (B) and transfer impedance (C) induced by activation of AMPARs and co-activation of AMPA and NMDARs at lemniscal and cortico-thalamic synapses.
Reference List


Redman S. Quantal analysis of synaptic potentials in neurons of the central nervous system. Physiol Rev 70: 165-198, 1990.


Proximal Part

Distal Part

<table>
<thead>
<tr>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 4</th>
<th>Cell 6</th>
</tr>
</thead>
</table>

Fit

\[
a \exp\left[ bx \right] + c
\]

\[
a = 3.66 \, \mu m
\]

\[
b = -0.0375 \, \mu m^{-1}
\]

\[
c = 0.652 \, \mu m
\]

Distance to soma (\(\mu m\))

Mean diameter (\(\mu m\))

Input impedance (M\(\Omega\))

Total membrane area (x 10^4 \(\mu m^2\))

\[
A = \left(\frac{\tau_m L}{C_m R_{in} \tanh(L)}\right)
\]

\[
\tau_m = 20 \, ms
\]

\[
L = 1.15
\]

\[
R^2 = 0.998
\]

Local diameter (\(\mu m\))

Local input impedance (M\(\Omega\))

Local amplitude (mV)

AMPA

NMDA

C1

C2

D

Local input impedance (M\(\Omega\))

Local input impedance (M\(\Omega\))

Local input impedance (M\(\Omega\))

Local diameter (\(\mu m\))
In vivo Medial lemniscus stimulation

Model 24 release sites stimulation

A

C

D

E

F

A_m = 73 073 \mu m^2
A_m = 67 046 \mu m^2
A_m = 52 895 \mu m^2
A_m = 52 895 \mu m^2
A_m = 35 008 \mu m^2

A

B

Without Na^+ and K^+ currents
Without T-current
Passive cell

Without Na^+ and K^+ currents

Control

Without T-current

Passive cell

Control

250 \mu m of maximal path length

200 \mu m of maximal path length

150 \mu m of maximal path length

0.2 mV

50 ms

0.2 mV

50 ms
**Figure A**

Comparison of synaptic currents and somatic voltage responses between Lemniscal and Corticothalamic pathways. 

**Figure B**

Bar graph showing voltage transfer for LM and CT pathways, with significant differences marked by asterisks.

**Figure C**

Bar graph depicting transfer impedance for LM and CT pathways, with significant differences marked by asterisks.