Subtype-Specific Dendritic Ca\textsuperscript{2+} Dynamics of Inhibitory Interneurons in the Rat Visual Cortex

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Cho KH, Jang JH, Jang HJ, Kim MJ, Yoon SH, Fukuda T, Tennigkeit F, Singer W, Rhie DJ. Subtype-specific dendritic Ca\textsuperscript{2+} dynamics of inhibitory interneurons in the rat visual cortex. J Neurophysiol 104: 840–853, 2010. First published June 16, 2010; doi:10.1152/jn.00146.2010. The Ca\textsuperscript{2+} increase in dendrites that is evoked by the backpropagation of somatic action potentials (APs) is involved in the activity-dependent modulation of dendritic and synaptic functions that are location dependent. In the present study, we investigated dendritic Ca\textsuperscript{2+} dynamics evoked by backpropagating APs (bAPs) in four subtypes of inhibitory interneurons classified by their spiking patterns: fast spiking (FS), late spiking (LS), burst spiking (BS), and regular-spiking nonpyramidal (RSNP) cells. Cluster analysis, single-cell RT-PCR, and immunohistochemistry confirmed the least-overlapping nature of the grouped cell populations. Somatic APs evoked dendritic Ca\textsuperscript{2+} transients in all subtypes of inhibitory interneurons with different spatial profiles along the tree: constantly linear in FS and LS cells, increasing to a plateau in BS cells and bell-shaped in RSNP cells. The increases in bAP-evoked dendritic Ca\textsuperscript{2+} transients brought about by the blocking of A-type K\textsuperscript{+} channels were similar in whole dendritic trees of each subtype of inhibitory interneurons. However, in RSNP cells, the increases in the distal dendrites were larger than those in the proximal dendrites. On cholinergic activation, nicotinic inhibition of bAP-evoked dendritic Ca\textsuperscript{2+} transients was observed only in BS cells expressing cholecystokinin and vasoactive intestinal peptide mRNAs, with no muscarinic modulation in all subtypes of inhibitory interneurons. Cell subtype-specific differential spatial profiles and their modulation in bAP-evoked dendritic Ca\textsuperscript{2+} transients might be important for the domain-specific modulation of segregated inputs in inhibitory interneurons and differential control between the excitatory and inhibitory networks in the visual cortex.

INTRODUCTION

An increase in dendritic Ca\textsuperscript{2+} concentration is involved in dendritic excitability, synaptic plasticity, restoring intracellular Ca\textsuperscript{2+} stores, and intracellular metabolic processes (Egorov et al. 1999; Haussser et al. 2001; Magee and Johnston 1997; Williams and Stuart 2000; Zhou et al. 2005). In cortical pyramidial neurons, backpropagating action potentials (bAPs) evoke dendritic Ca\textsuperscript{2+} transients depending on the distance from the soma (Waters et al. 2005). Spatial profiles of bAP-evoked Ca\textsuperscript{2+} transients in cortical pyramidal neurons differ between apical (Spruston et al. 1995; Waters et al. 2003) and basal dendrites (Cho et al. 2006; Hoogland and Saggau 2004; Nevian et al. 2007). Because many synaptic activities are regulated by \([\text{Ca}^{2+}]\), differential kinetics in bAP-evoked dendritic Ca\textsuperscript{2+} transients between restricted dendritic areas might be critical for local control of synaptic and dendritic activities (Koester and Sakmann 1998; Topolnik et al. 2009; Waters et al. 2003). Furthermore, a local change in dendritic activity might be involved in the pathway-specific regulation of synaptic inputs from different brain areas, which are relatively segregated and terminate in isolated dendritic areas (Binzegger et al. 2004; Feldmeyer et al. 2002; Peterein et al. 2009).

The bAP-evoked dendritic Ca\textsuperscript{2+} transients are cell type-specific for respective brain areas (see review by Waters et al. 2005). Furthermore, huge variation in dendritic arbors, active and passive membrane properties, and endogenous Ca\textsuperscript{2+} buffers responsible for dendritic Ca\textsuperscript{2+} dynamics (for review, see Ascoli et al. 2008) appear to be important in the subtype-specific function of inhibitory interneurons. An increase in dendritic Ca\textsuperscript{2+} causes excitatory postsynaptic potential (EPSP) depression (Zilberter et al. 1999), potentiation of L-type Ca\textsuperscript{2+} channels (Topolnik et al. 2009), and long-term synaptic potentiation (Laezza and Dingledine 2004) in a certain subtype of inhibitory interneurons. It has been reported that spatial profiles of bAP-evoked dendritic Ca\textsuperscript{2+} transients were not different among three subtypes of supragranular inhibitory interneurons from the mouse visual cortex (Goldberg et al. 2003a). However, they might be unable to resolve the differential spatial profile of dendritic Ca\textsuperscript{2+} transients in inhibitory interneurons by using a high concentration of Ca\textsuperscript{2+} dyes (Goldberg et al. 2003b, 2004).

In the present study, therefore we characterized the subtypes of inhibitory interneurons grouped by a classification scheme for the rat visual cortical interneurons used in our previous study (Rhee et al. 2003) with single-cell RT-PCR and immunohistochemistry for neurochemicals. We investigated the spatial profiles of dendritic Ca\textsuperscript{2+} transients evoked by somatic APs and their modulation using a low concentration of a high-affinity Ca\textsuperscript{2+} dye in four subtypes of inhibitory interneurons: fast spiking (FS), late spiking (LS), burst spiking (BS), and regular-spiking nonpyramidal (RSNP) cells. We found cell subtype-specific spatial profiles for dendritic Ca\textsuperscript{2+} dynamics and BS cell-specific nicotinic modulation, which might be important in the control of the dendritic function of inhibitory interneurons in cell subtype-specific and dendritic location-dependent manners.

METHODS

Slice preparation

Coronal slices of primary visual cortex were prepared from Sprague-Dawley (SD) rats (Orientbio) on their postnatal days 21–27.
Animal care and surgical procedures were conducted with the approval of the Catholic Ethics Committee of the Catholic University of Korea and were consistent with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Animals were anesthetized with chloral hydrate (400 mg/kg ip). After decapitation, the brains were quickly removed and then immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 25 NaHCO3, 1.25 NaH2PO4, 1 CaCl2, 2 MgSO4, and 10 d-glucose and aerated with a mixture of 95% O2-5% CO2. Coronal slices (300-μm thick) containing the visual cortex were prepared with a vibroslicer (HM650V, Microm, Walldorf, Germany) and allowed to recover in a submerged slice chamber for 30 min at 37°C. The slices were then maintained at room temperature (22–24°C) in the same ACSF before use. The slices were individually transferred to the recording chamber and superfused continuously (1–1.5 ml/min) with the same aerated solution except for the addition of 2 mM CaCl2 and 1 mM MgSO4. The temperature of the bath solution in the recording chamber was maintained at 31–32°C.

Whole cell patch clamp

A standard whole cell patch-clamp technique with a bridge amplifier (BVC-700A, Dagan, Minneapolis, MN) was used to record the membrane potential and to evoke somatic APs. The patch electrodes (4–6 MΩ) were pulled from borosilicate glass on a P-97 flaming-brown micropipette puller (Sutter Instrument, Novato, CA). Patch pipettes were filled with a solution containing (in mM) 130 K-gluconate, 10 KCl, 3 Mg-ATP, 1 Na2-phosphocreatine, 0.3 Na3-GTP, and 10 HEPES (pH 7.25/KOH), supplemented with 50 μM Oregon Green 488 BAPTA-1 (OG-1; 170 nM; Molecular Probes, Eugene, OR) as a Ca2+- indicator. Biocytin (0.5%) was added to the pipette solution when morphological reconstruction of the recorded cells was required. Under visual guidance, using IR-DIC video-microscopy with an upright microscope (BX51-W1 fitted with a ×40/0.80 NA water-immersion objective, Olympus, Tokyo, Japan), potential interneurons in the layer 2/3 of the primary visual cortex (Paxinos and Watson 1997) were distinguished from pyramidal neurons according to bipolar or multipolar dendritic processes as well as bipolar, oval, or rounded somata. Typical access resistance was 15–20 MΩ. Electrophysiological parameters were measured as previously described (Rhie et al. 2003). Membrane potentials were not corrected for ~14-mV junction potential. Input resistance (IR) and time constant (τ) were measured with a hyperpolarization response of ~5–15 mV from the resting membrane potential (RMP) by negative step current injection into the soma (40–50 pA, 300 ms). The AP was generated with a graded step-current injection of 10–20 pA for 980 ms from the RMP. With the exception of AP adaptation, all the parameters were measured at the first AP evoked by minimal current injection into the soma, were measured. The fluorescence signals were obtained using either the line-scan (every 1.4–1.6 ms) or area-scan mode (every 17–24 ms). Every 10 data points for the line-scanned data and dendritic areas of 10 μm in length for the area-scanned data, respectively, were averaged. The distance of the measured dendritic area from the soma was calculated from the center of the soma. Fluorescence signals were background-corrected, and traces were expressed as the relative change in fluorescence [ΔF/F0 = (F – F0)/F0], where F0 is background-corrected prestimulus fluorescence. The peak amplitude of the Ca2+ transients was determined at the largest ΔF/F0 value of the transients. When recording the BS cells or bath application of 4-AP, hyperpolarizing currents were intercalated between depolarizing current injection for AP firing to restrict the additional AP or AP burst firing. When a bath application of bicarbonate (CCh) depolarized cells (see Supplemental Table S1), the membrane depolarization was offset with hyperpolarizing current injection via the recording pipette.

Cytoplasm harvest and reverse transcription

Cytoplasm harvest and reverse transcription (RT) were performed as previously described with minor modification (Lambolez et al. 1992; Wang et al. 2004). In brief, patch pipettes were loaded with a 6.5 μl autoclaved internal solution consisting (in mM) of 142 K-gluconate, 10 KCl, 3 MgCl2, and HEPES (pH 7.25/KOH). Moreover, patch pipettes were also autoclaved to inactivate RNases. After a brief electrophysiological recording (~5 min), the cytoplasm of the neurons was aspirated into a patch pipette with gentle negative pressure under visual control. The cytoplasm in the pipette was then expelled into a 0.2 ml microtube containing 1.5 μl of nuclease-free water and 1 μl of RNaseOUT (40 U/μl), and then immediately frozen in liquid nitrogen and stored at ~80°C before use. After thawing of a frozen cytoplasm on ice, the tube was heated to 65°C for 5 min and placed on ice. The mixture of RT consisting of 2 μl 10× RT buffer, 1 μl mixed 10 mM deoxy NTPs, 4 μl 25 mM MgCl2, 2 μl 0.1 M DTT, 1 μl random hexamer (50 ng/μl), and 1 μl SuperScript III reverse transcriptase was subsequently added and resulted in a total reaction volume of 20 μl. This RT mixture was incubated at 25°C for 10 min, followed by 50 min at 50°C. The reaction was terminated by heating

Staining and confocal reconstruction

Following recording, the slices containing biocytin-loaded cells were fixed overnight at 4°C with 4% paraformaldehyde in 100 mM sodium phosphate buffer (pH 7.4). After three washes with 10 mM sodium phosphate-buffered saline (PBS), the slices were incubated overnight at 4°C with 0.5% Triton X-100 in PBS to permeabilize cell membranes. Subsequently, the slices reacted for 2 h at room temperature with Alexa Fluor 488-conjugated avidin (1 μg/ml in PBS, Molecular Probes) to detect intracellular biocytin. The slices were rinsed and mounted on glass slides using fluorescent mounting medium (DakoCytomation, Carpinteria, CA). The distribution of intracellular biocytin was then reconstructed under confocal microscopy (FV-300, Olympus).

Ca2+ imaging

Fluorescence Ca2+ imaging was performed by laser-scanning confocal microscopy (FV-300, Olympus) ≥25 min after obtaining the cells. Light from an argon ion laser (488 nm) was used for illumination, and the emitting fluorescence was filtered with a 510-nm long-pass filter. Dendrites were traced from the soma with the aid of the fluorescence signal of the calcium indicator. The laser intensity for excitation of the indicator was adjusted to minimize phototoxic damage. Dendritic Ca2+ transients evoked by bAPs, which were generated by a brief (5 ms) current pulse delivered by the recording pipette into the soma, were measured. The fluorescence signals were obtained using either the line-scan (every 1.4–1.6 ms) or area-scan mode (every 17–24 ms). Every 10 data points for the line-scanned data and dendritic areas of 10 μm in length for the area-scanned data, respectively, were averaged. The distance of the measured dendritic area from the soma was calculated from the center of the soma. Fluorescence signals were background-corrected, and traces were expressed as the relative change in fluorescence [ΔF/F0 = (F – F0)/F0], where F0 is background-corrected prestimulus fluorescence. The peak amplitude of the Ca2+ transients was determined at the largest ΔF/F0 value of the transients. When recording the BS cells or bath application of 4-AP, hyperpolarizing currents were intercalated between depolarizing current injection for AP firing to restrict the additional AP or AP burst firing. When a bath application of carbachol (CCh) depolarized cells (see Supplemental Table S1), the membrane depolarization was offset with hyperpolarizing current injection via the recording pipette.

1 The online version of this article contains supplemental data.
at 85°C for 5 min, followed either by chilling on ice or storage at −20°C. All reagents for these RT reactions were purchased from Invitrogen (Eugene, OR).

**Multiplex PCR**

The two steps of multiplex PCR were carried out as described elsewhere to amplify the cDNAs for neurochemical markers (Cauli et al. 1997). All of the resultant cDNA (20 μl) from the single-cell RT-reactions were first simultaneously amplified with primers for the following calcium binding proteins [calretinin (CR) and calbindin D28k (CB)], four neuropeptides [neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), somatostatin (SS), cholecystokinin (CCK)], three enzymes [glutamic acid decarboxylase 65 (GAD65), GAD67, and neuronal nitric oxide synthase (nNOS)], and house-keeping protein GAPDH (as a cytoplasm harvesting control). The PCR primers used are described by Cauli et al. (1997) with the exception of parvalbumin (PV) (Vysokanov et al. 1998), nNOS (Cauli et al. 2004), and GAPDH (Wang et al. 2004). The mixture of the PCR reaction was contained 1 μl of each primer (10 pmol/μl) and 10 μl 5× green GoTaq reaction buffer (Promega, Madison, WI), 1 μl mixed 10 mM deoxy NTPs, and 0.25 μl GoTaq DNA polymerase (5 U/μl, Promega) in a final volume of 50 μl. Thermal cycling conditions were as follows: 5 min of initial denaturation at 94°C, 20 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 35 s, and 7 min of final elongation at 72°C. In a separate amplification, the calcium binding protein PV was co-amplified only with GAPDH under the same PCR conditions as in the preceding text. A second round of PCR was performed using 3 μl of the first PCR products as an amplification template. In the second round of PCR, each cDNA was individually amplified using its specific primer pair in a new tube and 35 cycles of PCR under the same thermal conditions as in the preceding text were run. All primers were obtained from Bioneer (Daejeon). PCR products (10 μl) were run on a 1% agarose gel and stained with SYBR Safe DNA gel stain (Invitrogen) for visualization. The sensitivity of the single-cell RT-multiplex PCR was tested on 250 pg of total RNA without nonspecific amplification. Total RNA was prepared from the visual cortex of SD rats using an RNA STAT-60 (Tel-Test, Friendswood, TX) solution according to the manufacturer’s instructions. The predicted sizes for PCR fragments were 432 (CB), 309 (CR), 359 (NPY), 290 (VIP), 208 (SS), 216 (CCK), 391 (GAD65), 600 (GAD67), 548 (nNOS), 309 (PV), and 315 (GAPDH) bp. Introns-overlapping primer pairs and exclusion of the nucleus during cytoplasm harvesting eliminated contamination of genomic DNA in amplification. Complete removal of genomic DNA was confirmed by RT(−) control, which was performed in the absence of a reverse transcriptase enzyme during the normal RT processes. To rule out mRNA contamination from extracellular surrounding tissue, patch pipettes penetrated the slice without seal formation. After the release of positive pressure and the removal of the pipette from the slice, the pipette content was subjected to RT and multiplex PCR. No PCR product was obtained.

**Immunohistochemistry**

Following reconstruction of biocytin-loaded cells under confocal microscopy, slices were further processed for immunohistochemistry. After removal of the coverslips and thorough washing in PBS, the slices were incubated with PBS containing 1% bovine serum albumin (BSA) and 0.3% triton X-100 overnight, then were incubated with a mixture of mouse anti-PV (1:5,000; Swant, Bellinzona, Switzerland) and goat anti-nNOS (Herbison et al. 1996) antibodies in the same BSA solution for 7 days at 20°C, followed by incubation with rhodamine Red-conjugated donkey anti-goat IgG (1:100; Invitrogen) and Cy5-conjugated donkey anti-mouse IgG (1:200; Invitrogen) overnight. The slices were then mounted in Vectashield (Vector Laboratories) and examined with a confocal laser-scanning microscope (Leica TCS SP2). The long incubation period with primary antibodies was essential to obtain signals in the deep part of the 300-μm-thick slices. The specificity of the antibodies used was thoroughly confirmed elsewhere (Celio 1990; Fukuda and Kosaka 2003; Herbison et al. 1996; Nomura et al. 1997). Moreover, slices containing RSNP cells were used for negative controls in PV and nNOS immunoreactivities.

**Statistics**

All data are expressed as means ± SE. Student’s t-tests and ANOVA with a post hoc Tukey test were used for statistical comparisons. A P value of <0.05 was considered to be statistically significant. To classify visual cortical neurons into groups based on electrophysiological properties, the cluster analysis was used with nine electrophysiological variables (Table 1). Unsupervised clustering using Ward’s linkage with Euclidean distance was employed (Cauli et al. 2000). Before performing the cluster analysis all electrophysiological parameters were normalized to their z scores. Cluster analysis was performed using Systat 11 software (Systat Software, Chicago, IL).

**Drugs**

The manufacturers of chemicals and enzymes for the single-cell RT-PCR, fluorescent indicators are indicated in the preceding text. DNQX disodium salt, bicuculline methiodide, d-APV, and mecamylamine hydrochloride were obtained from Tocris bioscience (Bristol, UK). CCh, atropine, 4-AP, and all other chemicals were purchased from Sigma (St. Louis, MO).

**RESULTS**

**Classification of inhibitory interneurons**

We classified inhibitory interneurons primarily with spiking patterns evoked by step current injection, which should repre-

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**TABLE 1. Electrophysiological properties of layer 2/3 visual cortical neurons**

<table>
<thead>
<tr>
<th>Cell Subtype</th>
<th>Pyr (22)</th>
<th>FS (11)</th>
<th>LS (28)</th>
<th>BS (32)</th>
<th>RSNP (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP, mV</td>
<td>−78.56 ± 1.34bcde</td>
<td>−72.32 ± 2.15c</td>
<td>−69.59 ± 1.29w</td>
<td>−69.55 ± 1.19w</td>
<td>−63.87 ± 1.08bcd</td>
</tr>
<tr>
<td>IR, MΩ</td>
<td>125.2 ± 8.2</td>
<td>141.1 ± 7.3w</td>
<td>172.2 ± 13.5w</td>
<td>354.7 ± 16.2wbc</td>
<td>285.1 ± 28.1wbc</td>
</tr>
<tr>
<td>τ, ms</td>
<td>16.0 ± 0.7bc</td>
<td>7.0 ± 0.4ade</td>
<td>10.5 ± 0.5abc</td>
<td>18.1 ± 0.8bc</td>
<td>18.8 ± 1.4abc</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>−37.7 ± 1.3b</td>
<td>−30.8 ± 2.1id</td>
<td>−34.2 ± 0.9ed</td>
<td>−38.5 ± 1.0bc</td>
<td>−35.2 ± 1.3</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>67.8 ± 1.8bcde</td>
<td>38.3 ± 2.0ade</td>
<td>52.4 ± 1.4babc</td>
<td>45.9 ± 1.3abc</td>
<td>48.7 ± 2.3abc</td>
</tr>
<tr>
<td>AP width, ms</td>
<td>1.42 ± 0.06bcde</td>
<td>0.61 ± 0.04abc</td>
<td>0.97 ± 0.02ab</td>
<td>0.99 ± 0.03ab</td>
<td>1.04 ± 0.08ab</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>−13.3 ± 0.5bcde</td>
<td>−21.8 ± 0.9wde</td>
<td>−20.4 ± 0.8abcde</td>
<td>−0.2 ± 0.6bcde</td>
<td>−12.0 ± 0.8abcd</td>
</tr>
<tr>
<td>P-T, ms</td>
<td>55.3 ± 2.7</td>
<td>4.6 ± 0.7</td>
<td>11.3 ± 0.7</td>
<td>3.0 ± 0.2</td>
<td>23.7 ± 2.0</td>
</tr>
<tr>
<td>AP adaptation</td>
<td>1.18 ± 0.02c</td>
<td>0.97 ± 0.01c</td>
<td>1.02 ± 0.01c</td>
<td>NA</td>
<td>1.76 ± 0.22bc</td>
</tr>
</tbody>
</table>

ANOVA with Tukey test were used for statistical comparison. P < 0.05 was considered to be statistically significant. Numbers in parentheses are numbers of cells. NA, not applicable; RMP, resting membrane potential; IR, input resistance; AP, action potential; AHP, after hyperpolarization; Pyr, pyramidal; FS, fast spiking; LS, late spiking; BS, burst spiking; RSNP, regular spiking nonpyramidal; P-T, peak-trough. *vs Pyr; †vs FS; ‡vs LS; §vs BS; ¶vs RSNP.
sent passive and active membrane properties—one of the most important factors to specify Ca\(^{2+}\) dynamics by the influx of extracellular Ca\(^{2+}\) (Fig. 1). The group of interneurons based on frequently encountered spiking patterns was named with reference to previous reports from other cortical areas (Kawaguchi 1997; Kawaguchi and Kubota 1997) and from our previous study (Rhie et al. 2003). Quantitative analysis for membrane parameters was performed on cells successful for Ca\(^{2+}\) imaging. FS cells were characterized by abrupt nonadapting fast spikes (>40 Hz) after a slight depolarizing ramp by near-threshold current. The membrane time constant was short (7.0 ± 0.4 ms, \(n = 11\)) and the IR was low (114.1 ± 7.3 MΩ). LS cells exhibited slow nonadapting spikes (8–20 Hz) with slow depolarizing ramp potential before spike generation with an intermediate membrane time constant (10.5 ± 0.5 ms, \(n = 28\)) and an IR value (172.2 ± 13.5 MΩ). These LS cells constituted a large population in supragranular layers of rat visual cortex (32% of all inhibitory interneurons in this experimental set, \(n = 86\)). BS cells were characterized by a burst of two or three spikes at high frequency on a slow depolarizing hump at the beginning of plateau depolarization that were followed by few spikes, with a slow membrane time constant (18.1 ± 0.8 ms, \(n = 32\)) and a high IR (354.7 ± 16.2 MΩ). RSNP cells showed a generation of adapting slow spikes similar to pyramidal cells. They exhibited a similar membrane time constant (18.8 ± 1.4 ms, \(n = 15\)) and a twofold higher IR (285.1 ± 28.1 MΩ), compared with pyramidal neurons (16.0 ± 0.7 ms and 125.2 ± 8.2 MΩ, respectively, \(n = 22\)). We summarized all the analyzed membrane parameters in Table 1, which were consistent with results found in our previous study (Rhie et al. 2003). One difference was that the AP widths of the inhibitory interneurons in the present study were a little longer than those found in the previous study. Because capacitance compensation is critical for the recording of the fast events, individual variation for visual compensation and response time of the amplifiers seemed to cause this difference.

We compared our grouping of inhibitory interneurons by characteristic spiking patterns with groups segregated by unsupervised cluster analysis using all the electrophysiological dataset in Table 1. BS cells were separated from the other cells because they were clearly identified as a nonoverlapping group, and a variable for AP adaptation was missing in this cell subtype. Although some exceptions remained (5 of 108 cells), cluster analysis yielded well-suited segregation of inhibitory interneurons with our classification (Fig. 2). Thus grouping by spiking patterns is likely to be appropriate for studying cell subtype-specific properties of membrane excitability-related physiological events.

### Neurochemical markers for inhibitory interneurons

In a previous report of the mouse supragranular visual cortex, LS cells were not identified (Goldberg et al. 2003a). However, in the present study of rat visual cortex, FS and LS cells were clearly distinguished with electrophysiological features especially by spiking frequencies. Because FS and LS cells exhibited overlapping morphologic features, molecular markers were identified so they could be grouped as different cell subtypes with single-cell RT-PCR (Fig. 3). A different set of the recorded cells was used for this experiment. We also performed cluster analysis for quantitative electrophysiological parameters taken in these cells.

![FIG. 1. Spiking patterns and dendritic morphology of supragranular inhibitory interneurons in the rat primary visual cortex. Representative spiking patterns evoked by positive step current injection into the soma is shown in the top 2 rows for fast spiking (FS), late spiking (LS), burst spiking (BS), and regular spiking nonpyramidal (RSNP) cells. Current steps injected for 980 ms are shown in the 3rd row. Spikes generated by the largest current injection are shown in the top row for easy identification. Reconstructed cellular morphology obtained by biocytin staining is shown on the bottom row. Scale bars represent 100 μm.](http://jn.physiology.org/)

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**DENDRITIC CALCULUS DYNAMICS OF INHIBITORY INTERNEURONS**

**J Neurophysiol • VOL 104 • AUGUST 2010 • www.jn.org**
population characterized by weak immunoreactivity for nNOS was further confirmed by observations of the tissues surrounding the recorded cells (Fig. 4C, arrows). These cells had small, round soma and were scattered mainly in supragranular layers. Similar weakly nNOS-positive neurons have been described in the mouse visual cortex (Lee and Jeon 2005), which might correspond to a subpopulation of LS cells as demonstrated here in the rat visual cortex. As seen in Fig. 4C, there was another type of nNOS neurons that had a much larger soma and exhibited intense nNOS labeling. We could not obtain these cells in the present study, most likely because this subtype makes up a very small portion (0.5%) of GABAergic neurons in the rat visual cortex (Gonchar and Burkhalter 1997). Thus we concluded that weakly nNOS-immunoreactive nonpyramidal neurons having small soma represent a part of the LS cell population in the rat visual cortex. By contrast, PV immunoreactivity was found only in FS cells (3 of 8 cells), whereas the vast majority of LS cells (14 of 15 cells) were PV negative. Negative PV immunoreactivity in some FS cells might be due to the free diffusion of PV into the pipette during long recording sessions (Schmidt et al. 2007).

In addition to cluster analysis (Fig. 2 and Supplemental Fig. S1), these results from single-cell RT-PCR and immunohistochemical staining for neurochemicals verify that inhibitory interneurons in supragranular layers of the rat primary visual cortex can be classified by the free diffusion patterns into four main subtypes despite some overlapping electrophysio- and morphological features.

**Subtype-specific spatial profiles of bAP-evoked dendritic Ca\(^{2+}\) transients**

To study the spatial profiles of dendritic Ca\(^{2+}\) transients evoked by somatic APs in four subtypes of inhibitory interneurons, we used a low concentration (50 \(\mu M\)) of high-affinity Ca\(^{2+}\) dye, OGB-1 (Fig. 5). Single or burst APs at 20 Hz were evoked by step current injection (5–10 ms, 200–900 pA) into the soma. FS cells showed only small Ca\(^{2+}\) transients with a similar amplitude in all measured dendritic arbors \(\leq 105 \mu m\) from the soma (\(n = 11\) cells) (Fig. 5A). These Ca\(^{2+}\) transients increased with the number of somatic APs \(\leq 10\) APs (20 Hz). Although the length of targeted dendrites in FS cells was \(118 \pm 12 \mu m\) (\(n = 13\)), we could obtain dendritic Ca\(^{2+}\) transients \(\leq 72 \pm 6 \mu m\) from the soma, because of the low fluorescent signal in thin distal dendrites. LS cells showed a profile of dendritic Ca\(^{2+}\) transients that was similar to that of FS cells, with the exception of the amplitude: LS cells exhibited larger bAP-evoked Ca\(^{2+}\) transients of similar amplitude along the dendritic tree up to \(\approx 120 \mu m\) from the soma, which increased linearly with the number of somatic APs (\(n = 23\) cells).

Peak amplitude of bAP-evoked dendritic Ca\(^{2+}\) transients in BS cells increased as distance from the soma increased (\(n = 32\) cells) and reached a plateau at \(\approx 50–100 \mu m\) from the soma. A single exponential function analysis of peak amplitude to the dendritic distance yielded increasing length constants (the distance at 63% of maximal amplitude) with the number of APs (16.3 \(\pm 7.7\), 24.8 \(\pm 8.1\), and 30.1 \(\pm 11.5\) \(\mu m\) from the soma to single, 3, and 5 APs, respectively). Because we could
find no difference in the spatial profiles between apical and basal dendrites, we pooled the data in this analysis. A previous study has reported widespread but decreased AP-evoked Ca\(^{2+}\)/H\(_\text{11001}\) transients in the distal dendrites of bitufted interneurons in the somatosensory cortex (Kaiser et al. 2001). Although the bipolar nature of bitufted cells corresponds to that of the BS cells in the present study, they have no burst firing with current injection, which is one of electrophysiological characteristics of BS cells. Based on the regular spiking pattern of bitufted cells in previous studies (Kaiser et al. 2001; Reyes et al. 1998), they might be a subset of RSNP cells in the present study. The relatively younger age of animals in their study (P14–16) might be responsible for the discrepancy in AP firing and bAP-evoked Ca\(^{2+}\) transients (Okyt et al. 2009).

RSNP cells exhibited a bell-shaped profile in bAP-evoked Ca\(^{2+}\) transients along the dendritic length up to \(\sim 180 \mu\text{m}\) from the soma \((n = 19\) cells). Interestingly, the spatial profile of the RSNP cells was similar to that of pyramidal cells, as shown in our previous study (Cho et al. 2006, 2008) as well as in the study of others’ (Waters et al. 2003). However, the amplitude of the Ca\(^{2+}\) transients in RSNP cells was smaller than that of the pyramidal cells: the largest peak amplitude of \(\Delta F/F_0\) of Ca\(^{2+}\) transients evoked by five APs at 20 Hz from the baseline level was \(\sim 2.7\) in pyramidal neurons of our previous study (Cho et al. 2008), and it was \(\sim 1.8\) in RSNP cells in the present study.

Collectively, the dendritic Ca\(^{2+}\) transients evoked by bAPs were lower in inhibitory interneurons than in pyramidal neurons. When decay time constants of Ca\(^{2+}\) transients evoked by five APs were analyzed from single exponential fits in whole dendritic trees, the decay time constant of LS cells showed the smallest value (341 \pm 28 ms). FS and BS cells followed (406 \pm 38 and 478 \pm 31 ms, respectively) and RSNP cells showed the largest decay time constant (576 \pm 57 ms). These values were larger than those found in pyramidal cells (256 \pm 11 ms, \(P < 0.05\)). These results support that the presence of calcium-binding proteins such as PV, calbindin, and calretinin, might be responsible for the lower increase in Ca\(^{2+}\) in interneurons (Goldberg et al. 2003a; Kaiser et al. 2001). In addition, the lower increases might involve the smaller amplitude and shorter duration of APs in inhibitory interneurons (Vinet and Sik 2006).

Based on our visual inspection of the data, the amplitude of Ca\(^{2+}\) transients along the dendritic tree were fitted with the line function for FS and LS cells, with the exponential function for
These results clearly demonstrate that the spatial profiles of bAP-evoked Ca\(^{2+}\) transients along the dendritic trees in different interneuron subtypes have distinct characteristics. The cell subtype-specific location-dependent increase in dendritic [Ca\(^{2+}\)], might be involved in many cellular functions, endowing cell-specific function and modulation in the cortical circuit.

**Inhibition of A-type K\(^+\) channels on bAP-evoked dendritic Ca\(^{2+}\) transients**

Transient A-type K\(^+\) channels restrict invasion of APs into dendrites, and, thus limit the influx of Ca\(^{2+}\) into dendrites, which is significant for distal dendrites in hippocampal (Hoffman et al. 1997) and neocortical pyramidal neurons (Cho et al. 2008) and in neocortical inhibitory interneurons (Goldberg et al. 2003a). Thus we investigated the effect of the inhibition of A-type K\(^+\) channels on dendritic Ca\(^{2+}\) transients evoked by bAPs to characterize further cell subtype-specific dendritic Ca\(^{2+}\) dynamics (Fig. 6). On the bath application of 4-AP (1–2 mM), bAP-evoked Ca\(^{2+}\) transients were increased in all dendritic areas. However, whereas distal dendrites (154.3 ± 17.9% from the control) showed significantly larger increases in Ca\(^{2+}\) transients than proximal dendrites (58.5 ± 9.8% from the control) in RSNP cells (n = 5, P < 0.01), FS (n = 7), LS (n = 7) and BS cells (n = 4) showed a similar increase in Ca\(^{2+}\) transients between proximal and distal dendrites (Fig. 6). Thus we clearly demonstrated that A-type K\(^+\) channels significantly restricted the backpropagation of bAP into dendritic trees in all subtypes of interneurons with a greater effect in the distal dendrites of RSNP cells.

In this experiment, AP amplitude and width were increased markedly in all subtypes of inhibitory interneurons with the application of 1–2 mM 4-AP (Fig. 7A). Both A-type K\(^+\) channels and fast-activating delayed rectifier K\(^+\) channels assembled from Kv3.1 and Kv3.2 subunits are preferentially inhibited by 4-AP at this concentration. Because it has been reported that the blocking of fast-activating delayed rectifier K\(^+\) channels increases the AP amplitude and width in fast-spiking interneurons of mouse somatosensory cortex (Erisir et al. 1999), we addressed this issue further by examining the effect of a low concentration of 4-AP (0.1 mM). The amplitude and width of somatic APs were not changed significantly, except for a slight increase in the AP width of RSNP cells (122.6 ± 3.2% of the control, n = 3, P < 0.05). However, a low concentration of 4-AP did not increase bAP-evoked dendritic Ca\(^{2+}\) transients in all subtypes of inhibitory interneurons (Fig. 7D). A low concentration of TTX (1 mM), which blocks >80% of Kv3.1 and Kv3.2 subunits (Erisir et al. 1999), did not increase bAP-evoked dendritic Ca\(^{2+}\) transients, while both AP amplitudes and widths in FS, BS, and RSNP cells were slightly increased. These results suggest that fast-activating delayed rectifier K\(^+\) channels are minimally involved in the shaping of APs in visual cortical interneurons, which is consistent with the finding of 1 mM TTX by Goldberg et al. (2003a). Thus bAP-evoked dendritic Ca\(^{2+}\) transients were increased by the blocking of A-type, but not by fast-activating delayed rectifier K\(^+\) channels in inhibitory interneurons of the rat primary visual cortex. Our results also indicate that A-type K\(^+\) channels significantly limit the propagation of somatic transients.
APs into distal dendrites in all interneuron classes, and exhibit a greater effect in distal dendrites of RSNP cells. In addition, an increase in dendritic Ca\(^{2+}\) transients by the blocking of A-type K\(^+\) channels appears to be caused partly by an increase in the amplitude and width of somatic AP in inhibitory interneurons.

**FIG. 5.** Spatial profiles of dendritic Ca\(^{2+}\) transients evoked by somatic action potentials (APs) in inhibitory interneurons. Dendritic Ca\(^{2+}\) transients evoked by somatic APs were measured in inhibitory interneurons, which were grouped into FS (A), LS (B), BS (C), and RSNP (D) cells primarily by spiking patterns generated by step current injection into the soma just after obtaining cells. Left: the representative traces of Ca\(^{2+}\) transients at the numbered dendritic areas of the reconstructed cells with 100 \(\mu\)m scale bar insets. Ca\(^{2+}\) transients were evoked by single or bursts of 3 or 5 APs at 20 Hz (bottom). An additional 10 APs at 20 Hz were applied to the FS cells. Right: the spatial profiles of dendritic Ca\(^{2+}\) transients evoked by single APs and bursts of 5 APs at 20 Hz for each cell subtype. The length of the targeted dendrite, which was measured after the experiment.
Nicotinic modulation of bAP-evoked dendritic Ca\textsuperscript{2+} transients in BS cells

Balance between excitatory and inhibitory activities in cortical circuits is critical for proper functioning of the cortex. Both muscarinic and nicotinic receptors regulate excitability of inhibitory interneurons. Although a substantial amount of information on cholinergic modulation of dendritic Ca\textsuperscript{2+} transients in pyramidal neurons has been reported (Cho et al. 2008; Larkum et al. 2003; Nakamura et al. 2000), relatively little is known about the cholinergic modulation of dendritic Ca\textsuperscript{2+} transients in neocortical inhibitory interneurons. Thus we investigated the cell subtype-specific modulation of dendritic Ca\textsuperscript{2+} transients evoked by AP backpropagation. Cholinergic agonist CCh (20 \textmu M) in the bath (for 5 min at a flow rate 1 ml/min) activates muscarinic receptors, resulting in secondary Ca\textsuperscript{2+} release from the store via an IP\textsubscript{3}-dependent mechanisms in pyramidal neurons, as shown by the present experiment (Fig. 8A, Pyr), and in other reports (Cho et al. 2008; Nakamura et al. 2000). In contrast to excitatory pyramidal neurons, CCh failed to increase dendritic Ca\textsuperscript{2+} transients evoked by bAP in all subtypes of inhibitory interneurons (Fig. 8A). Rather one subtype of inhibitory interneurons, BS cells (18 of 21 cells), exhibited a constant decrease in Ca\textsuperscript{2+} transients along the dendritic tree (single AP: 72.6 \pm 2.9\% of the control; 3 APs: 70.7 \pm 2.2\% of the control, n = 18 cells, P < 0.01 each; Fig. 8B). Although changes in RMP were offset by negative current injection to the initial level after CCh application in this experiment, the decrease in Ca\textsuperscript{2+} transients might have resulted from the elevated baseline Ca\textsuperscript{2+} concentration by the nicotinic stimulation of BS cells in the present study (Supplemental Table S1), which was also reported previously (Gulledge et al. 2007). Thus we addressed this issue in another set of experiments. We found no changes in baseline Ca\textsuperscript{2+} levels after CCh application in BS cells, while bAP-evoked Ca\textsuperscript{2+} transients were attenuated with the application of CCh and recovered with a washout of CCh (n = 5, Supplemental Fig. S2).

We next examined which subtype of acetylcholine receptors is involved in the cholinergic decrease of dendritic Ca\textsuperscript{2+} transients in BS cells. As shown in Fig. 8C, the application of mecamylamine (30 \textmu M), a nicotinic receptor antagonist, abolished the CCh-induced decrease in bAP-evoked dendritic Ca\textsuperscript{2+} transients (100.6 \pm 2.8\% of the control in proximal dendrites, P < 0.05 vs. CCh only; 96.4 \pm 2.1\% of the control in distal dendrites, P < 0.05 vs. CCh only; n = 4), while atropine (10 \textmu M), a muscarinic receptor antagonist, did not (66.1 \pm 3.1\% of the control in proximal dendrites, P = 0.20 vs. CCh only; 71.5 \pm 7.7\% of the control in distal dendrites, P = 0.26 vs. CCh only; n = 5). The block by mecamylamine was not different between the proximal and distal dendrites of BS cells.
CCh application did not change the shape or parameters of somatic APs (AP threshold: $-37.4 \pm 1.7$ mV before CCh, $-37.1 \pm 1.9$ mV after CCh, $P = 0.59$; AP amplitude: $49.6 \pm 2.3$ mV before CCh, $49.4 \pm 2.4$ mV after CCh, $P = 0.78$; AP width at half-maximal amplitude: $1.0 \pm 0.1$ ms before CCh, $1.0 \pm 0.1$ ms after CCh, $P = 0.31; n = 22$). In accordance with nicotinic effect on Ca$^{2+}$ transients, CCh-induced depolarization was also blocked by a preapplication of mecamylamine ($n = 2$), but not atropine ($n = 2$), in BS cells (data not shown). Thus these results indicate that cholinergic activation increases bAP-evoked dendritic Ca$^{2+}$ transients via muscarinic receptors only in excitatory pyramidal neurons but decrease them via nicotinic receptors only in BS inhibitory interneurons. These results suggest that cholinergic activation might change the network function with differential action on the Ca$^{2+}$-mediated membrane excitability between excitatory and a subset of inhibitory interneurons.

**DISCUSSION**

Dendritic Ca$^{2+}$ dynamics were investigated in four subtypes of inhibitory interneurons (FS, LS, BS, and RSNP cells) in the supragranular layers of the rat primary visual cortex. There are three major aspects of the present study: cell subtype-specific spatial profiles of dendritic Ca$^{2+}$ transients evoked by bAPs, differential involvement of A-type K$^+$ channels in the backpropagation of somatic APs into distal dendrites among subtypes of inhibitory interneurons, and nicotinic inhibition of bAP-evoked dendritic Ca$^{2+}$ transients in BS cells.

**Subtypes of inhibitory interneurons in supragranular layers of the rat primary visual cortex**

In the present study, we did not find low-threshold spiking (LTS) cells in supragranular layers of rat primary visual cortex. Other studies have found LTS cells present as a small population in the supragranular layers of the agranular frontal cortex (Kawaguchi 1995) and abundantly present in layer IV (Beierlein et al. 2000; Gibson et al. 1999) and in the infragranular cortex (Bacci et al. 2003; Goldberg et al. 2004; Xiang et al. 1998). In those studies, some LTS cells showed burst spiking and bitufted dendritic arbors. In the present study, BS cells, which appeared frequently in rat primary visual cortex, had a distinct bipolar shape of the soma, bitufted dendritic arbor, and VIP as a specific neurochemical marker. Thus in the present study, BS cells constituted a different subset from LTS cells.

In a previous report on dendritic Ca$^{2+}$ dynamics of inhibitory interneurons in the supragranular layers of the mouse primary visual cortex, three subtypes were studied: FS, irregular spiking, and adapting cells (Goldberg et al. 2003a). Whereas we found LS cells more frequently than FS cells in the same region of rats, this aforementioned study did not report LS cells having slow nonadapting spikes. It seems that LS cells are the major population of interneurons in layer 1 (Chu et al. 2003; Gulledge et al. 2007). However, Gulledge et al. (2007) also found LS cells in layer 2/3 in the rat neocortex. LS cells have clearly been distinguished from FS cells by their spiking frequencies and characteristic neuronal markers in previous studies (Kawaguchi and Kubota 1996; Uematsu et al. 2008) as well as in the present study. Although a large proportion of LS cells in the present study might be due to the relatively large soma, it is likely that they constitute a major population of inhibitory interneurons in the rat visual cortex. This appears to be an apparent difference in subtypes of inhibitory interneurons among species (Zaitsev et al. 2004).
Non-FS cells are generally subdivided into BS, RSNP and irregular spiking cells. BS cells appear to be a relatively homogeneous population because of the characteristic bursting spikes on the hump and bipolar dendritic arbors. The bipolar dendritic morphology and bursting nature of irregular spiking cells of previous reports (Cauli et al. 1997; Goldberg et al. 2003a) appear to correspond to similar instances of BS cells in the present study. However, RSNP cells in the present study appeared to be relatively heterogeneous, and at least two subgroups could be distinguished (Fig. 2): one having adapting spikes with an initial slow burst and the other without the initial burst. Thus a subset of RSNP cells in the present study might also have been included in the irregular spiking cells in the Goldberg et al. study.

**Spatial profiles of bAP-evoked dendritic Ca\(^{2+}\) transients**

Individual synapses at different dendritic locations might be modulated with a differential amount of Ca\(^{2+}\) influx. In a previous study, dendritic Ca\(^{2+}\) accumulation was studied in three subtypes of interneurons in the mouse visual cortex (Goldberg et al. 2003a). They found barely detectable single AP-evoked Ca\(^{2+}\) transients even in proximal dendrites and no difference in high-frequency AP-evoked Ca\(^{2+}\) accumulation among different subtypes of interneurons, probably by using a high concentration of Ca\(^{2+}\) dye (200 µM calcium green or 400 µM Fluo-4). In their following study, they found global dendritic Ca\(^{2+}\) spikes in layer 5 LTS interneurons by using a lower concentration (100 µM) of Fluo-4 (Goldberg et al. 2004). In the present study, however, we clearly found subtype-specific spatial profiles of bAP-evoked dendritic Ca\(^{2+}\) transients by using a high-affinity dye at low concentration (50 µM OGB-1). Again, the discrepancy might have been due to the different ages of the animals (Isomura et al. 1999; Okaty et al. 2009). It is noteworthy that Ca\(^{2+}\) transients did not experience a decrease in distal dendrites in FS and LS cells but instead increase in BS cells, which differs from the apical dendrites of pyramidal cells. The spatial profiles were similar to those of basal dendrites from pyramidal cells (Cho et al. 2006, 2008). These results imply that the distal synapses in most inhibitory interneurons, with the exception of RSNP cells, might be regulated tightly as a single compartment, which is different from pyramidal neurons with multiple compartments (Antic 2003). Increases in bAP-evoked Ca\(^{2+}\) transients that were larger in distal, than in proximal, dendrites of BS cells might have resulted from a higher surface-to-volume ratio in distal fine dendrites (Schiller et al. 1995), less attenuated propagation of somatic AP into the dendritic tree by a high IR for BS cells (Table 1) and a differential expression of various voltage-gated ion channels along the dendrites (Migliore and Shepherd 2002), which requires further investigation.

**Dendritic propagation of somatic APs in inhibitory interneurons**

A-type K\(^+\) channels critically control the propagation of somatic APs into dendrites in cortical pyramidal neurons.
Nicotinic modulation of bAP-evoked dendritic Ca\(^{2+}\) transients

While it is well known that muscarinic activation increases dendritic Ca\(^{2+}\) transients via IP\(_3\)-dependent Ca\(^{2+}\)-induced Ca\(^{2+}\) release in pyramidal cells (Cho et al. 2008; Nakamura et al. 2000), cholinergic modulation of bAP-evoked dendritic Ca\(^{2+}\) transients in inhibitory interneurons is still lacking. In previous studies, FS cells were nonresponsive to the application of cholinergic agonists (Gulledge et al. 2007; Kawaguchi 1997). On the other hand, phasic nicotinic AChR-mediated excitation was found only in non-FS cells expressing VIP and CCK in layer 2/3 (Gulledge et al. 2007; Porter et al. 1999), LTS cells in layer 5 (Xiang et al. 1998), and layer 1 interneurons of the neocortex (Christophe et al. 2002). In accordance with the previous electrophysiological findings, we have found no cholinergic modulation on dendritic Ca\(^{2+}\) transients in FS cells in the present study. We have also found CCh-induced depolarization (25–30 mV; Supplemental Table S1) and spontaneous firing (data not shown) only in BS cells that expressed both VIP (10 of 10 cells) and CCK (9 of 10 cells). Thus the subset of non-FS inhibitory interneurons exhibiting excitatory response to nicotinic AChR activation is identical to the BS cells in the present study. However, in the present study, bAP-evoked dendritic Ca\(^{2+}\) transients were inhibited by tonic nicotinic activation, which was in contrast to the stimulatory effect on electrical excitability in these BS cells. A leaky membrane with a decreased IR (≈70 MΩ in the present study) might dampen the propagation of bAP into dendritic trees in BS cells. Although we observed no changes in the baseline dendritic Ca\(^{2+}\) levels throughout the CCh application, additional factors might not be excluded, such as the desensitization of dendritic calcium channels by local dendritic depolarization. In addition, although Gulledge et al. (2007) reported nicotinic excitation of two LS cells in layer 2/3, we observed no changes in Ca\(^{2+}\) transients in LS cells with tonic CCh application (n = 4). The lack of cholinergic modulation on dendritic Ca\(^{2+}\) transients in LS cells might be due to rapid receptor desensitization in the LS cells (Gulledge et al. 2007) or to the difference between electrical potential of the soma and the Ca\(^{2+}\) transients in dendrites. Taken together, we confirmed the previous findings on cholinergic modulation of interneurons and clarified that VIP(+) and CCK(+) BS cells were the major subset of inhibitory interneurons modulated by nicotinic activation in the visual cortex.

Functional implication of cell subtype-specific dendritic Ca\(^{2+}\) dynamics

The variability of inhibitory interneurons is very important in that they endow various functions to the cortical network. Subsets of inhibitory interneurons characterized by their electrophysiological, morphological, and molecular nature exert specific functions of the cortical network. Cell subtype-specific profiles of bAP-evoked dendritic Ca\(^{2+}\) transients in inhibitory interneurons in this study might be important for the domain-specific modulation of segregated input from different sources of input (Petreanu et al. 2009; Spruston 2008), which is yet to be studied. Furthermore, the function of the neocortex is modulated in a state-dependent manner by neuromodulators. In addition to cholinergic modulation of the excitatory pathway (Hasselmo 2006), cholinergic modulation of dendritic Ca\(^{2+}\) dynamics in a subtype of inhibitory interneurons could be involved in certain cortical functions.

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