Correlation of Physiological Subgroupings of Nonpyramidal Cells With Parvalbumin- and Calbindin$_{D_{28k}}$-Immunoreactive Neurons in Layer V of Rat Frontal Cortex

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SUMMARY AND CONCLUSIONS

1. To test the hypothesis that physiologically and morphologically different cortical nonpyramidal cells express different calcium-binding proteins, whole-cell current-clamp recording in vivo was combined with intracellular staining and double immunofluorescence in layer V of frontal cortex of rats 16–20 days old.

2. Nonpyramidal cells were first characterized as fast-spiking (FS) or low-threshold spike (LTS) cells, injected with biocytin, and subsequently stained immunohistochemically for parvalbumin and calbindin$_{D_{28k}}$.

3. FS cells were identified by input resistances <350 MΩ, spike width at half amplitude <0.8 ms, and virtually no spike frequency adaptation of spike trains by depolarizing pulses. LTS cells were identified by input resistances >350 MΩ, spike width at half amplitude >0.8 ms, and the discharge of low-threshold spikes from hyperpolarized potentials. Repetitive firing could be induced by a combination of stimulation-induced excitatory postsynaptic potentials with depolarization in FS cells. Repetitive firing was not observed in LTS cells under these conditions.

4. After biocytin injection of layer V cells characterized in this way, subsequent double immunostaining showed that all biocytin-labeled parvalbumin-immunoreactive cells (n = 18) belonged to the FS cells (FS-PV cells), whereas all biocytin-labeled calbindin$_{D_{28k}}$-immunoreactive cells (n = 10) belonged to the LTS cells (LTS-Calb cells).

5. FS-PV cells had smooth or sparsely spiny dendrites, whereas LTS-Calb cells had dendrites with a modest number of spines but fewer than pyramidal cells. FS-PV cells showed denser axonal branches near their somata and extended axons in a more horizontal direction. Some of them could be identified as basket cells by the presence of terminal boutons surrounding somata of other cells. LTS-Calb cells extended their main axons more vertically up to layer I.

6. Double immunofluorescent staining revealed that very few cells in layer V showed immunoreactivity for both calcium-binding proteins but that most cells immunoreactive for the calcium-binding proteins in layer V were also immunoreactive for γ-aminobutyric acid.

7. These results suggest that GABAergic nonpyramidal cells in layer V of neocortex can be divided into two functional groups on the basis of different firing modes, axonal distributions, and calcium-binding protein immunoreactivity: 1) FS-PV cells show repetitive firing by synaptic activation, have axonal arborizations that are more dense near their somata and oriented horizontally, and the cells exhibit parvalbumin immunoreactivity, and 2) LTS cells show low-threshold spikes, have more vertical axonal arborizations up to layer I, and exhibit calbindin$_{D_{28k}}$ immunoreactivity.

INTRODUCTION

The neocortex of the rodent contains pyramidal and nonpyramidal neurons, both classes of which can be divided into physiologically definable subclasses (Connors and Gutnick 1990). Pyramidal cells can be divided physiologically into regular spiking and intrinsically bursting cells on the basis of differences in the firing pattern in response to suprathreshold stimuli (Agmon and Connors 1989; McCormick et al. 1985). Recently, we have revealed that nonpyramidal cells that are mostly interneurons also show physiological heterogeneity (Kawaguchi 1993). Fast-spiking (FS) nonpyramidal cells fire repetitively at depolarized potentials in response to synaptic excitation, whereas low-threshold spike (LTS) nonpyramidal cells produce low-threshold spikes at hyperpolarized potentials. FS cells show more dense innervation near their somata and tend to extend their axons more horizontally along layers. Axons of LTS cells extend more vertically up to layer I. These observations suggest that subgroups of intrinsic neocortical cells with specific membrane biophysical properties show different innervation patterns that tend to follow either a layered or a columnar organization. For further understanding of the functional differences between these two classes of nonpyramidal cells, it is important to investigate the synaptic actions of the cells on their target cells and the neurotransmitters that they utilize.

Most nonpyramidal cells in neocortex contain the inhibitory transmitter, γ-aminobutyric acid (GABA) (Houser et al. 1983). In addition to GABA, many nonpyramidal cells in the neocortex show immunoreactivity for a number of other molecules such as calcium-binding proteins (Ccelio 1986, 1990) and neuropeptides (Hendry et al. 1984; Somogyi et al. 1984). These molecules are often differentially distributed among GABAergic nonpyramidal cells. For example, chandelier cells that mainly make synapses on initial axon segments of pyramidal cells, and basket cells that mainly make axosomatic synapses, possess one calcium-binding protein, parvalbumin (DeFelipe et al. 1989a; Hendry et al. 1989), whereas double bouquet cells that primarily innervate dendrites possess another calcium-binding protein, calbindin$_{D_{28k}}$ (DeFelipe et al. 1989b). These observations suggest that nonpyramidal cells in neocortex with different synaptic targets and making different contributions to local cortical circuitry may be chemically distinct.
In the hippocampus two physiological classes of nonpyramidal cells with specific spike-firing patterns also show different innervation patterns and distributions of parvalbumin immunoreactivity (Kawaguchi and Hama 1987; Kawaguchi et al. 1987; Lacaille and Schwartzkroin 1988). FS cells in the stratum pyramidale of the hippocampus innervate the stratum pyramidale horizontally and show parvalbumin immunoreactivity. Another type of nonpyramidal cell innervates the stratum pyramidale and the stratum radiatum vertically and lacks parvalbumin immunoreactivity (Kawaguchi and Hama 1988). Because the neo cortex contains subpopulations of GABAergic nonpyramidal cells, some of which show parvalbumin immunoreactivity and others immunoreactivity for calbindin-D28K (Demeulemeester et al. 1989, 1991; Hendry et al. 1989), these observations suggest that, in analogy to the hippocampal, neocortical FS cells may be parvalbumin-containing GABAergic cells, whereas LTS cells may lack parvalbumin and instead may be calbindin-D28K-containing GABAergic cells. The present study was undertaken to confirm the GABAergic nature of the two functional types of nonpyramidal cells and to extend further our understanding of the significance of parvalbumin and calbindin-D28K in relation to physiologically defined subclasses of neocortical cells.

**METHODS**

*In vitro slice preparation and solutions used*

The slicing and recording procedures used have been described in detail previously (Kawaguchi 1992, 1993). The experiments were performed on young Wistar rats (16–20 days postnatal). The animals were anesthetized deeply with ether and decapitated. The brains were quickly removed, submerged in ice-cold physiological Ringer, and hemisected. Sections (180 μm thick) were cut on a Microslicer (Dosska EM) through the medial frontal agranular cortex in a plane oblique to the horizontal along the line of radial fissure. Slices were then incubated in oxygenated Ringer solution at a temperature of 29–30°C. The standard Ringer solution had the following composition (in mM): 124.0 NaCl, 3.0 KCl, 2.4 CaCl₂, 1.3 MgCl₂, 26.0 NaHCO₃, 1.0 NaH₂PO₄, 10.0 glucose, and was continuously bubbled with a mixture 95% O₂-5% CO₂. After incubation for at least 2 h, a single slice was transferred to a recording chamber placed on the stage of an upright microscope (Edwards et al. 1989) and was totally submerged in the superfusing medium at 26–27°C.

*Whole-cell recording and stimulation*

Individual neocortical cells were visualized near the surface of the slice using a ×40 water immersion objective. Layer V cells that appeared smaller than typical pyramidal-shaped cells and that lacked obvious apical dendrites were preferentially targeted to obtain a higher sampling of nonpyramidal cells. Whole-cell recordings with a high seal resistance (>2 GΩ before break-in) were obtained with borosilicate glass pipettes by the use of an amplifier with bridge balance and capacitive compensation. Recorded cells were finally identified as pyramidal or nonpyramidal cells by intracellular staining.

Electrodes were filled with a solution containing (in mM) 120 K-methylsulfate, 0.6 ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N″-tetraacetic acid (EGTA), 2.0 MgCl₂, 4.0 ATP, 0.3 guanosine triphosphate (GTP), and 10 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES). The resistance of the electrodes filled with this solution was 5–6 MΩ. To identify the morphology of recorded cells, 0.5% biocytin (Sigma) was added to the electrode to stain cells by diffusion (Horikawa and Armstrong 1988). Electrical stimulation was applied by bipolar stimulating electrodes placed between the white matter and the lower part of the layer VI. Stimuli of 100-μs duration and 0.2- to 0.5-mA current intensity were delivered at 0.2–0.4 Hz.

After physiological identification, whole-cell recording was terminated as soon as possible to lower the possibility of reducing the somatic concentration of calcium-binding proteins by exchange of intracellular fluid with the pipette solution, which could potentially weaken the immunoreactivity of the soma. Most recordings were finished and the slices put into the fixative within 10 min after breaking the patched membrane.

*Immunohistochemical procedure for immersion-fixed slices*

Slices containing biocytin-loaded cells were fixed by immersion in 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB) for 1–2 h at room temperature and incubated in PB containing 30% sucrose for 30 min. The tissue was frozen with dry ice and thawed at room temperature twice. The slices, without resectioning, were then washed with Trius-buffered saline (TBS) and incubated with the streptavidin-conjugated 7-amino-4-methyl-

*Immunochemistry in perfusion-fixed brains*

Eight male Wistar rats (16 and 20 days postnatal) were used. The animals were given an overdose of pentobarbital sodium
NONPYRAMIDAL CELLS AND CA\(^{2+}\)-BINDING PROTEINS

Identification of two physiological classes of nonpyramidal cells

Two classes of nonpyramidal cells were easily distinguished by responses to intracellular current injection and synaptic stimulation (Kawaguchi 1993). Input resistances were determined by hyperpolarizing current pulses (duration, 500–600 ms) inducing voltage shifts of 6–15 mV negative to rest. Spike widths were determined by depolarizing current pulses (duration, 50 ms) and faster sweeps. FS cells were identified by input resistances <350 MΩ, spike widths at half amplitude <0.8 ms, and almost no spike-frequency adaptation of spike trains to depolarizing pulses (Fig. 1, A1–A3). In contrast, LTS cells were identified by input resistances >350 MΩ, spike widths at half amplitude >0.8 ms, and the firing of low-threshold spikes from depolarized potentials. LTS cells showed spike-frequency adaptation during depolarizing current pulses (Fig. 1, B1–B3). They also could be identified by firing patterns induced by synaptic activation. Repetitive firing could be induced in FS cells by a combination of stimulation-induced excitatory postsynaptic potentials (EPSPs) and depolarization (Fig. 1, A4 and A5). Repetitive firing was not observed in LTS cells under these conditions. Low-threshold spikes could be induced in LTS cells by synaptic activation at depolarized potentials (Fig. 1, B4 and B5).

Immunohistochemical identification of recorded nonpyramidal cells

Nonpyramidal cells injected with biocytin were visualized by AMCA-conjugated avidin (blue fluorescence; Fig. 2, A and C). Parvalbumin and calbindin\(_{\text{D28K}}\) immunoreactivity in recorded cells was visualized by TRITC-conjugated (red fluorescence, Fig. 2B) and FITC-conjugated (green fluorescence, Fig. 2D) secondary antibodies, respectively. Among 44 nonpyramidal cells identified as either FS cells (n = 26) or LTS cells (n = 18), 28 showed immunoreactivity for one or other of the calcium-binding proteins. Among these, 18 cells showed parvalbumin immunoreactivity (Fig. 2, A and D), and 10 cells showed calbindin\(_{\text{D28K}}\) immunoreactivity (Fig. 2, C and D). Nonpyramidal cells showing both parvalbumin and calbindin\(_{\text{D28K}}\) immunoreactivity were not encountered in this study. All identified nonpyramidal cells with parvalbumin immunoreactivity belonged to the group of FS cells (FS-PV cells), whereas all identified nonpyramidal cells with calbindin\(_{\text{D28K}}\) immunoreactivity belonged to the group of LTS cells (LTS-Calb cells; Fig. 3, Table 1).

FS-PV cells (n = 18) had input resistances <350 MΩ (131–295 MΩ) and spike widths at half amplitude <0.8 ms (0.48–0.81 ms), whereas LTS-Calb cells (n = 10) had input resistances >350 MΩ (372–890 MΩ) and spike widths >0.8 ms (0.98–1.52 ms; Fig. 3, Table 1). Resting membrane potentials of FS-PV cells (−66 to −79 mV) were more negative than those of LTS-Calb cells (−56 to −65 mV; Table 1). Membrane time constants of LTS-Calb cells (29–69 ms) were four times longer than those of FS-PV cells (7–14 ms). After hyperpolarizations (A1Ps) following spikes were monophasic in FS-PV cells but showed two components with early and late peaks in LTS-Calb cells.
Comparison of morphological characteristics between FS-PV cells and LTS-Calb cells

The DAB reaction product in injected cells was weaker than in FS and LTS cells reported previously (Kawaguchi 1993) probably because of reduced concentration of biocytin due to the shorter recording times and the added immunohistochemical procedures. In addition, parts of the cells were lost by sectioning off the slice surface to facilitate penetration of immunoreagents. Nevertheless, morphological differences between FS-PV cells and LTS-Calb cells comparable with those previously reported in FS and LTS cells could still be visualized (Figs. 4 and 5). Distributions of somatic diameters as measured across the long and short axes of the somata were similar in the two groups (Table 1). In the long axis, somatic diameters of FS-PV cells ranged from 12.0 to 18.0 μm. Somatic diameters in the long axis of LTS-Calb cells ranged from 13.1 to 18.7 μm. FS-PV cells had dendrites that were smooth or possessed very few dendritic spines. Among six FS cells with dendrites close to layers II/III, dendrites of three cells avoided entering layer II/III by curving or running along the border, and those of the other three cells extended into layers II/III (Fig. 4A). LTS-Calb cells had dendrites with few or a modest number of spines, although they were always less spiny than those of pyramidal cells. Axons of FS-PV cells originated from somata (9 cells) and from dendrites (3 cells). Axons of LTS-Calb cells originated from somata (2 cells) and from dendrites (6 cells). The LTS-Calb cells extended dendrites more widely in layers V and VI than FS-PV cells (Fig. 5). FS-PV cells had axonal branches and boutons at the same depth as the somata (5 cells; Fig. 4. A and B) or above the level of the somata (4 cells; Fig. 4C). In both cases, FS-PV cells had denser axonal arborizations and made more boutons near to or just above the somata, and the principal axonal branches tended to run in a horizontal direction. At a distance, these branches of FS-PV cells formed terminal boutons. Because the sections were embedded in Epon in these experiments, it was easier to see the relationship of stained axons and unstained cell bodies by interference microscopy than in the previous study. Some boutons were observed surrounding somata of other cells (Fig. 6). The injected cells forming such terminals were therefore considered as basket cells (Jones and Hendry 1984). By contrast, LTS-Calb cells extended their main axons upward in a more vertical orientation, in two cells up to layer I (Fig. 5). In one LTS-Calb cell, axonal branches also distributed vertically downward in addition to upward (Fig. 5A). These morphological differences between FS-PV cells and LTS-Calb cells were similar to those of FS cells and LTS cells described previously (Kawaguchi 1993). Taken together
with the previous morphological data on well-stained cells without added immunohistochemical staining, the new findings indicate that both FS cells with axonal branches at the same vertical level as the somata and FS cells with axonal branches at levels superficial to the somata show parvalbumin immunoreactivity, and that LTS cells with ascending or with ascending and descending axonal branches both show calbindin_{D28K} immunoreactivity for both calcium-binding proteins. In the 16-day-old rats, parvalbumin- or calbindin_{D28K}-immunoreactive layer V cells showed similar characteristics. The proportion of parvalbumin- and calbindin_{D28K}-immunoreactive cells that showed immunostaining for GABA was also investigated (Fig. 7, C–F). In 20-day-old rats, all 216 parvalbumin-stained cells in layer V showed modest to strong staining for GABA. Among 256 calbindin_{D28K}-stained cells in layer V, 136 cells (53.1%) showed modest to strong staining for GABA, and 130 cells (48.9%) showed weak staining. Cells in 16-day-old rats also showed similar characteristics.

DIGESTION

Our results show that physiologically and morphologically differentiated subgroups of neocortical nonpyramidal cells in layer V are also chemically distinct in regard to calcium-binding protein immunoreactivity but that both contain GABA (Fig. 8). In the following paragraphs we discuss the significance of differential expression of parvalbumin and calbindin_{D28K} in layer V nonpyramidal cells and the different roles of these two groups of cells in neocortical
local circuitry. FS cells lacking parvalbumin and LTS cells lacking calbindin\textsubscript{D28k} may exist in layer V of neocortex, but it is difficult to ascertain their existence by the techniques used in this study.

**Differential expression of two types of calcium-binding proteins in FS cells and LTS cells in layer V**

The present findings are consistent with the results previously obtained in the hippocampus. Nonpyramidal cells in hippocampus are physiologically and morphologically differentiated into two groups (Kawaguchi and Hama 1987, 1988; Lacaille and Schwartzkroin 1988), and one type of nonpyramidal cells (FS cells) shows parvalbumin immunoreactivity (Kawaguchi et al. 1987). Taken together, these studies suggest that, both in neocortex and hippocampus, cells exhibiting a constant higher frequency of spike firing under the particular experimental conditions used have parvalbumin immunoreactivity. In other parts of the CNS, parvalbumin has also been correlated with neurons that are thought to be functionally more active (Celio 1990). Parvalbumin-immunoreactive neurons are found, for example, in the thalamic reticular nucleus, globus pallidus, and substantia nigra pars reticulata in which cells all show higher-frequency discharges than other neurons in the thalamus and basal ganglia. Functional roles of parvalbumin in FS cells of the neocortex may therefore be related to their being more active than other cells. FS cells fire repetitively under the influence of N-methyl-D-aspartate (NMDA) receptor-mediated EPSPs (Kawaguchi 1993). NMDA receptor activation induces not only depolarization but also an increase in intracellular calcium concentration (Nicoll et al. 1990). In addition, long-duration depolarizations and repetitive firing of sodium spikes may activate voltage-dependent calcium channels that also tend to cause a net flow of calcium into the cell. Accumulation of intracellular calcium passing through NMDA receptor channels and voltage-gated channels may activate in turn calcium-activated potassium channels that will cause firing-rate adaptation due to hyperpolarization, probably because of reduced input impedance. Parvalbumin may therefore serve to buffer against intracellular calcium increase after synaptic excitation and thus maintain a nonadapting high-frequency firing rate. When synaptic excitation is above the threshold for NMDA receptor activation, the firing pattern of FS cells can switch from single spike firing mode to repetitive high-frequency firing mode that is dependent on NMDA recep-

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**TABLE 1. Properties of FS-PV cells and LTS-Calb cells**

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<tr>
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<th>FS-PV Cells</th>
<th>LTS-Calb Cells</th>
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<tbody>
<tr>
<td>Soma diameter (long)</td>
<td>13.8 ± 1.6</td>
<td>15.5 ± 2.5</td>
</tr>
<tr>
<td>Soma diameter (short)</td>
<td>10.6 ± 1.6</td>
<td>11.0 ± 1.7</td>
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<tr>
<td>Resting potential, mV</td>
<td>≈73.0 ± 3.9</td>
<td>≈59.7 ± 3.4</td>
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<tr>
<td>Spike height, mV</td>
<td>95.8 ± 7.9</td>
<td>80.7 ± 10.9</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>204 ± 52</td>
<td>618 ± 197</td>
</tr>
<tr>
<td>Spike width at half amplitude, ms</td>
<td>0.59 ± 0.09</td>
<td>1.24 ± 0.22</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>10.9 ± 1.9</td>
<td>44.5 ± 12.2</td>
</tr>
<tr>
<td>Time to peak of first AHP, ms</td>
<td>3.9 ± 1.2</td>
<td>8.3 ± 3.1</td>
</tr>
<tr>
<td>Amplitude of first AHP, mV</td>
<td>156.6 ± 4.3</td>
<td>18.6 ± 3.5</td>
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<tr>
<td>Time to peak of second AHP, ms</td>
<td>8 ± 1.2</td>
<td>26.7 ± 5.0</td>
</tr>
<tr>
<td>Amplitude of second AHP, mV</td>
<td>8 ± 1.2</td>
<td>-16.3 ± 2.9</td>
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Data are means ± SD; number of FS-PV cells is 18 and LTS-Calb cells is 10. FS-PV, fast-spiking parvalbumin; LTS-Calb, low-threshold spike calbindin\textsubscript{D28k}; AHP, afterhyperpolarization. *Significant differences by Mann-Whitney U test (P < 0.01). Input resistance and time constant were determined by hyperpolarizing current pulses inducing voltage shift of 6–15 mV negative to rest. †Time to peak and amplitude of AHP were measured from the spike onset. ¶FS cells had 1 component of AHP.
tor-mediated EPSPs (Kawaguchi 1993). Thus NMDA receptor-mediated EPSPs in FS cells may trigger an all-or-none transition to a high-frequency discharge mode that may be supported by parvalbumin.

LTS cells showed immunoreactivity for calbindinD28k rather than for parvalbumin. CalbindinD28k is expressed not only in GABAergic nonpyramidal cells but also in layer II/III pyramidal cells that are excitatory (Celio 1990; DeFelipe and Jones 1992). This means that calbindinD28k is not solely related to inhibitory systems in the neocortex. Electrophysiologically, the discharge of low-threshold spikes is characteristic of LTS cells. However, this physiological property does not seem to be correlated with calbindinD28k immunoreactivity in the CNS. For example, in the neostriatum, both calbindinD28k-positive and calbindinD28k-negative cells with similar somatic and dendritic morpholo-

FIG. 5. Reconstruction of 3 LTS-Calb cells. Axons (Axon) and dendrites (Dend.) are separately drawn. Note the dendrites with a few spines and axon branches running mainly vertically. Dendrites are mainly confined to layers V and VI.

FIG. 6. A: a photomicrograph of an intracellularly stained FS parvalbumin-immunoreactive cell (FS-PV cell). B: higher magnification of A. Note the horizontal orientation of axon branches and boutons surrounding an unstained cell body (*).
gies (Gerfen et al. 1985) have similar electrophysiological properties and neither fires low-threshold spikes (Kawaguchi et al. 1989). This suggests that neurotransmitter types and basic electrophysiological properties recorded in the soma such as input resistance and firing pattern are not directly related to the presence of calbindin<sub>D28k</sub>. Calbindin<sub>D28k</sub>-immunoreactive cells such as LTS-CAb cells may have dendritic physiological properties that differ from those of calbindin<sub>D28k</sub>-negative cells and that the somatic recordings used here could not reveal. For example, a sub-type of calcium channels may be correlated with calbindin<sub>D28k</sub> immunoreactivity (Celio 1990), and these calcium channels might be concentrated on dendrites.

Functional differentiation of GABAergic nonpyramidal cells in neocortex

Two physiological subtypes of nonpyramidal cells in layer V, FS cells and LTS cells, are immunoreactive either for parvalbumin or for calbindin<sub>D28k</sub>. Because most layer V
neurons containing these calcium-binding proteins also contain GABA, both FS cells and LTS cells can be considered GABAergic. This suggests that GABAergic neurons in cortex are functionally differentiated into at least two subtypes by firing and innervation patterns. Similar subtypes of GABAergic nonpyramidal cells have not yet been morphologically characterized in other layers but similar morphological differences are found. GABAergic inhibitory postsynaptic potentials (IPSPs) are mediated by both $\alpha_1$ and GABA$_B$ receptor subtypes, and IPSPs mediated by both receptor subtypes are produced in neocortical cells by intrinsic GABAergic receptors (Connors et al. 1988; Wagauchi 1992; McCormick 1989). These two types of IPSPs are differentially activated in rat frontal cortex by local stimulation, which raises the possibility that the two types of IPSPs may be conveyed by different groups of GABAergic cells (Kawaguchi 1992). Thus the relation of FS-PV and LTS-Calb cells to the putative two populations of GABAergic cells that induce the two types of IPSPs could be clarified.

In addition to calcium-binding proteins, certain neuropeptides are selectively expressed in some GABAergic cortical cells (Jones et al. 1987). Somatostatin (SRIF) is largely localized with calbindinD$_{28k}$ in rat neocortex (Rogers 1992). This suggests that the two types of nonpyramidal cells described here may also release different neuronal substances additional to GABA.

FS-PV cells show denser axonal arborizations near the somata, more preferential innervation in the horizontal direction, and basket terminals surrounding the somata of other neurons, whereas LTS-Calb cells extend their axons more vertically upward toward layer I (see also Kawaguchi 1993). Because both types of nonpyramidal cells in layer V contain GABA, the preferential distributions of their axons suggest that the inhibitory circuits of which they form part can be considered to consist of separate local, horizontal, and vertical systems. Hence local, intralaminar, and intracolumnar inhibition may be structurally separated. GABA-mediated inhibition can be divided into at least three categories (Nicoll et al. 1990): postsynaptic GABA$_A$, presynaptic GABA$_B$, and presynaptic GABA$_D$ inhibition. Differential expression of GABA$A$ receptor subtypes in neocortex may add further complexity to the system (Wisden et al. 1992). Thus neocortical excitability may be regulated in a complex manner with the use of structural diversity of GABAergic innervation, differential expression of GABA receptor subtypes, and different firing modes among subgroups of GABAergic cells with different calcium-binding proteins.

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