Previous analyses of the spiny layer IV neurons have almost exclusively focused on spiny stellate cells. Here we provide detailed morphological data characterizing three subpopulations of spiny neurons in slices of adolescent rats: (i) spiny stellate cells (58%), (ii) star pyramidal cells (25%) and (iii) pyramidal cells (17%), which can be distinguished objectively by the preferential orientation of their dendritic stems. Spiny stellate cells lacked an apical dendrite and frequently confined their dendritic and axonal arbors to the respective column. Star pyramidal and pyramidal cells possessed an apical dendrite, which reached the supragranular layers. Their axonal arbors were similar, showing both a columnar component and transcolumnar branches with direct transbarrel projections. However, a small fraction of star pyramidal cells possessed few or even no transcolumnar branches. Electrophysiologically, all three types of neurons were either regular-spiking or intrinsically bursting without a significant relation to the morphological subtypes. The basic synaptic properties of thalamic inputs were also independent of the type of target layer IV spiny neuron. All remained subthreshold and showed paired-pulse depression. In conclusion, the columnar axonal arborization of spiny stellate cells is supplemented by a significant oblique to horizontal projection pattern in pyramidal-like neurons. This offers a structural basis for either segregation or early context-dependent integration of tactile information, in a cell-type specific manner.

Keywords: barrel cortex, cortical circuits, pyramidal neuron, spiny stellate neuron, star pyramidal neuron, thalamo-cortical slice

Introduction

Excitatory interneurons are characteristic for layer IV of sensory cortices (Jones, 1975; Lund, 1984). They sample the majority of sensory input relayed by thalamic nuclei (Benshalom and White, 1986; Staiger et al., 1996) and thus initiate cortical information processing which leads to major transformations in the neuronal representations of sensory stimuli (Simons and Carvell, 1989; Reid and Alonso, 1996; Miller et al., 2001).

The contribution of layer IV neurons to this processing is strongly constrained by their intrinsic physiology and synaptic input-output relationship. The physiological properties define the temporal characteristics of the spike output whereas morphology outlines the circuits which are, however, further determined by their spatiotemporal dynamics (Gilbert and Wiesel, 1979; Connors and Gutnick, 1990; Callaway, 1998). It is therefore of major importance for the understanding of cortical circuits to characterize the firing patterns and the axonal projections of the spiny layer IV neurons and to identify any systematic relationship between the two.

The barrel cortex has become a model system for unraveling cortical circuits and sensory information processing because it offers the advantage that punctuate receptor organs (mystacial vibrissae) possess circumscribed cortical processing units (layer IV barrels) which are the morphological correlate of a functional column spanning all cortical layers (Woolsey and van der Loos, 1970). Recent studies have shown that layer IV spiny neurons form a highly interconnected local excitatory network which is capable to amplify the numerically weak thalamic input before it is mainly relayed to the supragranular layers (Stratford et al., 1996; Feldmeyer et al., 1999, 2002). Horizontal interactions across barrels were not observed (Petersen and Sakmann, 2000), a finding which is in agreement with many in vitro imaging or in vivo recording studies. This led to the hypothesis that barrels are independent processing units (Goldreich et al., 1999; Petersen and Sakmann, 2001; Laaris and Keller, 2002). Independent processing implies that the integration of the separate receptor organs into a unified representation takes place at hierarchically higher layers or areas. However, recent studies (Fox et al., 2003; Schubert et al., 2003) suggest that the necessary circuitry to process tactile information in context of what is going on in neighboring receptors is present at the level of layer IV. A main finding of the latter study is that spiny stellate cells participate in intracolumnar circuits, whereas pyramidal-like neurons are also part of transcolumnar circuits. This poses the question of whether multiple circuits operating in parallel already exist in layer IV of barrel cortex, which would allow, in a context-dependent manner, to either separate or combine the tactile information acquired by the vibrissae. Such context-dependent information processing has been found to be critical for any sensory perception (Gilbert et al., 2001). In the present study we characterize three morphological cell types — spiny stellate, star pyramidal and pyramidal cells — and provide objective measures to differentiate between them. Whereas these cell types clearly differ in their dendritic and axonal characteristics, they all contain subpopulations of both regular-spiking (RS) and intrinsically burst-spiking (IB) neurons and cannot be differentiated electrophysiologically.

Materials and Methods

Electrophysiology

Male Wistar rats, 19–21 days old, were anesthetized with enflurane, decapitated and the brain quickly removed. Thalamocortical slices of 500 µm thickness were prepared in a manner similar to the method...
described previously (Agmon and Connors, 1991). Slices were placed in an interface-type recording chamber, kept at 31 ± 0.5 °C and continuously superfused with artificial cerebrospinal fluid (aCSF), saturated with 95% O₂-5% CO₂ and containing (in mM): NaCl, 124; NaHPO₄, 1.25; MgCl₂, 1.8; CaCl₂, 1.6; KCl, 5; glucose, 10; NaHCO₃, 26.

After at least 1 h of incubation, ‘blind’ whole-cell current-clamp recordings in layer IV were performed as described by Blanton et al. (1989). Electrodes were pulled from glass capillaries (GB 200 F8P, Science products, Hofheim, Germany) on a vertical microelectrode puller (PP-830; Narishige Tokyo, Japan) and had a final resistance of 7–12 MΩ. The pipette solution contained (in mM): K-gluconate, 130; NaCl, 6; CaCl₂, 1; MgCl₂, 2; EGTA, 11; K-HEPES, 10; Na₂ATP, 2; NaGTP 0.5; pH was adjusted to 7.5 with KOH. The osmolarity of the solution was 270 mOsm. Biocytin (10 mg per ml; Sigma, Deissenhofen, Germany) was added to the pipette solution. The patch pipettes were connected to a discontinuous voltage-clamp amplifier (SEC-05LX; npi, Tamm, Germany), operated in bridge mode. For thalamic stimulation, a bipolar tungsten electrode was positioned in the ventrobasal thalamic nucleus. Data acquisition and analysis were performed using Tida software (HEKA Elektronik, Lambrecht, Germany).

For each cell, we determined the electrophysiological and morphological characteristics as well as the exact laminar location. The resting membrane potential (Vₘₑₐₚ) was measured immediately after obtaining the whole-cell configuration and not corrected for liquid junction potential. The input resistance (Rᵢ) was calculated according to Ohm’s law from the voltage change following application of a small hyperpolarizing current pulse. Spike amplitude and duration were measured from spike threshold. Spike duration was calculated at half maximal amplitude.

EPSP onset latency after thalamic stimulation was calculated from the beginning of the stimulus artifact. Stimulation intensity, which caused the largest EPSP amplitude, was defined as 100%. This stimulation intensity was also used for the paired-pulse experiments.

In order to address the question, whether the action potential discharge pattern (e.g. intrinsically burst-firing) of layer IV neurons depends on the in vitro recording conditions or on the intracellular pipette solution, we performed a number of control experiments on animals of the same sex and age. (i) In one set of experiments, we used a pipette solution containing 130 mM K-methylsulphate instead of K-gluconate (Zhang et al., 1994). (ii) In another set of control experiments we tested a frequently used pipette solution (Feldmeyer et al., 1999; Petersen and Sakmann, 2000) containing (in mM): K-gluconate, 105; KCl, 30; K-HEPES, 10; MgATP, 4; NaGTP, 0.3; phosphocreatine, 10; pH was set to 7.5 with KOH. Osmolarity was adjusted to 300 mOsm by adding sucrose. Biocytin (2 mg per ml; Sigma, Deisenhofen, Germany) was added to the pipette solution. (iii) ‘Blind’ whole-cell recordings were also performed from layer II/III cells, since these neurons are generally considered as non-bursting (Connors and Gutnick, 1990). (iv) In an additional set of experiments, whole-cell recordings from visually identified layer IV neurons in coronal slices were performed in a submersed-type slice chamber. The technical details of these experiments and electrophysiological characteristics of these layer IV spiny neurons have recently been published (Schubert et al., 2005). Neurons from these experiments were evaluated here only for their morphology (see below).

**Morphology**

Slices containing biocytin-filled cells were fixed for 12–24 h in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at 4°C. Then, they were rinsed extensively with PB including an intermediate step with 1% H₂O₂ (in PB) to block endogenous peroxidase activity. After three rinses in PB, the slices were incubated in a cryoprotectant (25% saccharose, 10% glycerol in 0.01 M PB) for 1 h. Then, the slices were freeze-thawed three times over liquid nitrogen. After three rinses in PB, the slices were incubated overnight with ABC (1:200; Vector, Burlington, CA) at 4°C. Thereafter, 1 mg/ml 3,3′-diaminobenzidine (DAB; Sigma, Deisenhofen, Germany) was preincubated for 10 min and the peroxidase reaction was revealed by starting the reaction with 0.01% H₂O₂. The reaction was stopped by rinsing with PB. After incubation in the cryoprotectant, the slices were resectioned at 100 µm on a freezing microtome.

These sections were subjected to cytochrome oxidase (CO) histochemistry to visualize the barreled field. For this purpose the sections were first rinsed in PB, then incubated (at 39°C in a water bath) under gentle agitation in a solution of 6 mg cytochrome C, 5 mg DAB and 44 mg saccharose per 10 ml with 0.3% catalase (all from Sigma, Deisenhofen, Germany) included. The reaction was stopped by rinsing with PB when sufficient staining was achieved. Finally, sections were intensified with 0.1% OsO₄ (in PB) for 10 min, air-dried, mounted on glass slides, immersed in xylene and coverslipped in Depex (Merck, Darmstadt, Germany). It should be realized that histological processing of the tissue leads to a shrinkage which we have determined to be up to 15% in the x and y-axes and up to 75% in the z-axis. In order to be comparable to previous studies (Lübke et al., 2000; Brecht and Sakmann, 2002; Schubert et al., 2005), we provided the original uncorrected data.

For morphological analysis, the cells derived from thalamocortical or from coronal slices were pooled because pre-testing with multivariate statistics (SPSS Inc., Chicago, IL) showed that the two sets of cells were indistinguishable in all observed variables. The only obvious difference was that CO worked much better in coronal than thalamocortical slices, leading to a clear demarcation of individual large barrels (∼300–500 µm in diameter and up to 300 µm in depth).

Only neurons with an unambiguous localization in a layer IV barrel were reconstructed for the present study. It has to be noted that the use of CO-stained barrels to delineate layer IV most likely overestimates the thickness of this layer since it was noted that CO staining extends into layer III (Land and Simons, 1985).

The biocytin-labeled cells were examined with a light microscope (Eclipse 800; Nikon, Ratingen, Germany) connected to the computerized reconstruction system Neurolucida (Microbrightfield, Colchester, VT). Neurons were digitally photographed by using the capability of NeuroLucida to acquire z-stacks. Such stacks were processed with MetaView 4.5 (Universal Imaging Corporation, West Chester, PA) using the minimum projection in order to achieve an ‘extended-focus view’. In addition, a subset of neurons selected for the most complete dendritic (n = 65) and axonal arbors (n = 18) was reconstructed three-dimensionally with Neurolucida using the 40° objective (numerical aperture, 0.75) and analyzed quantitatively with Neuroexplorer (Microbrightfield, Colchester, VT). For the somatodendritic part of the neurons, the largest vertical and horizontal diameter of the soma, vertical and horizontal dendritic field span, soma area, number of dendrites, number of dendritic ends, total dendritic length, minimum and maximum diameter of dendrites and the trunk diameter of apical dendrite were determined. For the axonal portion of the neurons, the total number of boutons, length of the axon, bouton density and the number of boutons for various area compartments (intracolumnar, extracolumnar, supragranular, granular and infragranular) were analyzed.

In a further attempt to provide unbiased quantitative measurements, which characterize the three groups as proposed by qualitative criteria, the origin of primary dendrites was measured as ‘angle-of-descent’ on a circle fitted around the neurons. This was done manually on printouts of Neurolucida tracings. First, the approximate gravity center of the neuron was marked. Then, a cross hair was fitted on this center, with the x-axis being perpendicular to the pial surface and with the aid of a set square the ‘angle-of-descent’ was determined in binocular view (5°). Note that these measurements were mirrored in the vertical axis to compensate for the asymmetric dendritic organization in a subpopulation of the spiny stellate and star pyramidal cells. Data were pooled for each type and visualized by histograms.

**Statistical Analyses**

Quantitative analyses of group differences between the electrophysiological and morphological cell types were performed using multivariate analysis of variance (MANOVA) followed by pairwise comparisons of cell types with Bonferroni adjustment for multiple comparisons separately for 12 dendritic, 12 axonal and 11 electrophysiological variables, as well as four experimental conditions (pipette solutions) (SPSS Inc., Chicago, IL). In addition, individual comparisons between two conditions of particular interest (occurrence of RS and IB cells, respectively in each of the experimental conditions; proportion of...
neurons responding to thalamic stimulation) were performed using Pearson $\chi^2$ test for more than two variables and verified by Fisher’s exact test for two variables without correction for multiple comparisons.

Exploratory cluster analysis of morphological cell types was performed using multidimensional scaling (MDS) and hierarchical clustering analysis (HCA) to explore, without a priori assumptions, whether specific classes of neurons existed in our sample (for methodological details see Köttter et al., 2001). In the HCA the Euclidean distances between groups were calculated based on the centroids, a measure which uses the average value of all objects in a cluster as the reference point to other objects or clusters. Based on the resulting classification, discriminant analysis was used to identify in a backward procedure the variables that contributed most strongly to the separation. All data are given as mean ± standard deviation.

Results

Morphology

The morphological analysis was based on a sample of 112 biocytin-labeled layer IV spiny neurons. These consisted of 44 symmetric and 20 asymmetric spiny stellate (Figs 1A–C and 2A), 19 symmetric and nine asymmetric star pyramidal (Figs 1D and 2B) as well as 20 pyramidal cells (Figs 1E and 2C). This classification was based on the following cellular features. (i) Spiny stellate neurons were characterized by the absence of an apical dendrite extending out of the barrel into supragranular layers; symmetric and asymmetric refers to the missing or existing polarization of the dendritic tree toward the center of a barrel.

Figure 1. Photographic documentation of cytochrome oxidase-stained layer IV barrels and the main types of layer IV spiny neurons labeled with biocytin. (A) Low-power micrograph showing the location of a symmetrical spiny stellate cell in the dorsolateral aspect of a large barrel. Separated by a narrow septum, a second barrel is partially visible. (B) High-power micrograph of the same neuron. Apart from the spherical dendrites, also the axon (arrow) is clearly labeled. Note the numerous axonal boutons visible in this and all other subsequent micrographs. (C) Asymmetric spiny stellate cell, located at the most lateral aspect of a barrel. (D) Centrally located star pyramidal cell. (E) Peripherally located pyramidal neuron. Roman numerals mark cortical layers. Scale bars: 250 μm (A, B); 50 μm (C, D).
a hallmark of dendritic organization in barrel cortex (Simons and Woolsey, 1984; Lübke et al., 2000). The presence of an apical dendrite led to the classification as either (ii) a star pyramidal cell, in the case that the other primary dendrites were not polarized (except toward the barrel center for asymmetric neurons), or (iii) a pyramidal cell, in the case that the non-apical primary dendrites were polarized as a skirt of basal dendrites preferentially originating at the basal circumference of the soma. This qualitative classification is fully in accordance with the report by Jones (1975). It is further substantiated by observer-independent quantitative measurements and statistical procedures (see below). Nevertheless, a minor proportion of cells were not easily classifiable into these categories, and these probably represent border cases between spiny stellate and star pyramidal cells on the one hand and star pyramidal and pyramidal cells on the other hand. They clearly were not numerous enough to assume a continuum of spiny layer IV neurons. It is important to keep in mind that the somatodendritic domains were completely or nearly completely retained within the slice, whereas probably all the axonal arbors were compromised by the slicing or reslicing procedure. Thus, an undeterminable proportion of the axonal arbor is not represented by the data. Especially in the infragranular layers, the orientation of the axon often caused it to run out of the slice before reaching layers Vb or VI.

**Spiny Stellate Neurons**

**Somatodendritic Features.** The round or ellipsoid soma of these cells possessed a largest diameter in the vertical dimension of 18.5 ± 2.2 μm and in the horizontal dimension of 14.3 ± 2.0 μm (Table 1). Sixty-nine percent (44/64) of all spiny stellate cells were symmetrical and 31% asymmetrical (20/64). Symmetrical neurons were inevitably found in the center of barrels, whereas asymmetrical cells were restricted to the outer third of the cytochrome oxidase-stained patches. This

<table>
<thead>
<tr>
<th></th>
<th>Spiny stellates (n = 31)</th>
<th>Star pyramids (n = 16)</th>
<th>Pyramids (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma diameter, vertical</td>
<td>18.5 ± 2.2</td>
<td>19.6 ± 2.4</td>
<td>19.0 ± 1.7</td>
</tr>
<tr>
<td>Soma diameter, horizontal</td>
<td>14.3 ± 2.0</td>
<td>15.1 ± 2.3</td>
<td>14.9 ± 2.7</td>
</tr>
<tr>
<td>Dendritic span, vertical</td>
<td>254 ± 45 μm***</td>
<td>510 ± 117</td>
<td>510 ± 176</td>
</tr>
<tr>
<td>Dendritic span, horizontal</td>
<td>206 ± 43 μm***</td>
<td>271 ± 57</td>
<td>283 ± 77</td>
</tr>
<tr>
<td>Primary dendrites (n)</td>
<td>4.1 ± 1.5</td>
<td>3.5 ± 1.1</td>
<td>3.9 ± 1.2</td>
</tr>
<tr>
<td>Dendritic ends (n)</td>
<td>23.7 ± 4.7</td>
<td>25.5 ± 6.4</td>
<td>26.8 ± 7.1</td>
</tr>
<tr>
<td>Dendritic length (total)</td>
<td>2112 ± 899 μm***</td>
<td>2792 ± 793</td>
<td>2813 ± 809</td>
</tr>
<tr>
<td>Dendritic length (BD)</td>
<td>2112 ± 899</td>
<td>1818 ± 659</td>
<td>1812 ± 613</td>
</tr>
<tr>
<td>Dendritic length (AD)</td>
<td>n.a.</td>
<td>974 ± 400</td>
<td>1001 ± 381</td>
</tr>
<tr>
<td>Minimum diameter (BD)</td>
<td>1.6 ± 0.6</td>
<td>1.7 ± 0.6</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Maximum diameter (BD)</td>
<td>2.9 ± 0.9</td>
<td>3.2 ± 1.1</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>Trunk diameter (AD)</td>
<td>n.a.</td>
<td>2.5 ± 0.7 μm*</td>
<td>2.9 ± 0.8</td>
</tr>
</tbody>
</table>

Largest somatic diameter and dendritic span in the vertical and horizontal plane are given. Apical dendrite (AD) and basal dendrites (BD, as all non-apical dendrites) were distinguished. In spiny stellates an AD is not available (n.a.). All values are μm unless otherwise indicated.

*Comparison between spiny stellates and star pyramids.
**Comparison between spiny stellates and pyramids.
***Comparison between star pyramids and pyramids.

*P < 0.05; **P < 0.01; ***P < 0.001.

**Figure 2.** Reconstructions of layer IV spiny neurons showing the variations of their somatodendritic morphology. (A) Spiny stellate neurons range from entirely asymmetric (Aa), over partially asymmetric (Ab) to symmetric (Ac), depending on their relative location within in a barrel (not individually shown). (B) Star pyramidal cells show a similar diversity with clearly asymmetric (Ba) or symmetric dendritic arbors (Bc), with longer or shorter (Bc) apical dendrites extending into layers II/III. (C) Pyramidal cells also possess an apical dendrite of varying length (Ca-c) which is located opposite to a skirt of basal dendrites. These display also various configurations (Ca-c); however, an asymmetric polarization toward the barrel center was never observed. Roman numerals mark cortical layers. Scale bar: 100 μm.
shows that in the large barrels representing the mystacial vibrissae both varieties coexist (Fig. 2A). In both types, the dendrites, which had a total length of 2112 ± 889 µm (Table 1), were virtually limited to their home barrels (Figs 1 and 3).

**Axonal Features.** The axonal arbor of spiny stellate cells was largely confined to its related column. The axon always originated from the basal aspect of the soma (Fig. 1) and was directed toward but not into the white matter (Fig. 3). Within layer IV or mostly within layer Va, the main stem of the axon emitted numerous recurrent collaterals which formed an extremely dense plexus within layers IV and II/III (Fig. 3). The axon was studded with boutons throughout its course, except for the most proximal portions of the main stem in layer V. The number of boutons per unit length of axon (‘density’) averaged over all layers was 16.8 ± 1.0 per 100 µm axon (Table 2). The total number of boutons ranged from 2252 to 3179 (2586 ± 317). Within the home column, the majority of boutons was located in the granular layer IV barrel (1060 ± 225), followed by the supragranular layers (939 ± 233). Qualitatively, neither adjacent barrels nor their related supragranular layers were reached to a significant extent (Fig. 3). Quantitatively, in four out of six reconstructed neurons (and in many of the just inspected cells) only an ‘aberrant’ collateral reached a neighboring barrel, bearing between 6 and 37 boutons (Table 2). Thus most boutons, which represent synapses (Lübke et al., 2000), by their location are either devoted to local signal processing in layer IV, or to relaying processed information to supragranular layers (Feldmeyer et al., 2002).

**Star Pyramidal Neurons**

**Somatodendritic Features.** Their often irregularly shaped soma possessed a largest diameter in the vertical dimension of 19.6 ± 2.4 µm and in the horizontal dimension of 15.1 ± 2.3 µm (Table 1). Sixty-eight percent (19/28) of all star pyramids were symmetrical, 32% asymmetrical (9/28), with a barrel-related distribution similar to spiny stellate cells. This demonstrates that in barrels representing the mystacial vibrissae also asymmetrical star pyramids exist (Fig. 2B). In both types, the non-apical dendrites were virtually confined to their home barrels (Fig. 4). By definition, the apical dendrite left the barrel in all cases. It showed few side branches, rarely reached layer I and never formed an extensive terminal tuft (Figs 2B and 4).

**Axonal Features.** The axonal arbor of star pyramidal cells showed the largest variability of all three cell classes (Fig. 4). Concerning its initial parts and its bouton density it was comparable to spiny stellate cells. However, in its overall distribution it ranged from strictly columnar (Fig. 4A) to extensively transcolumnar (Fig. 4O) with some gradations in between (e.g. 19.6 ± 0.4 µm axon (Table 2). The number of boutons per unit length of axon (‘density’) averaged over all layers was 16.8 ± 1.0 per 100 µm axon (Table 2). The total number of boutons ranged from 2252 to 3179 (2586 ± 317). Within the home column, the majority of boutons was located in the granular layer IV barrel (1060 ± 225), followed by the supragranular layers (939 ± 233). Qualitatively, neither adjacent barrels nor their related supragranular layers were reached to a significant extent (Fig. 3). Quantitatively, in four out of six reconstructed neurons (and in many of the just inspected cells) only an ‘aberrant’ collateral reached a neighboring barrel, bearing between 6 and 37 boutons (Table 2). Thus most boutons, which represent synapses (Lübke et al., 2000), by their location are either devoted to local signal processing in layer IV, or to relaying processed information to supragranular layers (Feldmeyer et al., 2002).

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Horizontal diameter of 14.9 neurons showed a polarization of the dendritic tree in the soma possessed a largest vertical diameter of 19.0

Pyramidal Neurons

Axonal Features. The axonal arbor of pyramidal cells always showed a substantial transbarrel component, in addition to their columnar plexus, which was more or less elaborate (Fig. 5). As a minimum the two neighboring columns, as a maximum five barrel-related columns (including the home column) were reached by a single pyramidal cell. Bouton density was determined as 15.4 ± 2.8 per 100 µm axon (Table 2). The total number of boutons ranged from 2020 to 3052 (2505 ± 378). These data show that pyramidal cells are the main type enabling direct horizontal transbarrel interactions. Such a projection pattern was also recognized in an even more extensive manner for supragranular pyramidal cells (Lübke et al., 2005), layer V IB cells (Chagnac-Amiat, et al., 1990; Staiger et al., 2002) and a subpopulation of layer VI pyramidal cells as well (Kaneko et al., 1995). This strongly indicates a common principle of axonal arborization for the pyramidal cell type across different layers and cortical areas.

Distinguishing Features Between the Cell Types

Somatodendritic Measures. For the somatodendritic variables, the maximum dendritic span was smaller with high significance in the vertical and the horizontal dimension for spiny stellate cells compared to star pyramidal or pyramidal cells (Table 1). The difference in the vertical direction was caused by the absence of an apical dendrite. It is likely that the difference in the horizontal direction can largely be related to the numerous asymmetric spiny stellate cells which do not show a compensatory enlargement of their polarized dendrites. Also the total dendritic length was significantly smaller for spiny stellate cells in comparison to star pyramidal or pyramidal cells (P < 0.001). Together, these features point to the possibility that star pyramidal and pyramidal cells sample qualitatively and quantitatively differing inputs in comparison to spiny stellate cells, a feature mostly attributable to the existence of an apical dendrite in the former types.

When the quantitative data for the somatodendritic portion of the neurons were normalized by pairwise correlation and subjected to cluster analysis, a clear separation of spiny stellate from star pyramidal and pyramidal cell occurred (Fig. 6A). This separation did not depend on any a priori assumption about the number of groups or cell types, neither on the inclusion of apical dendrites as a separate category (not shown). Discriminant analysis based on the dendritic data showed a 100% correct jackknifed classification of all 32 spiny stellate cells, whereas the 17 star pyramidal and the 16 pyramidal neurons overlapped to a large extent (Fig. 6B). The most important distinguishing parameters were: vertical dendritic span; number of basal and total number of dendritic ends; and basal and total dendritic length.

As a further observer-independent analysis of the characteristics of the apical and basal dendritic distributions, we plotted the distribution of the ‘angle-of-descent’ for primary dendrites of the three types of neurons (Fig. 6C). This very clearly shows a pattern for star pyramidal cells that is different from

Pyramidal Neurons

Somatodendritic Features. Their ellipsoid to pyramidal soma possessed a largest vertical diameter of 19.0 ± 1.7 µm and horizontal diameter of 14.9 ± 2.7 µm (Table 1). All pyramidal neurons showed a polarization of the dendritic tree in the vertical dimension. By definition, the apical dendrite left the barrel. It showed more side branches than in star pyramids, rarely reached layer I and never formed an extensive terminal tuft (Fig. 2C). The dendrites had a total length of 2813 ± 809 µm (Table 1). The basal dendrites extended slightly more extensively across the barrel boundaries than in the other two types (not quantified).

**Figure 4.** Axonal arbor of star pyramidal cells and their topographical relation to barrels and layers. (A) Asymmetric star pyramidal neuron which distributes virtually all dendritic and axonal branches toward its ‘home’ barrel column. (B) Symmetric neuron possessing a single direct ‘transbarrel’ collateral. Note that the horizontal infragranular collateral was truncated to save space. It continued for one and a half further barrels. (C) Symmetric neuron showing numerous ‘transbarrel’ collaterals. Medial is to the left, lateral to the right. The barrels, as seen with cytochrome oxidase-staining, are shaded gray. Roman numerals mark cortical layers. Upper and lower black line delineates pial surface or layer VI-white matter border, respectively.

Fig. 4B). It has to be noted that the columnar variant was an exception and that neurons with direct transbarrel projections prevailed. As a maximum, four barrel-related columns (including the home column) were reached by a single star pyramid (Fig. 4C). The density of boutons, averaged across layers, was 17.3 ± 4.7 per 100 µm axon (Table 2). The total number of boutons ranged from 2444 to 5020 (3386 ± 1074), which was the highest value of all three types. These axonal patterns suggest that star pyramidal cells are at the interface between two types of circuits either formed by spiny stellate neurons (organized primarily in an intracolumnar fashion) or established by pyramidal cells (displaying a strong transcolumnar component).
pyramidal neurons. Thus, star pyramidal cells differ from pyramidal cells in the angular direction of their primary dendrites. They do not share the typical pattern of pyramidal cells by having significantly more dendrites emanating between 15 and 30° (i.e. close to the apical dendrite) and lack the exuberant preference for the (‘skirt’) sector of 120–135°. Interestingly, it was common for all types of neurons to emit dendrites at 0, 90 and 135° very frequently. This may point to their putative shared ontogenetic history (Vercelli et al., 1992).

**Axonal Measures.** For the axonal parameters, star pyramids showed more boutons than pyramids ($P < 0.05$) and, although not statistically significant, also more than spiny stellates. Another significant difference was that spiny stellate axons sent more boutons to supragranular layers than star pyramidal or pyramidal cells ($P < 0.05$). This fits well with their assumed role as the main relay cell for translaminar intracolumnar processing. Along the same line, also the second major difference can be interpreted (Table 2). Spiny stellate cells displayed fewer boutons in extracolumnar granular (i.e. transbarrel) areas than pyramids with a high significance ($P < 0.001$).

**Electrophysiology**

We recorded from 101 layer IV spiny neurons in P19–21 rat barrel cortex. Eighty-three of these neurons were recorded in an interface-type slice chamber with a patch pipette containing the standard K-gluconate solution. The remaining 18 cells served for control experiments using two other intracellular solutions (see Materials and Methods).

**Action Potential Discharge Pattern**

Two different action potential firing patterns were observed upon injection of suprathreshold depolarizing current pulses. In 18 neurons, the initial response to just suprathreshold stimulation consisted of a single action potential followed by a small depolarizing afterpotential (DAP; Figs 7A1 and B1). In agreement with previous studies in rodent cerebral cortex (Connors et al., 1982; Chagnac-Amitai and Connors, 1989), we...
classified this group of layer IV neurons as RS cells. In 65 neurons, just suprathreshold stimulation elicited an initial spike ‘doublet’ (Fig. 7A2, B2) or a burst consisting of at least three action potentials placed on a prominent DAP. The first ISI was always <20 ms. In agreement with previous reports (see above), we classified these neurons as IB neurons. RS and IB neurons were comparable in both their basic passive and most of their active membrane properties. However, they differed significantly in their action potential firing patterns. The first and second ISI were significantly shorter in IB neurons as compared to RS cells (Table 3). By plotting the first versus the second ISI, these distinct differences in the initial firing pattern between RS and IB neurons became apparent (Fig. 7B).

Since action potential discharges in high frequency ‘doublets’ or bursts may have special importance in cortical processing (for review see Lisman, 1997), we determined in eight IB neurons whether these cells are capable to fire bursts repetitively (data not shown). At interstimulus intervals of ≤80 ms, all neurons revealed a single spiking pattern following the initial burst. Repetitive ‘doublets’ or bursts could be reliably elicited at intervals of ≥200 ms, indicating that layer IV spiny neurons are capable to discharge in this mode preferentially when driven at frequencies of ≤5 Hz.

In order to further characterize the range of conditions under which intrinsic burst discharges may be detectable in layer IV spiny neurons, we carried out experiments using different intracellular patch pipette solutions and recording conditions. (i) Nine layer IV spiny neurons were recorded with a pipette solution containing 130 mM K-methylsulfate instead of K-glucuronate. Under these conditions, four of nine cells showed the typical intrinsic burst discharges and the others

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Figure 6. Statistical methods to classify neurons in an observer-independent manner. (A) Cluster analysis (HCA) showing the complete separation of spiny stellate cells (blue) from star pyramidal (green) and pyramidal cells (red). (B) Canonical scores plot of discriminant analysis of the dendritic parameters of 32 spiny stellate (blue), 17 star pyramidal (green) and 16 pyramidal neurons (red). The plot shows the first two canonical variables and the confidence ellipses centered on the centroids of each group. Note that spiny stellate cells separate completely from the two types of pyramidal cells, whose morphological parameters show a higher degree of variability. (C) Graphical representation of the frequency of the dendritic ‘angles-of-descent’ from the somatic circumference. This shows a statistically significant separation of star pyramidal from pyramidal cells, (i) by the relative abundance of dendritic origins between 16 and 30° (*b) and (ii) by a highly significant relative lack of primary dendrites between 121 and 135° (**b) in star pyramidal cells. Note that in the latter segment also spiny stellate cells are significantly different from pyramidal neurons (*a).
were identified as RS cells. (ii) A modified intracellular solution, as used in previous studies on layer IV neurons (Feldmeyer et al., 1999; Petersen and Sakmann, 2000), also elicited burst firing in three of the nine investigated cells. Fisher’s exact test revealed that the proportion of neurons firing an initial burst was significantly higher with our pipette solution containing K-gluconate and a physiological concentration of calcium (Zhang et al., 1997). At present, it cannot be determined which proportional value of IB neurons among the entire population of layer IV spiny cells comes closest to the natural in vivo conditions.

**Thalamocortical Synaptic Inputs**

Thalamic stimulation (n = 65) elicited monosynaptic EPSPs in 80% of the investigated cells (Fig. 8A). Pyramidal cells showed the lowest incidence of thalamic inputs (56%), whereas star pyramidal (83%) and spiny stellate cells (86%) revealed more frequently an EPSP after thalamic stimulation. However, the statistical comparison between the incidence of such inputs on spiny stellate versus pyramidal cells revealed no statistically significant difference (P = 0.096). In none of these cells did thalamic stimulation reach the threshold to elicit an action potential (at V_{thp}). There was no detectable difference in EPSP amplitude, rise time (20–80%) and onset latency between the different morphologically and electrophysiologically defined groups (Table 4). At V_{thp} increasing stimulus intensities caused a gradual increase in EPSP amplitude to a given maximum (Fig. 8A). In 23 of 31 neurons tested, a reduction in EPSP amplitude was observed by a further increase in stimulus intensity (see 110% trace in Fig. 8A). Depolarization of the cells by current injection unmasked a monophasic fast IPSP (Fig. 8B, n = 17).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>RS cells [n = 13]</th>
<th>IB cells [n = 57]</th>
</tr>
</thead>
<tbody>
<tr>
<td>V\textsubscript{thp} (mV)</td>
<td>(-71 \pm 1.3)</td>
<td>(-70 \pm 0.5)</td>
</tr>
<tr>
<td>R\textsubscript{thp} (\text{\Omega})</td>
<td>148 \pm 19.9</td>
<td>155 \pm 7.8</td>
</tr>
<tr>
<td>Spike threshold (mV)</td>
<td>(-37 \pm 1.2)</td>
<td>(-39 \pm 0.5)</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>73 \pm 3.6</td>
<td>66 \pm 1.4</td>
</tr>
<tr>
<td>Spike duration (ms)</td>
<td>1.1 \pm 0.1</td>
<td>1.1 \pm 0.1</td>
</tr>
<tr>
<td>mAHP amplitude (mV)</td>
<td>(-12.7 \pm 0.9)</td>
<td>(-11.9 \pm 0.3)</td>
</tr>
<tr>
<td>sAHP amplitude (mV)</td>
<td>(-6.6 \pm 0.5)</td>
<td>(-6.6 \pm 0.2)</td>
</tr>
<tr>
<td>First ISI (at low current, ms)</td>
<td>115 \pm 8.1</td>
<td>8.2 \pm 3.1\textsuperscript{**}</td>
</tr>
<tr>
<td>Second ISI (at low current, ms)</td>
<td>295 \pm 27.1</td>
<td>111 \pm 10.6\textsuperscript{**}</td>
</tr>
<tr>
<td>Spiny stellate cells (n)</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Star pyramidal cells (n)</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Pyramidal cells (n)</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

Statistically significant differences obtained with pairwise comparisons (Bonferroni corrected) are indicated by \(*\)*\(\textsuperscript{P < 0.05}\), \(**\)\(\textsuperscript{P < 0.01}\), \(**\)\(\textsuperscript{P < 0.001}\).

**Figure 8.** Postsynaptic responses recorded in a layer IV bursting spiny stellate cell upon electrical stimulation of the thalamocortical input in the ventrobasal thalamus. (A) Increasing stimulus intensities from 20 to 100% (100% = stimulus intensity to elicit the maximal response) evoke an EPSP of increasing amplitude. A further augmentation to 110% elicits a smaller EPSP due to recruitment of a postsynaptic inhibition. (B) Depolarization of the membrane by continuous positive current injection unmasks a prominent monophasic IPSP, which curtails the monosynaptic EPSP. (C) Paired-pulse stimulation of a layer IV spiny stellate cell causes depression of the second EPSP. (D) Paired-pulse depression was highly significant (\(P < 0.01\); 30 ms interstimulus interval, \(n = 7\)).
indicating that thalamocortical inputs rapidly activated intracortical inhibitory circuits. Hyperpolarisation increased the EPSP amplitudes in all investigated cells (Fig. 8A, n = 14).

Further investigation of the thalamocortical inputs by paired-pulse stimulation showed a reduced EPSP amplitude as response to the second stimulus (Fig. 8C) in 16 out of 17 recorded neurons (RS = 4, IB = 13). The amplitude of the second EPSP was depressed on average by 35% (P < 0.01; Fig. 8D, n = 7), at an interstimulus interval of 80 ms. Paired-pulse depression was observed at all interstimulus intervals tested (20–150 ms).

Discussion

The present results suggest that layer IV spiny neurons are more diverse than previously thought. The morphological analyses presented here show qualitative and quantitative features which lead to an unambiguous classification of spiny stellate, star pyramidal and pyramidal cells. The physiological analyses of these three classes show that they consist of subpopulations, which either fire in trains of single spikes or an initial burst. Burst firing may help to entrain local recurrent excitatory circuits more efficiently and to secure the transfer of tactile information to target structures after local processing in the thalamic recipient layer IV. The high incidence of monosynaptic thalamic input to spiny stellate neurons together with the columnar organization of their axonal tree implies a function of local sensory signal processing for this cell type. A presumably lower incidence of thalamic inputs to pyramidal cells together with their extensive transcortical axonal branches assigns a function of a more global integration to this cell type. In this scheme, star pyramidal cells seem to stand in between. On the one hand they frequently receive thalamic inputs, on the other hand their axonal arbors are more of the pyramidal type, i.e. with a substantial transcolumnar portion. Thus, different circuits which are organized in a cell-type specific manner may already be present at the first stage of sensory information processing in cortical layer IV.

Methodological Aspects

In vitro studies inherently suffer from the severance of neuronal processes. At least concerning the axon, none of the reconstructed neurons was completely retained within its slice. This limitation is not to overcome at present, but there is reason to believe that at least for the investigated (excitatory) interneurons, a substantial portion of the neuronal processes can still be revealed (cf. present results with Gilbert and Wiesel, 1979; Lübke et al., 2000). In fact, that the cell type with the most extensive projections, i.e. pyramidal cells, shows the most homogeneous pattern argues against a strong bias caused by the use of slice preparations. Thus, the three types of neurons presented here are likely to be genuinely different cells.

This study showed a large population of intrinsically-bursting cells in layer IV (cf. Connors and Gutnick, 1990). We exhaustively controlled this intrinsic physiology whether it could have been caused by our intracellular solution or other factors peculiar to our slices. However, all measures taken confirmed the finding that burst firing is a physiological property of a subset of spiny layer IV neurons, in agreement with Schubert et al. (2003).

Physiological Diversity in Layer IV

Several studies have recently examined the physiological properties of layer IV spiny neurons. It was realized that synapses between spiny stellate cells show a high reliability and thus are suited to secure transmission of thalamus-derived sensory information to target layers (Stratford et al., 1996; Gil et al., 1999; Feldmeyer et al., 1999). A second candidate mechanism to amplify the numerically weak thalamic input is burst-firing (Lisman, 1997) of a substantial population of layer IV spiny neurons. Although most previous studies have recorded RS cells only (Hirsch, 1995; Feldmeyer et al., 1999; Petersen and Sakmann, 2000), our results are not without antecedent (Connors and Gutnick, 1990). To prevent runaway excitation in this highly interconnected excitatory network, mechanisms to restrict recruitment of too many excitatory cells have to be present. Inhibitory circuits are one such means (Gibson et al., 1999). As a complement to inhibition, Egger et al. (1999) reported the existence of a LTD-like behavior of synapses between spiny stellate cells. We have now shown the presence of a depressing synaptic mechanism also for the thalamic input to excitatory layer IV neurons, in general agreement with previous findings in mice (Gil et al., 1999). Such diverse mechanisms which balance excitation and inhibition in layer IV circuits are of great functional consequence for sensory signal processing (Moore and Nelson, 1998; Pinto et al., 2000; Miller et al., 2001).

Morphological Diversity in Layer IV

Recent research has revealed that there is a large diversity in the proportion of cell types present in layer IV, related to species and modality of sensory cortex (reviewed in Smith and Populin, 2001). There is a general agreement, however, that in principle three excitatory cell types can be found (Jones, 1975) and the best characterized of these is the spiny stellate neuron. More problematic is the differentiation of star pyramidal and pyramidal cells, of which one or the other has not been described in recent studies (Simons and Woolsey, 1984; Lübke et al., 2000), or which have been lumped together (Tarcey-Hornoch et al., 1998; Schubert et al., 2003). This can be justified according to the specific aim of each study. However, to recognize the full diversity of cell types and circuits formed by them, it is important to find ways to distinguish these cell types when necessary.

With our qualitative separation of the cell types we are in full accord with Jones (1975). We add quantitative and statistical measures to aid an observer-independent classification of the different types of layer IV spiny neurons. Somatodendritic features of the neurons allow the distinction of spiny stellate versus star pyramidal and pyramidal cells by hierarchical cluster analysis. The latter can be separated by quantitatively

<table>
<thead>
<tr>
<th>Investigated cells (n)</th>
<th>35</th>
<th>18</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responsive cells (n)</td>
<td>30</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>EPSP amplitude (mV)</td>
<td>5.74 ± 2.02</td>
<td>5.74 ± 2.90</td>
<td>4.32 ± 2.17</td>
</tr>
<tr>
<td>Rise time 20–80% (ms)</td>
<td>1.77 ± 1.45</td>
<td>2.05 ± 1.34</td>
<td>2.21 ± 1.53</td>
</tr>
<tr>
<td>Onset latency (ms)</td>
<td>2.78 ± 0.52</td>
<td>3.11 ± 0.67</td>
<td>3.13 ± 1.40</td>
</tr>
</tbody>
</table>

No statistically significant differences were determined.

Table 4

Electrophysiological properties of thalamic input onto layer IV spiny neurons

<table>
<thead>
<tr>
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No statistically significant differences were determined.
plotting the origins of non-apical primary dendrites from the soma, reflecting their characteristic gap of origin in the vicinity of the apical dendrite which results in the appearance of a skirt-like grouping at the basal circumference of the soma (Peters and Kara, 1985).

Furthermore, this is the first study showing significant differences in the number of boutons carried by the axons of the different cell types in different layers and columns. This is similar to inhibitory interneurons where it is now accepted that the axonal arbor offers the most reasonable parameters for distinguishing the subpopulations and interpreting their functional specialization (Kawaguchi and Kubota, 1997; Somogyi et al., 1998; Wang et al., 2002). Whereas spiny stellate neurons reveal significantly more boutons in supragranular layers within their home column than star pyramidal and pyramidal neurons, pyramidal cells showed significantly more boutons in granular layer IV barrels of neighboring columns. The first suggests for spiny stellate cells a nearly exclusive local, within-column function in sensory signal processing, the second a more global transcolumnar role for pyramidal cells. As in nearly all measures, star pyramidal cells stand in between the two other groups of cells. Although the full spectrum of strictly columnar to exhaustively transcolumnar axonal patterns was observed in the present study, a bias toward pyramidal-like patterns was noticed: four out of six fully reconstructed star pyramidal neurons sent axonal collaterals at least to the two neighboring barrels. A possible explanation why this was not noted by Lübke et al. (2000) is that they were biased by their aim to record from pairs of neurons located closely together in cell-dense sides of small barrels (Feldmeyer et al., 1999). No such preselection was performed in the present study.

The Controversy About Direct Transbarrel Connections: Are Barrels Functionally Independent or Not?

Transbarrel and other transcolumnar projections as described in the present and a recent study (Schubert et al., 2003) can explain several far unresolved findings. They may account for the consistent finding that in intracellular in vitro studies layer IV neurons in barrels of adjacent whiskers show the shortest latency of activation, right after the neurons in the principal whisker’s barrel (Carvell and Simons, 1988; Moore and Nelson, 1998). Furthermore, they may reflect a necessary circuit for the construction of multi-whisker receptive fields in layer IV (Fox et al., 2003).

Why has this type of connectivity only rarely been observed? In single-unit in vivo studies, the reason may be that the effect of the transbarrel projection remains subthreshold for the stimulation parameters used (Goldreich et al., 1999). In studies using voltage-sensitive dyes and electrical stimulation of either thalamus or cortex (Laaris et al., 2000; Petersen and Sakmann, 2001), the effect of transbarrel connections might have been below the detection limits of the method (Laaris and Keller, 2002). An alternative explanation is that concurrent activation of inhibitory circuits might have masked the effects of transbarrel connections. Such lateral inhibition has been demonstrated in vitro (Simons and Carvell, 1989) and in vitro, specifically for pyramidal cells (Schubert et al., 2003). This does not imply that there is no natural behavioral situation where the pattern of cortical activity, triggered by thalamic relay of sensory information or by cortical feedback projection, is engaging direct transbarrel connections for early horizontal integration. This horizontal integration could be computation-

ally as important as the one shown for the hierarchically higher supragranular layers (Feldmeyer et al., 2002).

Functional Diversity in Layer IV may be Expressed by Cell-type-specific Circuits

Sensory circuits are thought to subserve two basic functions: (i) extraction of physical parameters of the stimulus encoded in the spike trains and (ii) combination of the representations of an object sensed by different sets of receptors into a coherent percept. The first needs to keep information separate, as it is observed in the labeled-line pathways formed by all sensory systems. The second needs a substrate for convergence of these separate pathways. We suggest that the differential patterns of axonal projections of the three types of spiny layer IV neurons, together with their specific input patterns of functional connectivity (Schubert et al., 2005) form the basis of cell type-specific circuits with a still unknown locus of interaction. Whether star pyramidal cell circuits could form the interface of spiny stellate and pyramidal cell circuits, is a matter of speculation. To date only homotypical connections between spiny stellate or (star) pyramidal cells have been reported (Feldmeyer et al., 1999). Cell-type-specific circuits would be a means to enable co-representations of different tasks within the same functional unit. Circuits dominated by spiny stellate neurons could mediate on-line sensory signal processing. Under the necessary behavioral conditions, pyramidal cells, additionally or exclusively, could mediate an early horizontal transcolumnar integration and based on our mapping studies also top-down modulation of these local spiny stellate circuits. It is known from all sensory system that signal processing depending on the context of what is going on in neighboring receptors is critical for sensory perception and learning (Gilbert et al., 2001).

Notes

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References
