Morphology, Electrophysiology and Pathophysiology of Supragranular Neurons in Rat Primary Somatosensory Cortex

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Abstract

Intracellularly biocytin-labelled neurons in layers II/III of adult rat primary somatosensory cortex were analysed for their morphological and electrophysiological properties and studied for their response pattern to transient hypoxia under in vitro conditions. The largest dendritic region is formed by the basal dendrites, which constitute an average area of 0.08 mm² and which can receive synaptic inputs over horizontal distances of more than 300 μm. The dendritic territories formed by the oblique dendrites situated on the apical trunk and by the apical tuft are much smaller. The spine density is highest on the apical trunk, suggesting that large numbers of excitatory synapses are present in this region of the cell. All neurons revealed intrinsic membrane properties of typical regular spiking cells and received an excitatory and a strong biphasic inhibitory input. Whereas a significant correlation could be detected between the cell's input resistance and soma area, no correlation existed between the cell's total dendritic length and input resistance or membrane time constant/input resistance. Neurons responded to transient hypoxia either with an anoxic hyperpolarization with an apparent reversal potential of −82.4 mV, or with a gradual anoxic depolarization which reversed at −56 mV. Oxygen deprivation caused a significant reduction in the extent of axonal collaterals, whereas dendritic proportions and spine density were unaffected. The present study indicates that the dendritic tree is well preserved under in vitro conditions, whereas axonal connections are diminished by oxygen deprivation. Our results further suggest that certain structural properties correlate with the cellular physiology, but that the cell's morphology does not determine its responsiveness to hypoxia.

Introduction

The spatial and temporal information processing capabilities of a single neuron are determined by its morphological and physiological properties (for review see Thomson and Deuchars, 1994). The extent and geometry of the dendritic tree defines the territory over which afferent information can be received and the complexity of the axonal projection specifies the divergence of the cell's output to other regions. The cell's structural characteristics also determine its 'computational' capability, e.g. the propagation of postsynaptic potentials generated in the remote dendritic region towards the soma (for review see Holmes and Rall, 1992). The functional properties of a neuron are also characterized by the excitatory and inhibitory synaptic inputs and by its intrinsic membrane properties (for review see Connors and Gutnick, 1990). The question of whether morphological features correlate with certain physiological properties is fundamental for the understanding of information processing algorithms and has been addressed in several central nervous structures. In the neocortex of different mammalian species, the morphology of single cells has been successfully correlated with functional features, both in in vivo (Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984a) and in vitro preparations (McCormick et al., 1985; Chagnac-Amitai et al., 1990; Larkman and Mason, 1990; Mason and Larkman, 1990). Intracellular recordings and labelling techniques in rodent neocortical slices have revealed a close correlation between the cell's morphological and electrophysiological features (for review see Connors and Gutnick, 1990): (i) pyramidal neurons respond to suprathreshold depolarizing current injection with a train of action potentials and pronounced spike-frequency adaptation (McCormick et al., 1985) (regular spiking cells); (ii) a subpopulation of layer V pyramidal cells with a large soma and a thick apical dendrite respond to current injection with intrinsic burst discharges (Chagnac-Amitai et al., 1990; Mason and Larkman, 1990) (intrinsic bursting neurons); and (iii) inhibitory interneurons are characterized by brief action potentials, discharge frequencies up to 600 Hz and a lack of spike-frequency adaptation (McCormick et al., 1985) (fast spiking cells). Each of these cell groups and different subcategories has recently been studied in more detail in rat frontal cortex (Kawaguchi, 1993, 1995), rat visual cortex (Larkman, 1991a, b, c) and layers V/VI of rat sensorimotor cortex (Van Brederode and Snyder, 1992). However, quantitative analyses are still incomplete and further comparative investigations on possible correlations between anatomical and...
electrophysiological properties are required. In addition, pathophysiological patterns of single cells are examined only rarely, although neocortical neurons respond very sensitively to hypoxia–ischaemia (Rosner et al., 1986; Lin et al., 1990) and undergo prominent structural and functional modifications in a variety of neuronal disorders, e.g. stroke, trauma, and seizures (for review see Meyer, 1989). A quantitative analysis of morphological, normal physiological and pathophysiological features in the same cell may provide insights into possible structure–function relationships between these different parameters. The aim of the present study was (i) to give the first detailed description of the structural properties of intracellularly labelled neurons in layers II/III of rat SMI, (ii) to determine the neuron’s electrophysiological characteristics and to correlate them with its morphological parameters, and (iii) to correlate the cell’s morphology and electrophysiology with its response pattern to transient hypoxia under *in vitro* conditions. Some of these results have been presented in abstract form (Schröder and Luhmann, 1992).

**Materials and methods**

**Preparation of brain slices, recording techniques and induction of hypoxia**

The methods for preparing and maintaining neocortical slices in *in vitro* were similar to those described previously (Luhmann and Heinemann, 1992). Adult (4–12 weeks) rats were deeply anaesthetized with i.p. sodium pentobarbital (50 mg/kg body weight) and decapitated. Coronal 400 µm thick slices of the primary somatosensory cortex (SMI) (Zilles and Wree, 1985) were cut in cold oxygenated artificial cerebrospinal fluid (aCSF) with a Dosaka vibratome. The bathing solution (aCSF) contained (in mM): 124 NaCl, 3 KCl, 1.25 NaH2PO4, 1.8 MgSO4, 1.6 CaCl2, 26 NaHCO3, 10 glucose, pH 7.4 when saturated with 95% O2–5% CO2. Intracellular microelectrodes were filled with 2 M potassium acetate and 1–2% biocytin (60–120 MW). Intracellular recordings were obtained from cells located in layers II/III of SMI. Only cells with a stable resting membrane potential (V<sub>rest</sub>) more negative than −65 mV and overshooting action potentials were included in the data analysis. De- and hyperpolarizing current pulses of 200 ms duration were injected into the cell at V<sub>rest</sub> (Fig. 1A) to determine the input resistance (R<sub>i</sub>) and the membrane time constant. The membrane time constant was determined by a monoexponential fit of the membrane response to injection of a small negative current pulse (see inset in Fig. 9C). Spike frequency adaptation and repetitive firing pattern were investigated by injecting a depolarizing supra-threshold current pulse of 1 s duration into the cell (Fig. 1B). Besides the analysis of these intrinsic membrane properties, cells were examined in their excitatory and inhibitory synaptic input. A bipolar tungsten stimulating electrode (3–5 MΩ; FHC, Brunswick, NJ) was positioned below the recording site at the border between layer VI and the white matter. Voltage pulses of 200 µs duration and 5–20 V in amplitude were delivered to the stimulating electrode at a frequency of <0.1 Hz. The stimulus intensity was gradually increased and adjusted to a value which elicited an excitatory postsynaptic potential (EPSP) of 5–15 mV in peak amplitude (in Fig. 1C). To elicit an inhibitory postsynaptic potential (IPSP), stimulus intensity was increased to twice the threshold value for orthodromically evoking an action potential at resting membrane potential (in Fig. 1C and 1D).

Hypoxia was induced by switching the aerating gas in the recording chamber from 95% O2–5% CO2 to 95% N2–5% CO2. In order to monitor hypoxia-induced changes in membrane conductance, alternating hyper- and depolarizing current pulses of 300 ms in duration were injected into the cell at the resting membrane potential. The reversal potential of the hypoxia-induced response (E) was calculated by linear regression from a plot of the response amplitude versus membrane potential (Fig. 1D). On the basis of the estimated reversal potential of the hypoxic response, cells were categorized into two groups showing either an anoxic hyperpolarization (E more negative than −78 mV) or a gradual anoxic depolarization (E more positive than −70 mV) (for details see Luhmann and Heinemann, 1992).

**Intracellular staining and histological procedures**

Neurons were labelled intracellularly by injecting depolarizing current pulses of variable duration (120–1000 ms) and amplitude (0.1–2 nA) into the cell. Slices containing biocytin-labelled neurons were kept in the recording chamber for at least 1 h to optimize the quality of the staining. After that period slices were transferred to a small piece of tissue paper to flatten the slice and immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for >24 h. After washing and cryoprotection, slices were resectioned at 60–120 µm on a freezing vibratome, processed overnight with ABC peroxidase reagent (PK-4000 kit, Vector Labs, Burlingame, CA) and air-dried before mounting them on gelatin-coated slides. We used the protocol of Tseng et al. (1991) with the exception that the dianaminobenzidine reaction product was intensified with OsO4. This protocol yielded intense staining of the majority of the dendritic tree including spines and a portion of the axonal projections within the 400 µm thick slice (Fig. 2).

**Reconstructions, morphological analyses and terminology**

Camera lucida reconstructions of intracellularly labelled neurons were made at ×500 magnification using a Zeiss Orthoplan or an Olympus BH-2 microscope equipped with a drawing tube. Analyses of spine distribution, spine counts, measurements of the diameter of the apical dendrite and drawings of the soma were made at ×1250 magnification using a ×100 oil immersion objective. The somatic shape factor was calculated by dividing the soma height by the soma width (Larkman and Mason, 1990). The spine density was determined by counting all visible spines on a 40 µm long dendritic segment at a distance of approximately 100 µm from the soma centre. Changes in spine density on the apical and basal dendrites were estimated by counting all visible spines in a 20 µm long segment at a distance of 20–160 µm from the soma. A digitizing tablet (SigmaScan, Jandel Scientific, Erkrath, Germany) was used to measure soma areas, lengths of dendritic branches and areas of dendritic field from the drawings of the cells. The terminology used in the present report is similar to that used previously by Larkman and Mason (1990) and Larkman (1991a). Dendrites originating from the soma were termed basal with the exception of the main apical dendrite or apical trunk which may give off oblique branches and which terminates in an apical tuft (Fig. 3A). The endpoint of a dendrite is called its tip and the initial dendritic segment adjoining the soma or the apical trunk is termed the stem. The area of the basal, oblique and apical tuft dendritic field was determined by connecting the tips of the corresponding dendrites and measuring the area of the resulting polygon with the digitizing tablet (see also Fig. 7). The maximal horizontal extent of the dendritic field was estimated by measuring the tangential distance between the tips of the two dendrites showing the longest medio–lateral extent.

All quantitative data given in this report were not corrected for two-dimensional projection errors or dehydration-induced shrinkage, which according to measurements in rat hippocampal slices may amount to 20% (Trommald et al., 1995).
Fig. 1. Electrophysiological properties of layers II/III cells in adult rat neocortex. (A) Current-voltage relationship and typical firing pattern of a regular spiking cell at a resting membrane potential of -87 mV. (B) Response to a suprathreshold depolarizing current pulse (upper trace) of 1 s duration. (C) Synaptic responses to electrical stimulation of the afferent pathway at the border between layer VI and the white matter at low (•) and high (••) stimulus intensity. Low stimulus intensity evokes an EPSP that is followed by a small IPSP. An increase in stimulus intensity elicits an action potential followed by a larger and longer IPSP. (D) Same recording as in (C) but at a higher resolution. All recordings in (A)–(D) were obtained from the same cell.

Statistics
Statistical data processing was performed on a personal computer using the CSS software program (StatSoft Inc., Tulsa, OK). The Mann-Whitney U-test and the non-parametric Kendall tau test were used for statistical analyses. If not otherwise noted, values throughout this report are expressed as mean ± SEM.

Results
Data pool
From a large population of intracellularly labelled cells (n = 150) located in layers II/III of adult rat SMI only those with a complete dendritic tree (Fig. 2A, C) were selected for quantitative analysis (n = 30). The triangular-shaped cell bodies of these neurons (Fig. 2B) were located in the inner segment of the slice in a relative depth between 100 and 300 μm from the surface of the slice. The main apical trunk of the cells could be followed over its entire length up to its termination zone in layer I. Spines could be detected on dendritic processes with highest magnification at the light microscopic level (Fig. 2D).

Morphology of layers II/III neurons
Soma size and shape
The height of the somata varied between 12.5 and 36.3 μm (20.1 ± 4.8 μm, n = 26) and the width of the somata ranged from 11.2 to 28 μm (16.4 ± 4.3 μm). The somatic shape factor (height/width) ranged from 0.92 to 1.83 (1.24 ± 0.21, n = 26), indicating that the somata showed a slight elongation in the vertical direction. The perimeter of the cell body varied between 41.4 and 103.3 μm (61.2 ± 15 μm, n = 26) and the area of the somata ranged from 108.4 to 743.9 μm² (269.8 ± 146.5 μm², n = 26).

Properties of the dendritic tree
The area of the basal dendritic tree (59 ± 18.2 × 10⁻³ mm², n = 26) was on average 2.3 times larger than the area of the apical dendritic tuft (25.9 ± 20.4 × 10⁻³ mm², n = 24) (Fig. 7). A significant correlation between these two parameters could not be detected (Fig. 3B), indicating that cells with a large basal dendritic field do not necessarily possess an extensive apical dendritic field and vice versa. The average total length of all basal dendrites (2.63 ± 0.5 mm) was larger as compared to the entire extent of the oblique branches (1.31 ± 0.44 mm) and the apical tuft (1.06 ± 0.59 mm, n = 23) (crossed columns in Fig. 3C). We estimated an average combined dendritic length of all dendrites, including the apical trunk, of 5.08 ± 0.93 mm (n = 26). The length of the apical trunk obviously depended on the relative depth of the cell’s soma from the pial surface and varied between 42.5 and 378 μm (196.2 ± 98 μm, n = 26). Our data suggest that the basal dendrites show the most elaborate bifurcation and complexity. This result is also supported by the analysis of other dendritic parameters. The maximal horizontal extent of the basal dendrites (313.2 ± 40.3 μm, n = 26) was larger than the maximal horizontal range of the oblique (247.5 ± 60.6 μm, n = 26) and apical tuft dendrites (252.6 ± 129.4 μm, n = 23) (filled columns in Fig. 3C; Fig. 7). These variations in the dendritic spatial dimensions in distinct regions of the cell were accompanied by prominent differences in the complexity of the dendritic tree (Fig. 3D). On average, 4.96 ± 1.06 basal stems, exclusively the main apical dendrite, divided into 28.7 ± 4.9 tips (n = 26). In contrast, 7.7 ± 2.9 oblique dendrite stems segregated into 15.5 ± 5.7 tips (n = 26), indicating that basal stems divided into more branches (5.9 ± 1.4 tips/stem) as compared with the oblique dendrites (2.2 ± 0.8 tips/stem). The main apical dendrite diverged into 10 ± 4.3 dendritic tips (n = 23). Differences in the complexity between the basal, oblique and apical tuft dendrites could be also detected in the Sholl analysis (Fig. 4). At a distance between 20 and
120 μm from the soma, basal dendrites showed the largest number of intersections (9–21), whereas oblique and apical tuft dendrites ramified to a much smaller extent (1.3–8). This result is further supported by the dendrograms shown in Figure 5. The dendrogram in Figure 5A was constructed from a typical pyramidal cell with a complex dendritic tree. In contrast, the dendrogram illustrated in Figure 5B was obtained from a supragranular neuron with only minor dendritic ramification. However, in both neurons the basal dendrites showed a higher...
Fig. 3. Quantitative analyses of the dendritic tree. (A) Schematic diagram of the cell’s dendritic morphology to illustrate the terminology. (B) Area of the basal dendritic field plotted versus the area of the apical dendritic tuft in 27 neurons. (C) Total length (crossed columns) and maximal horizontal extent (filled columns) of the basal (left, n = 30 cells) and oblique dendrites (middle, n = 30) and of the apical tuft (right, n = 27). (D) Number of dendritic stems (crossed columns) and dendritic tips (filled columns) of the basal (left, n = 30), oblique (middle, n = 30) and of the apical tuft dendrites (right, n = 30). Bars in C and D indicate mean ± SEM.

Fig. 4. Sholl analysis of dendritic arborization (n = 19 neurons). The number of intersections in dependence of the distance from the soma is plotted separately for the basal (□), oblique (■) and apical tuft dendrites (△).

complexity as compared to the oblique and apical tuft dendrites. Measurements of the diameter of the main apical dendrite indicate that the apical trunk on average becomes thinner from 2.91 ± 1.28 μm measured at 40 μm from the soma center to 1.38 ± 0.26 μm at 200 μm (Figs 6A, 7).

Fig. 5. Dendrogram of a supragranular neuron with a relatively complex (A) and simple (B) dendritic structure.
Dendritic spine distribution
The spine density counted in a 40 μm long dendritic segment was highest at the apical trunk (30.4 ± 9.1, n = 7) (Fig. 6B). Basal and oblique dendrites revealed a density of 25.2 ± 6.5 (n = 27) and 21.8 ± 5.2 (n = 22) visible spines/40 μm segment respectively. The lowest spine density was measured in the terminal apical tuft, with 14.4 ± 6.9 spines/40 μm (n = 15). These numbers on the spine density on different dendritic compartments were used to calculate the total number of dendritic spines per cell. This value ranged from 1896 to 3612 visible spines per cell (2859 ± 467, n = 26). An analysis of the mean spine distribution along the basal and apical dendrite is shown in Figure 6C. Whereas the spine density on basal dendrites is relatively constant at a distance of 20–160 μm from the soma and varies in average between 9 and 13.8 visible spines/20 μm segment, the mean spine density on apical dendrites increased from 3.8 measured at 20–40 μm from the soma to 16.3 spines/20 μm at 120–140 μm from the soma.

Axonal ramification
The thickness of in vitro brain slices is usually limited to 400 μm. Therefore, a detailed quantitative analysis of axonal collaterals in in vitro preparations is confined to relatively short-range connections [for a discussion of this issue see Li et al. (1994)]. In all neurons, the main axon descended towards the white matter (Fig. 2C) and gave off between 1 and 17 collaterals (5.6 ± 4.6, n = 16). The axonal ramification pattern for five typical layers II/III pyramidal neurons is shown schematically in Figure 8. Axon collaterals were predominantly located in layers II–V and extended up to 418 μm in the horizontal direction from the main axon (188.8 ± 126.6 μm, n = 15). The total length of all axonal ramifications estimated up to 6.06 mm (1.2 ± 1.55 mm, n = 16).

Electrophysiological properties
After obtaining a stable intracellular recording, supragranular neurons were functionally analysed in their intrinsic membrane properties (Fig. 1A, B) and their excitatory and inhibitory synaptic inputs (Fig. 1C, D). The results of theses analyses will be presented separately.
Intrinsic membrane properties

The resting membrane potential varied from -67 to -94 mV (±80.5 ± 6.3 mV, n = 29). Injection of a small hyperpolarizing current pulse allowed the calculation of the neuronal input resistance (\(R_i\)) and estimation of the membrane time constant. \(R_i\) ranged from 12.9 to 69.5 MΩ (36.2 ± 14.5 MΩ, n = 19) and the membrane time constant varied from 4.5 to 16.6 ms (10.1 ± 3.1 ms, n = 15). The current-voltage (I-V) relationships (Fig. 1A) and the injection of a suprathreshold depolarizing current pulse of 1 s duration (Fig. 1B) allowed the categorization of the cell into one of the three basic cortical cell types. As can be expected from the cells' localization in layers II/III, intrinsic burst firing could never be detected, since bursting cells are predominantly or exclusively located in layer IV and V (Connors et al., 1982; McCormick et al., 1985; Chagnac-Amitai et al., 1990). Although fast spiking response patterns from presumably inhibitory interneurons could be occasionally observed (see e.g. Luhmann et al., 1993), none of the 30 neurons analysed in this study could be classified as a typical fast spiking cell. All neurons showed the firing pattern of regular spiking neurons with different degrees of spike frequency accommodation (Fig. 1B). In addition, the maximum rate of spike rise over the maximum rate of spike fall (action potential dV/dt ratio) was higher than 2.5 (3.39 ± 0.39, n = 19), supporting the assumption that the recordings were obtained from pyramidal neurons (see also Fig. 7 in McCormick et al. (1985)).

Synaptic inputs

Electrical stimulation of the afferent pathway in layer VI to the border of the white matter elicited at low stimulus intensities a monophasic EPSP of up to 80 ms duration and of various amplitudes (Fig. 1C). Only two cells showed a biphasic EPSP at the resting membrane potential, indicating the activation of a second component in these neurons. Increasing the stimulus intensity to double the threshold for evoking an action potential elicited in all neurons an IPSP (Fig. 1D).

These data indicate that the neurons analysed in this study received a powerful inhibitory synaptic input, as has been described previously for layer II/III cells (Howe et al., 1987; Connors et al., 1988; Luhmann and Prince, 1991).

Correlation between structural and functional properties

The analyses of the intrinsic membrane properties and the subsequent morphometrical investigations allowed a correlation between the cell's structural and functional properties (Fig. 9). The area of the soma was inversely correlated with the neuronal input resistance \(R_i\) (Kendall's tau = 0.46, P < 0.002, n = 23), indicating that neurons with a large cell body have a smaller input resistance.
Structure–function relationships of cortical neurons

(Fig. 9A). No significant correlation could be detected between the cell’s input resistance and its total dendritic length (Kendall’s tau = 0.068, P > 0.05, n = 23) (Fig. 9B). The quotient of membrane time constant and neuronal input resistance showed the tendency to rise with increasing dendritic length, but this relationship was not significant at P < 0.05 (Kendall’s tau = 0.064, n = 19) (Fig. 9C).

Influence of hypoxia

Hypoxia-induced functional modifications

Previous studies from the same laboratory have shown that supragranular neocortical neurons respond to transient hypoxia with two different tetrodotoxin (TTX)-insensitive response patterns (Luhmann and Heinemann, 1992; Luhmann et al., 1993). In the present study neurons showed within 2–3 min after the onset of hypoxia either an initial anoxic hyperpolarization with an apparent reversal potential of E = –82.4 ± 3.1 mV (n = 8) (Figs 10C, 11C) or a gradual anoxic depolarization, which on average reversed polarity at E = –56 ± 15.9 mV (n = 7) (Fig. 12C). During the anoxic hyperpolarization the membrane potential hyperpolarized by dV = –1.3 ± 3.2 mV (n = 8) and the gradual anoxic depolarization depolarized the membrane by dV = 8.4 ± 3.9 mV (n = 8). No significant correlation between the cell’s response pattern to hypoxia and any of the investigated structural parameters could be detected. These results indicate that the response pattern to hypoxia does not depend on the cell’s morphology, but rather on the presence or lack of certain membrane channels like ATP- or calcium-sensitive potassium conductances and certain inward currents [for a discussion of this topic see Knehr and Leblond (1989), Rosen and Morris (1991) and Luhmann and Heinemann (1992)].

Beside these initial slow responses to transient hypoxia, cortical neurons also expressed a pronounced depolarization upon prolongation of oxygen deprivation. This spreading depression-like response is termed sudden anoxic depolarization and is characterized by a massive conductance increase and a membrane depolarization by 30–75 mV (Fig. 11C) (Luhmann et al., 1993). The extent of functional recovery from hypoxia depended critically on the duration of oxygen deprivation. Brief hypoxic episodes, which were insufficient to cause a sudden anoxic depolarization, cells fully recovered after 2–5 min of re-oxygenation (Figs 10C, 12C). Longer hypoxic intervals generally elicited a sudden anoxic depolarization (Fig. 11C), which had a strong impact on the cell’s functional properties. However, cells tolerated even repetitive episodes of sudden anoxic depolarizations when they were oxygenated between these hypoxic periods for >10 min [Fig. 6B in Luhmann et al. (1993); Fig. 4 in Luhmann (1996)]. In contrast to the cell’s intrinsic membrane properties, synaptic responses were more sensitive to transient hypoxia. As reported previously (Luhmann and Heinemann, 1992; Khozajiev et al., 1993; Luhmann et al., 1993; Rosen and Morris, 1993), stimulus-evoked IPSPs and EPSPs were suppressed early during hypoxia and only slowly recovered upon re-oxygenation.

Hypoxia-induced structural modifications

Cells (n = 21) were exposed to 1 to 8 (2.7 ± 2.1) episodes of hypoxia, which varied in duration from 1.8 to 10.2 min (4.3 ± 2.5 min). The total exposure time of the cells to hypoxia ranged from 1.8 to 26.2 min (8.9 ± 6.4 min). Nine cells were not exposed to hypoxia and served as controls (’0 min hypoxia’). An unexpected result of the correlation analyses between the duration of hypoxia and the morphological properties of the cell was an inverse correlation between the exposure time to hypoxia and the extent of the axonal collaterals. The number of axonal collaterals decreased with the total duration of hypoxia (Kendall’s tau = 0.371, P < 0.05, n = 16) (Fig. 13A). A similar relationship could be detected for the total length of all axonal collaterals which declined with longer exposure times to hypoxia (Kendall’s tau = 0.438, P < 0.02, n = 16) (Fig. 13B). Cells with no hypoxia exposure under in vitro conditions revealed on average 9.5 ± 5.8 axonal collaterals and a total length of axonal branches of 2.8 ± 2.1 mm (n = 4). In hypoxic cells (n = 12), these values were 4.25 ± 3.2 (P > 0.05) and 0.66 ± 0.75 mm (P < 0.05) respectively, suggesting that the extent of axonal collaterals was significantly reduced by hypoxia. In contrast to the sensitivity of the axonal projections to oxygen deprivation, the total dendritic length (Fig. 13C) and the number of dendritic spines (Fig. 13D) was not significantly affected by long exposure times to hypoxia.

Discussion

This study has investigated layer II/III neurons in SMI of the adult rat. These cells were analysed under three different aspects. (i) A detailed quantitative description of the structural properties is a prerequisite to understand the role of these neurons in the neocortical microcircuitry. (ii) The functional analyses of the intrinsic membrane properties and the synaptic inputs allow subsequent structure–function correlations and assumptions on the functional connectivity. (iii) The exposure of these neurons to hypoxia allows conclusions on a possible relationship between the cell’s morphological properties and its response to this type of pathophysiological stimulus.

Structural properties of supragranular neocortical neurons

Our quantitative data on the morphological properties of supragranular neocortical neurons in SMI of the adult rat are in good agreement with previous in vivo and in vitro investigations. Our measurements and calculations on the morphology of the soma of pyramidal neurons resemble those reported for layers II/III pyramidal cells in adult rat visual cortical slices (Larkman and Mason, 1990). Larkman and Mason (1990) estimated an average soma height and soma width of 18.4 and 14.7 μm respectively. These values are very close to our measurements in the rat somatosensory cortex (20.1 and 16.4 μm respectively). Consequently, the somatic shape factor (height/width) reported by Larkman and Mason (1990) (1.26) also corresponds to our data (1.24). Our measurements on the average perimeter of the cell body (61.2 ± 15 μm) compares well to the somatic perimeter of lower layer III/upper layer IV callosal pyramidal neurons in cat primary visual cortex (54.8 ± 11.3 μm reported by Farinas and DeFelipe (1991)). Our estimation on the average soma membrane area (269.8 μm²) is larger than that reported for layer IV pyramidal cells in striate cortex of normal cats (144 μm² in Kossel et al. (1995)). This result may be explained by the layer-specific differences in the sizes of the cell bodies in neocortical areas (Sholl, 1956). Most of our quantitative observations on the complexity of the dendritic tree are in agreement with previous reports. The mean length of the apical trunk measured in our sample (196.2 μm) corresponds well to the measurements of Larkman (1991a) performed on layers II/III pyramidal neurons in rat visual cortex (180 μm). A good agreement could be also obtained for the number of basal stems per cell (4.96 versus 4.3 in Larkman (1991a)), the average number of basal tips per stem (5.9 versus 6.7), the number of oblique dendritic stems per cell (7.7 versus 5.7), and the number of oblique tips per stem (2.2 versus 1.9). Our data on the horizontal extent of the basal dendrites (243–395 μm) correspond well to the width of the basal dendritic fields estimated by Martin and Whitteridge (1984b) in
visual cortical neurons labelled intracellularly under *in vivo* conditions (up to approximately 410 μm in their Fig. 5). Our measurements on the dendritic length, bifurcation and area indicate that the region of the basal dendrites is about 1.6 times larger than the territory formed by the oblique dendrites and that the smallest dendritic area is formed by the terminal tuft (Fig. 7). The average lateral range of the dendritic field was also largest at the basal region and about the same for the oblique and terminal tuft dendrites (Fig. 7), indicating that layer II/III pyramidal cells possess the largest recipient zone for incoming synaptic activity in their basal dendritic territory. The total length of all basal dendrites and the combined length of all dendrites were smaller in our sample (2.63 and 5.08 mm respectively) as compared to the measurements by Larkman and Mason (1990) (3.33 and 5.86 mm respectively), indicating that the extent of dendritic processes is 13-21% smaller in our sample. This difference may be related to pruning of dendrites due to the slicing procedure. However, since the thickness of the slice (400 μm) and the plane of sectioning (coronal) used by Larkman and co-workers was identical to our preparation, we consider this possibility as rather unlikely. A more likely explanation for this discrepancy is the use of different histological protocols. Larkman and Mason (1990) used an electron-based fixation technique and osmicated the sections prior to dehydration and embedding in epoxy resin. Using this protocol, the authors anticipated a shrinkage of <1%. It cannot be excluded that the histological procedures used in the present study and in particular the air drying of the sections caused a tissue shrinkage of approxi-
mately 20% (Trommald et al., 1995). Using this correction factor, our data on dendritic lengths would be in close agreement with the observations by Larkman and Mason (1990).

The diameter of the apical trunk has a strong influence on the propagation of postsynaptic potentials, predominantly EPSPs, from the site of generation in the distal dendrite towards the soma [for review see Holmes and Rall (1992), Rall et al. (1992) and Spruston et al. (1994)]. Our measurements indicate that the diameter of the apical trunk decreases over a distance of 160 \( \mu \text{m} \) from 2.91 to 1.38 \( \mu \text{m} \) (Fig. 7). These observations are in good agreement with previous data by Larkman and Mason (1990) on layer V pyramidal neurons in rat visual cortex (see values measured at 100 and 200 \( \mu \text{m} \) in their Fig. 3). This thinning of the apical trunk may be advantageous for the propagation of remote signals (Holmes, 1989). Wolf et al. (1992) have shown in their computer simulations of frog motoneurons that thick proximal dendrites and thin distal dendrites profoundly increased cellullipetal impulse propagation and synaptic efficiency. Another factor which strongly influences the 'computational' properties of a neuron is the dendritic spine distribution, since spines represent as much as 50% of the total membrane area (Holmes and Rall, 1992). In accordance with the observations by Feldman and Dowd (1975) and Larkman (1991c) in rat visual cortex, our quantitative data on the distribution of light microscopiclly visible spines indicate that the terminal tuft shows the lowest spine density. Larkman (1991c) estimated in the terminal tuft segment of layer II/III pyramidal cells an average spine density of 0.56 spines/\( \mu \text{m} \). This value is higher than our estimated average spine density of 0.36 spines/\( \mu \text{m} \). In agreement with Parnavelas et al. (1973; 0.73 spines/\( \mu \text{m} \)), Feldman and Dowd (1975; 1.43 spines/\( \mu \text{m} \)) and Larkman (1991c; 2.54 spines/\( \mu \text{m} \)), we also observed the highest spine density on the apical trunk segment (0.76 spines/\( \mu \text{m} \)). Since Larkman used a correction factor for obscured spines between 1.2 and 2.2, his values are much higher than ours and the data of Parnavelas et al. (1973). In particular, spines on the thick apical trunk are obscured and uncorrected numbers for this dendritic compartment are certainly underestimated [for a further discussion of this issue see Larkman (1991c and Trommald et al. (1995)].

Our quantitative data on the projection pattern of the main axon and the axon collaterals are in agreement with previous reports in other neocortical areas. All layer II/III pyramidal cells analysed in the present study sent their main axon towards the white matter and gave off in average 5.6 collaterals within the cortex. In the non-
hypoxic pyramidal neurons, 9.5 collaterals/axon could be detected. A similar axonal ramification has been described for layer II/III pyramidal cells in the visual cortex of young squirrel monkeys (Tigges and Tigges, 1982; six collaterals/axon) and in adult rat visual cortex (Paldino and Harth, 1977; eight collaterals/axon). The maximal horizontal extent of the axon collaterals in supragranular layers in our sample (418 μm) was much smaller as reported for the lateral projections following extracellular HRP injections in area 17 of the rat in vitro (Burkhalter, 1989). This difference can be explained by the much larger sample of injected neurons in extracellular tracer studies. In agreement with Burkhalter (1989), we also did not detect any input from layers II/III to layer VI. However, in contrast to the observations by Burkhalter in rat visual cortex (1989), we saw axonal terminations in layer IV.

Structure–function correlations of neocortical neurons

Our electrophysiological measurements on the intrinsic membrane properties are in accordance with previous data obtained from supragranular rat neocortical neurons. The average resting membrane potential and the neuronal input resistance (Rm) in our sample (−80.5 mV and 36.2 MΩ) is very similar to the results of Connors et al. (1988) in layers II/III of rat SMI (−79 mV and 30 MΩ), the data of Sutor and Hablitz (1989) in layers II/III of rat medial frontal cortex (−79.3 mV and 21.6 MΩ) and observations of Luhmann and Prince (1991) in layers II/III of rat somatosensory and visual cortex (−77.6 mV and 43.2 MΩ). Orthodromic synaptic stimulation at threshold intensities elicited at resting membrane potential with the exception of two cells a monophasic EPSP, as it has been described previously in guinea pig and rat neocortex (Gutnick et al., 1982; Deisz et al., 1991). The delayed EPSP observed in two neurons resembled the component previously described by Sutor and Hablitz (1989) in rat frontal cortex. Increasing stimulus intensities evoked a biphasic IPSP as reported by Howe et al. (1987), Connors et al. (1988) and Luhmann and Prince (1991). These data clearly indicate that the neurons analysed in the present study received the characteristic excitatory, and a powerful inhibitory, input.

The relationship between the cells’ morphological characteristics and their passive membrane properties gave some insights into the structure–function relationships of neocortical neurons. The neuronal input resistance correlated significantly with the soma size due to the increase in membrane area. No correlation was found between input resistance and total dendritic length. A weak, but significant negative correlation between neuronal input resistance and total membrane area has been previously described by Larkman et al. (1992), suggesting that the inclusion of other parameters, like dendritic diameter and spine area, produced a better correlation. Although the quotient of membrane time constant and input resistance tended to increase with the cell’s dendritic length, this relationship was not significant at P < 0.05. Larkman et al. (1992) observed only a weak positive correlation between these parameters, but obtained a highly

![Diagram](image-url)
significant correlation when the time constant/input resistance was plotted against the total membrane area.

Pathophysiology of neocortical neurons

In agreement with previous reports on the response pattern of neocortical neurons to hypoxia (Rosen and Morris, 1991; Luhmann and Heinemann, 1992; Luhmann et al., 1993) we observed two distinct TTX-insensitive response types to transient oxygen deprivation. Within 2–3 min after induction of hypoxia, cells either hyperpolarized to an average membrane potential of −82.4 mV (anoxic hyperpolarization) or slowly depolarized to an average potential of −56 mV (gradual anoxic depolarization). In the present study we were interested in the question of whether these two basic response types correlated with a specific morphology or electrophysiology of the cell. With the data analysed so far, we were unable to detect any correlation between these parameters. However, we noticed a significant correlation between the duration of hypoxia and the extent of the axonal ramification. Both the number of axonal collaterals per cell and the total length of all axonal projections decreased significantly with longer exposure times to hypoxia. In addition, the total axonal length was significantly smaller in neurons exposed to transient hypoxia as compared to non-hypoxic controls. This hypoxia-induced decline in axonal complexity may be caused by a loss of axonal collaterals, a reduced anterograde axonal transport, which crucially depends on oxidative metabolism, and/or by a deterioration of the histological demonstration of the biocytin reaction product. Since the total dendritic length and the spine number was not influenced by hypoxia, we consider the latter explanation as unlikely. A more likely explanation is an impairment of axonal transport during and after hypoxia. However, since the cells completely recovered in their intrinsic membrane properties even after repetitive hypoxia, active axonal transport may be fully restored during the re-oxygenation period. Therefore it may well be that transient hypoxia causes a rapid degeneration of axonal collaterals. This hypothesis is supported by in vivo experiments which revealed significant hypoxia-induced degenerative changes (Pokorny and Trojan, 1986). Further support for this hypothesis comes from observations on cultured hippocampal pyramidal neurons, which showed a shrinkage of dend-
ritic spines within 1-2 h after repetitive brief applications of the glutamate agonist N-methyl-D-aspartate, indicating that excessive synaptic stimulation under these conditions may induce short-term modifications at the ultrastructural level (Segal, 1995). If hypoxia causes structural damage to axonal ramifications, synaptic transmission may be impaired and the resulting modifications in cellular function may cause pathophysiological activity [for review see Luhmann (1996)].

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Abbreviations
aCSF artificial cerebrospinal fluid
E reversal potential
EPSP excitatory postsynaptic potential
IPSP inhibitory postsynaptic potential
IR neuronal input resistance
SMI primary somatosensory cortex
TTX tetrodotoxin
Vrest resting membrane potential

References


