Review Article

Neocortical Inhibitory System

(cortical interneurons / GABAergic neurons / calcium-binding proteins / neuropeptides)

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Abstract. The neocortex contains two neuron types, excitatory (glutamatergic) pyramidal cells and inhibitory nonpyramidal (GABAergic) cells. GABAergic, inhibitory interneurons are morphologically distinct from excitatory pyramidal cells and account for 20–25 % of all neocortical neurons. Recent studies discovered that besides morphological features, inhibitory interneurons are molecularly and physiologically heterogenous and differ significantly in arrangement and terminations of their axonal endings. In neocortical interneurons, GABA is also co-localized with calcium-binding proteins (parvalbumin, calbindin, calretinin), with neuropeptides and nitric oxide synthase. Axons of GABAergic neurons target distinct domains of pyramidal neurons. Double-bouquet, Martinotti and neurogliaform cells (CB-IR, CR-IR) target distal dendrites of pyramidal neurons and probably regulate the vertical integration of synaptic input along the dendritic tree of pyramids. Basket cells (PV-IR) innervate soma and proximal dendrites, and Chandelier cells (PV-IR) exhibit synaptic contacts on the axon initial segment of pyramidal neurons. GABAergic neocortical interneurons are interconnected by gap junctions. Most often coupling is bidirectional and occurs between interneurons of the same type. Cortical pyramidal neurons derive from the dorsal telencephalon while the majority of interneurons derive from the ganglionic eminences of the ventral telencephalon, and tangentially migrate into cortex. Adult mammalian neurogenesis is not restricted to the hippocampus, but a small number of the new neurons is also generated in the neocortex. New cortical neurons are GABAergic and co-express calbindin and calretinin. Quantitative analysis of selected areas of the neocortex (neuropsychiatric diseases, models of epilepsy, aging) demonstrate a decrease in density of PV-IR and CB-IR neurons but not CR-IR neurons.

Introduction

The development of mammalian neocortex represents one of the most important events in the history of the vertebrate brain. This complicated process peaked in the human brain. In the human neocortex are deposited structural and functional mechanisms of language, thinking, planning and other cognitive functions that significantly differentiate human beings from other mammals. Mammalian neocortex consists of a plethora of neuronal types, each exhibiting specific structural, molecular and functional features (Ramón y Cajal, 1937).

The proper functioning of the cerebral cortex is dependent on two classes of neurons:

a) excitatory, projecting neurons, with pyramidal somatodendritic morphology using glutamate as a neurotransmitter, which typically send their axons to distant cortical as well as subcortical targets;
b) inhibitory local-circuit interneurons, whose axonal arborization is typically restricted to the neocortex and does not project into the white matter. These neurons primarily use GABA as a neurotransmitter. The majority (ca 70 %) of cortical neurons belong to the category of pyramidal cells. Cortical GABAergic interneurons represent about 20–30 % of the total number of neocortical neurons. The comparison with subcortical telencephalic structures is striking. While the majority of neocortical neurons are excitatory, glutamatergic elements, the predominant neurons in the

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Abbreviations: BCs – basket cells, CB – calbindin, CBPs – calcium-binding proteins, CCK – cholecystokinin, CGE – caudal ganglionic eminences, ChCs – Chandelier cells, CR – calretinin, C-Rs – Cajal-Retzius cells, DBCs – double-bouquet cells, fMR – functional neuroimaging, GABA – gamma-aminobutyric acid, GAD – glutamic acid decarboxylase, IR – immunoreactive, LBCs – large basket cells, LGE – lateral ganglionic eminences, MCs – Martinotti cells, MGE – medial ganglionic eminences, NADPH-d – nicotinamide adenine dinucleotide phosphate-diaphorase, NBCs – nest basket cells, NgCs – neurogliaform cells, nNOS – neuronal nitric oxide synthase, NO – nitric oxide, NOS – nitric oxide synthase, NPY – neuropeptide Y, PV – parvalbumin, SBCs – small basket cells, SOM – somatostatin, SP – substance P, TLE – temporal lobe epilepsy, VIP – vasoactive intestinal peptide.

striatopallidal complex, are inhibitory, GABAergic cells. Despite their smaller number, the inhibitory neocortical neurons play a key role in modulating neocortical functions. It was already Santiago Ramón y Cajal who recognized that they are particularly abundant in the cortex of higher primates and in human neocortex, and therefore were likely to be responsible for higher brain functions (Ramón y Cajal, 1911).

The specific functions of cortical GABAergic interneurons are accomplished through a remarkable diversity of subgroups distinguished by somatodendritic morphology, chemical and genetic markers, functional properties and connectivity (Ramón y Cajal, 1911; Del Río and DeFelipe, 1994; Kubota et al., 1994; Gabbott et al., 1997a,b; Kawaguchi and Kubota, 1997; Fabri and Manzoni, 2004). Cortical interneurons are found in all neocortical areas and layers. Cell bodies of cortical interneurons are oval, spindle or multipolar and dendrites are smooth, aspiny or sparsely spiny. These elements are generally called aspiny or sparsely spiny non-pyramidal neurons.

The research of cortical interneurons was at the beginning (primarily) oriented to their somatodendritic and axonal characteristics, with exploiting the staining capacity of various modifications of the metallic Golgi impregnation based on "reazione nera" (Golgi, 1879; Valverde, 1965). Later was introduced electron microscopy demonstrating ultrastructural features and synaptology of these elements. With recent advent of molecular markers and electrophysiological studies, rich data were generated indicating enormous phenotypical diversity of cortical interneurons (Kawaguchi and Kubota, 1997; DeFelipe et al. 2002).

An important stimulus in research of interneurons was the discovery that these elements express an inhibitory neurotransmitter, GABA, and enzymatic systems of glutamic acid decarboxylase GAD 65 (65 kDalton) and GAD 67 (67 kDalton) synthesizing GABA from glutamic acid. It was thus demonstrated that the majority of cortical interneurons belong to the category of GABAergic inhibitory elements, which are on the basis of the expression of other molecules (neuropeptides, calcium-binding proteins (CBPs), nitric oxide (NO)) divided into several subpopulations with different synaptic relations and functional properties. In rat neocortex, the largest groups of GABAergic interneurons have been identified by expression of CBPs parvalbumin (PV) and calretinin (CR), and neuropeptide somatostatin (SOM). Application of triple immunostaining recently revealed 13 groups of GABAergic neurons co-expressing CBPs and several neuropeptides in various combinations (Gonchar and Burkhalter, 1997; Kawaguchi and Kubota, 1997; Gonchar et al. 2008).

GABA released on presynaptic endings produces hyperpolarization of the postsynaptic membrane and has thus an inhibitory effect on matured postsynaptic elements. The basic function of this mechanism is suppression and modulation of the activity of pyramidal cortical neurons which represent the cortical output (Cherubini and Conti, 2001). It is well established that GABAergic inhibition is necessary for normal cortical function, including shaping of the sensory receptor fields, modulation of the sharpness of frequency tuning and oscillatory cortical activities. In the embryonic period and shortly postnatally GABA produces depolarization of the postsynaptic membrane with subsequent excitation of postsynaptic elements. The excitatory effect of GABA in this period is associated with developmental cortical processes. In the rodent neocortex, GABA is already present at embryonic day 14 together with GABAA receptors (Ben Ari, 2002; Xu et al., 2004; Cancedda et al., 2007).

A challenge of recent research in this field is distinctly defined by subtypes of cortical interneurons using a systematic correlation between their morphology, molecular characteristics and electrophysiological features. It appears that a valuable contribution will also be the correlation between the expression of particular genes and the firing pattern of interneurons (Toledo-Rodriguez et al., 2004).

In an effort to overcome the problems associated with the lack of consensus on the classification and nomenclature of cortical interneurons, a meeting devoted to this topic was organized in 2005 in S. Ramón y Cajal's birthplace, Petilla de Aragon (Navarra, Spain). At this meeting a group of 39 interneuron researchers representing the leading laboratories accepted a common list of terms and characteristics (the "Petilla convention") that describe the morphological, physiological and molecular features of neocortical inerneurons. It is expected that this proposal could in future improve the methodology of study of cortical interneurons. In the further text the interneurons will be classified on the basis of their morphology as well as their imunocytochemical properties.

A. Morphological, functional and molecular characteristics of cortical interneurons

Basket cells

The term "basket cells" (BCs) comes from the basket-like appearance of their preterminal axonal segments around the somata of target neurons. In his original description Ramón y Cajal noticed large multipolar cells with long horizontal axonal collaterals, which were considered the source of the terminal axonal arborizations around the somata and proximal dendrites of pyramidal neurons (Ramón y Cajal, 1911). On the basis of their somatodendritic morphology, axonal arborization and expression of CBPs and neuropeptides these cells are divided into three distinct subclasses: large, small and nest basket cells (Marin-Padilla, 1969; Wang et al., 2002). BCs represent approximately one half of all inhibitory (GABAergic) neurons in the supragranular cortical layers. Their boutons target the somata and proximal dendrites of pyramidal neurons and other interneurons. BCs typically express the two CBPs, PV and calbindin (CB), and many neuropeptides (Kisvarday,

1992; Wang at al., 2002). The majority of BCs belong to the category of fast-spiking cells (Kawaguchi and Kubota, 1993, 1997; Zaitsev et al., 2005). BCs are mutually interconnected by chemical synapses as well as by electrical synapses (gap junctions) (Hestrin and Galaretta, 2005). Perisomatic inhibition ensured by BCs has a regulatory effect on synchronization and oscillatory activity of large populations of pyramidal neurons (Freund and Katona, 2007). By means of this mechanism pyramidal neurons may be integrated within one layer of extensive cortical area.

Large basket cells

Large basket cells (LBCs) are multipolar cell bodies (20–25 μm) from which originate three or more primary smooth aspiny dendrites radiating in all directions. Their axons usually originate from the pial aspect of the soma and in the majority of cells give rise to many long horizontally and vertically oriented axonal collaterals, which can extend up to the distance of 900–1000 μm from the cell body. Their smaller side branches terminate in pericellular baskets around somata and proximal dendrites of other neurons (perisomatic inhibition). LBCs are the primary source of horizontal inhibition across cortical columns, obviously within the layer that contains their somata. Together with neurogliaform cells, LBCs are the sole inhibitory interneurons under direct thalamic (excitatory) influence. LBCs prevail in III–V layers and approx. 50 % of them are located in layer IV. In addition to GABA, 50 % of the LBCs express PV and 25 % CB. They express neuropeptide Y (NPY), cholecystokinin (CCK) and occasionally SOM. They never express vasoactive intestinal peptide (VIP) (Krimer and Goldman-Rakic, 2001; Markram et al., 2004).

Small basket cells

Small basket cells (SBCs) are aspiny interneurons with cell bodies up to 20 μm. Their somatodendritic morphology is multipolar but can also be bi-tufted or bipolar in dependence of the cortical layer. Axonal arborizations are dense and obviously limited to one layer. The horizontal extent of axonal ramifications is more limited than in LBCs (up to 300 μm). About 20–30 % of their synaptic contacts terminate on cell bodies of postsynaptic neurons. They are GABAergic but 30 % of SBCs co-express CB. All SBCs co-express VIP partly co-localized with CCK and SOM (Wang et al., 2002; Markram et al., 2004).

Nest basket cells

Nest basket cells (NBCs) are small (up to 20 μm), irregularly shaped cell bodies, giving rise to radially projecting aspiny dendrites. Axonal arborization is local, more compact than in previous types and forms a nest-like plexus around the cell body. About 2/3 of the axonal arborization lies within 150 μm from the soma, indicating that the inhibitory effect of the NBCs is local and probably intracolumnar. A substantial portion of their synaptic endings (23 %) terminate on cell bodies of postsynaptic elements (Gupta et al., 2000; Wang et al., 2002).

NBCs are GABAergic neurons and half of them contain PV, while one third contain CB. Each of the neuropeptides NPY, SOM, CCK is co-expressed uniformly in 30 % of NBCs. To activate one NBC, a larger number of pyramidal neurons is necessary than for the activation of other interneurons (Gupta et al., 2000; Wang et al., 2002). NBCs prevail in layers II–III and constitute a major fraction in layer IV.

Chandelier cells

Chandelier cells (ChCs) represent a type of aspiny interneurons with smooth radially oriented dendrites, oval, multipolar or bi-tufted cell body and with a unique arrangement of its axon terminals. ChCs have been found in layers II–VI. The axon exhibits extensive branching of its preterminal segments, which form short vertically oriented rows of boutons resembling rows of candles in a chandelier (Szentágothai and Arbib, 1974; DeFelipe, 1999). These terminal boutons have been shown to innervate only the axon initial segments of pyramidal cells on which they form symmetrical (inhibitory) synapses (Fig. 1). ChCs never innervate other interneurons (Jones, 1975; del Río and DeFelipe, 1994, 1997a, b; Gabott and Bacon, 1996).

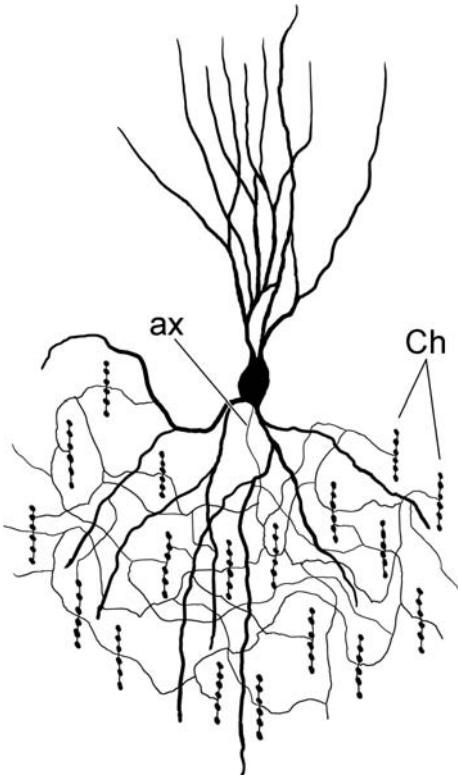

Fig. 1. Schematic drawing of a chandelier cell in the human neocortex according to DeFelipe (1999).
ax – axon, Ch – terminal portion of the axon, which forms a short vertical row of boutons resembling candlesticks. Each Ch terminal innervates a single initial segment of a pyramidal cell.

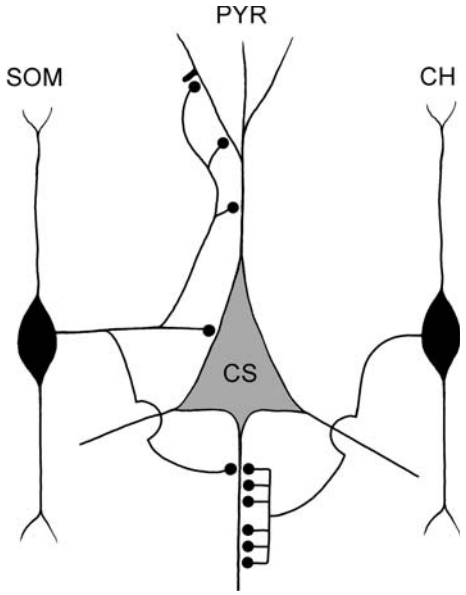

Fig. 2. Synaptic contacts of somatostatin-IR neuron (SOM) and chandelier (CH) axo-axonic cell onto postsynaptic domains of pyramidal neuron (PYR). Modified according to Gonchar et al. (2002)

Because these cells are inhibitory and distributed in the majority of cortical layers and because the axon initial segment of pyramidal neurons appears to be the strategical region where the action potential is generated, ChCs are considered to be the most powerful cortical inhibitory neurons. On the basis of immunocytochemistry, ChCs are defined as GABAergic cells which typically express CBPs PV and CB, but not CR. The expression of neuropeptides is denied with the exception of corticotropin-releasing factor (DeFelipe and Fariňas, 1992). Recently it was demonstrated that ChCs target the distal region of the axon initial segment containing voltage-gated Kv1.2 channels associated with the adhesion molecule Caspr2. The proximal region of the axon initial segment is innervated by other types of GABAergic interneurons. SOM-IR neurons appear to be a candidate (Gonchar et al., 2002; Howard et al., 2005 ; Inda et al., 2006) (Fig. 2). ChCs represent a part of the population of fast-spiking neurons (Kawaguchi and Kubota, 1993, 1997; Kawaguchi and Kondo, 2002).

Martinotti cells

Martinotti cells (MCs) are found in layers II–V, but less frequently in layer VI. They represent about 15 % of interneuronal population. The cell body is most frequently ovoid or spindle and less frequently, different morphology is described (Ramón y Cajal, 1911; Gabbott and Bacon, 1996). Dendrites are vertically oriented (bi-tufted morphology) and the majority of them extend to the infragranular cortical layers. The dendritic arborization is the most extensive of all interneurons. The lateral expanse of dendritic arborization was usually about 300 μm in diameter. Such arrangement suggests that MCs receive inputs from several layers, but within the diameter of a cortical column (Wang et al., 2004). Their axons project towards layer I, where they form a cluster of collaterals spreading horizontally and projecting as far as 2000 μm. These horizontally distributed plexuses inhibit distal dendritic tufts of pyramidal neurons. Synaptic contacts are symmetrical and in all cortical layers their majority (70 %) is located on dendritic shafts of pyramidal neurons, less frequently on dendrites of other interneurons. The majority of MCs are functionally low threshold "regularly spiking neurons" (Kawaguchi and Kubota, 1993). Other data indicating layer-specific differences in electrophysiological properties have been described by Wang et al. (2004). MCs are GABAergic and belong to the group of SOM-positive neurons. SOM is expressed by all MCs regardless of their morphological and electrophysiological differences. In 50 % of MCs SOM is the solely expressed peptide and in the remaining expression of SOM together with CBPs (CB, CR) or other peptides (NPY, CCK) has been described. Most common co-expression patterns were SOM + CB, SOM + NPY and SOM + CCK. No MCs express PV or VIP (DeFelipe, 1997; Wang et al., 2004). MCs are rarely connected by chemical (GABAergic) synaptic contacts, but frequently by electrical synapses (Hestrin and Galarreta, 2005).

Double-bouquet cells

Double-bouquet cells (DBCs) occur in layers II–V, although they are preferentially distributed in the supragranular layers. They frequently exhibit a bi-tufted dendritic morphology. Axons are descending, vertically oriented and tightly coupled into fascicles resembling a horse tail. DBCs innervate dendritic spines and shafts and are therefore dendritic-targeting cells. DBCs participate in interlayer and probably in intracolumnal inhibition. They express CR and CB and can also express VIP or CCK but not PV, SOM or NPY (Markram et al., 2004). They functionally belong to the non-fast-spiking neurons (Kawaguchi and Kondo, 2002).

Bipolar cells

Bipolar cells are small cells with ovoid or spindle-cell bodies and bipolar or bi-tufted dendrites that extend vertically towards layer I and down to layer VI. They occur in layers II–VI. Axons and axon collaterals form a narrow band that crosses all layers. The number of their boutons is relatively low and bipolar cells contact dendrites of only a few cells, mainly pyramidal neurons. They typically express CR and VIP. Bipolar cell can be excitatory (VIP-positive) or inhibitory (GABAergic) (Markram et al., 2004).

Bi-tufted cells

Bi-tufted cells have ovoid somata and bi-tufted morphology of dendrites. They are distributed in layers II–VI. In contrast to bipolar cells and DBCs their vertically oriented axons are distributed in a wider space, but only to neighbouring layers. The majority of them are dendritic-targeting cells. Bi-tufted cells express CB and CR

and several neuropeptides (NPY, VIP, SOM and CCK) (Markram et al., 2004).

Neurogliaform cells

Neurogliaform cells (NgCs) are small cells with round somata that give rise to a large number of short, fine and radiating dendrites forming a spherical structure. NgCs were first described by Ramón y Cajal (1911) as spider-web cells according to their morphology. Their dendritic field is 100–200 μm and their dense axonal arborization extends up to 400 μm. Axons are thin and densely branched. NgCs were described in all cortical layers (Kawaguchi and Kubota, 1997; Krimer et al., 2005). NgCs have a specific position among neocortical interneurons because they establish electrical synapses not only with each other, but also with other interneuron types in the neocortex. So far, electrical synapses have been described between NgCs and BCs and between NgCs and ChCs (Simon et al., 2005). These GABAergic cells form inhibitory synapses mainly on the dendrites of target cells. Part of NgCs co-express CB and NPY. Functionally they belong to the category of "late-spiking cells" (Markram et al., 2004).

Cajal-Retzius cells

Cajal-Retzius cells (C-Rs) have large ovoid perikarya with long horizontal dendrites and axonal arbors that are restricted to layer I. The axonal collaterals of C-Rs form a dense horizontally oriented plexus in layer I, projecting over millimetres of cortical surface. C-Rs synthesize and secrete reelin, an extracellular matrix protein necessary for cortical lamination. Reelin acts as a signal in the marginal cortical zone, to regulate the migration of cortical plate neurons. Loss of reelin activity causes severe malformations of the cortex. The properties of C-Rs have seemed controversial and only recently their expression of transcription factors, neurotransmitters and CBPs has been defined (Hevner et al., 2003; Kirmse et al., 2007). Identification of their transcription factors suggests that C-Rs are derived from pallial progenitors. In contrast to previous reports that C-Rs may be GABAergic, recent data indicate that C-Rs contain high levels of glutamate. In addition, C-Rs express CR and less frequently CB. C-Rs receive direct excitatory synaptic input from the thalamus and from brain-stem serotoninergic fibres. Their axons appear to establish synaptic contacts on the apical dendrites of pyramidal cells (Soriano and del Rio, 2005; Kirmse et al., 2007).

B. Classification of neocortical interneurons

1. Cortical GABAergic cells are divided into distinct classes on the basis of CBP expression

Parvalbumin (PV), calbindin (CB) and calretinin (CR) belong to the large family of EF-hand CBPs, which are characterized by the presence of a variable number of helix-loop-helix motives binding Ca^{2+} ions with high affinity. It is generally accepted that the major function of these three CBPs is buffering of intracelular Ca^{2+}. In some neurons CB and CR are bound to cellular structures while PV is freely mobile in axons, neuronal somata and nuclei. Data from different brain regions suggest that these proteins are involved in regulating calcium pools important for synaptic plasticity. Although many studies demonstrated their neuroprotective function, this concept is not generally supported (Schwaller et al., 2002; Schmidt et al., 2007; Mojumder et al., 2008).

Previous analyses have demonstrated that neurochemical markers such as CBPs represent relevant tools to define the chemo-architecture of the neocortex and that they are observed in distinct neuronal subpopulations. GABAergic inhibitory neurons that express CBPs PV, CB and CR form three largely non-overlapped subpopulations. In primate neocortex, it was demonstrated that 20–25 % of all GABAergic cells co-express PV, 45–50 % CR and 20–25 % co-express CB (Lund and Lewis, 1993; Gabbott and Bacon, 1996; Zaitsev et al., 2005). The density of CB-immunoreactive (CB-IR) and CR-IR neurons is greater in layers II–III, while PV-IR interneurons were most often encountered in the middle cortical layers (Zaitsev et al., 2005). CB- and CR-expressing interneurons share many morphological similarities and are mainly bipolar, bi-tufted and double-bouquet neurons, with minimal overlap among these subpopulations in the rodent and primate neocortex. PV-IR neurons prevail in layers II–V and are classified as BCs and ChCs (DeFelipe, 1997; DeFelipe et al. 1999). In the majority of mammalian representatives (including primates) balanced representation of the three CBPs was demonstrated (Hof and Sherwood, 2005).

1.1. PV-positive neurons

PV-positive neurons are located in layers II–VI but prevail in layers III and IV (Fig. 3, Fig. 4). They repre-

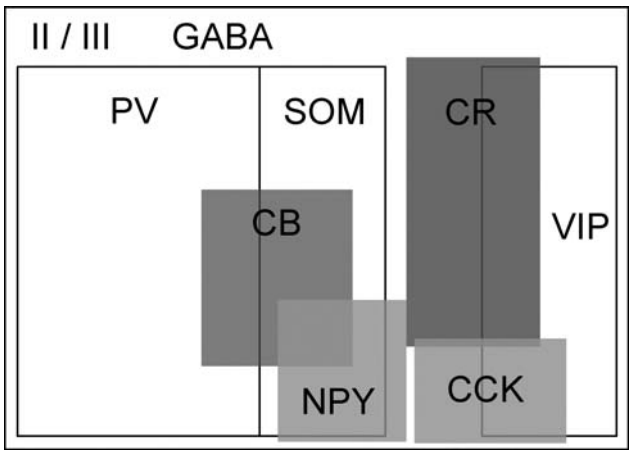

Fig 3. Proportion of GABAergic interneurons expressing calcium-binding proteins and neuropeptides in rat frontal cortex (layer II/III). Modified according to Kawaguchi and Kubota (1997)

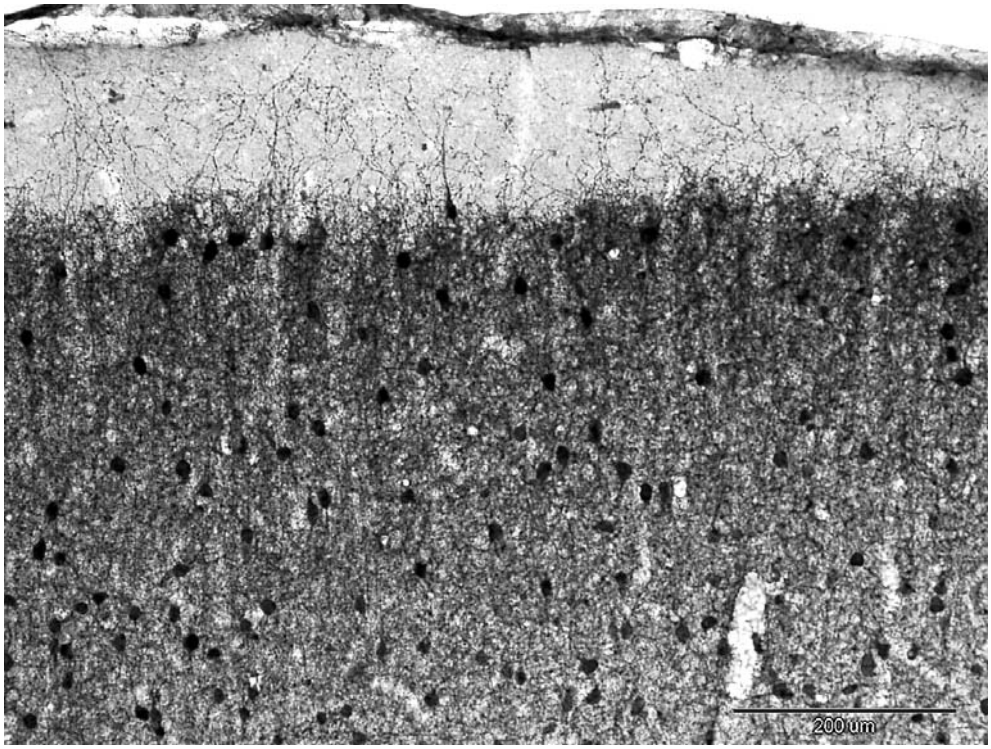

Fig. 4. PV-immunoreactive neurons in the supragranular layers of the rat visual cortex. Bar = 200 μm

sent about 25 % of all inhibitory GABAergic neurons. The density of excitatory synaptic contacts on dendritic arborizations of PV-IR neurons is larger than on CR-IR neurons (Melchitzky and Lewis, 2003). Their axonal terminations are distributed in II–VI layers (del Río and DeFelipe, 1997a, b). Axonal ramifications of the PV-IR neurons are oriented rather horizontally (Henry and Jones, 1991; Lund and Lewis, 1993). PV-IR neurons as a rule do not express peptides with the exception of corticotrophin-releasing factor, which is expressed by some of the ChCs (Lewis et al., 1989). Co-localization experiments showed that PV was expressed by a separate subpopulation of GABAergic neurons that did not co-localize CR, SOM and NOS. However, a small number of PV-IR co-localize CB (Kawaguchi and Kubota, 1993, 1997; Gonchar and Burkhalter, 1997; Vruwink et al., 2001; Gabbot et al., 2006; Gonchar et al., 2008). The majority of PV-positive cells morphologically resembled BCs, while a smaller number of them showed morphology of the ChCs. Both cell types innervate pyramidal cells and BCs innervate other interneurons, too (Szentagothai and Arbib, 1974). Functionally, BCs and ChCs belong to the category of fast-spiking neurons. PV-IR neurons are the main target of feedforward and feedback connections between visual areas of the rat cortex. This finding indicates the preference of these connections for PV-IR neurons over other types of interneurons (CR-IR, SOM-IR) (Gonchar and Burkhalter, 2003).

1.2. CB-positive neurons

These cells are distributed in all cortical layers with the exception of layer I and significantly prevail in supragranular layers (II–III). The majority of CB-IR neurons exhibit vertically oriented morphology of DBCs and bipolar-bitufed neurons (58 %), while neurons with multipolar cell bodies and radiating dendrites are less frequent (31 %) (Henry and Jones, 1991; Staiger et al., 2004). Their axonal ramifications form dense plexuses in the vicinity of cell bodies and in layer I. Besides strongly immunoreactive neurons, weakly positive cells partly showing pyramidal morphology were demonstrated in layers II and III. CB-IR neurons represent a heterogeneous population containing DBCs, MCs and NgCs (Kawaguchi and Kubota, 1993; Gabbott and Bacon, 1996, DeFelipe et al. 1999). About 80 % of CB-IR neurons are innervated from the CR-IR neurons, while 30 % of CB-IR neurons from the PV-IR neurons. The proportion of 10–30 % of CB-positive neurons are synaptically influenced from both PV-IR and CR-IR subpopulations (DeFelipe et al., 1999). CB-IR neurons express PV (12 %), SOM (cca 70 %) and NPY, but never VIP, CCK and CR (Gonchar and Burkhalter, 1997).

1.3. CR-positive neurons

This population of nonpyramidal GABAergic neurons is distributed in layers I–VI, prevailing in the supragranular layers (I–III) (Fig. 5). Supragranular layers contain ca 50 %–85 % of the whole population of CR-IR neurons in dependence on the species analysed. The majority of the CR-IR neurons were demonstrated in the layer II. CR-IR neurons represent in the rat cortex 17–24 % of GABAergic neurons while in the primate cortex about 50 % of GABAergic neurons and about 8 % of the total neuron population of human frontal cortex (Gabbot et al., 1997; Gonchar and Burkhalter, 1999; Gonchar et

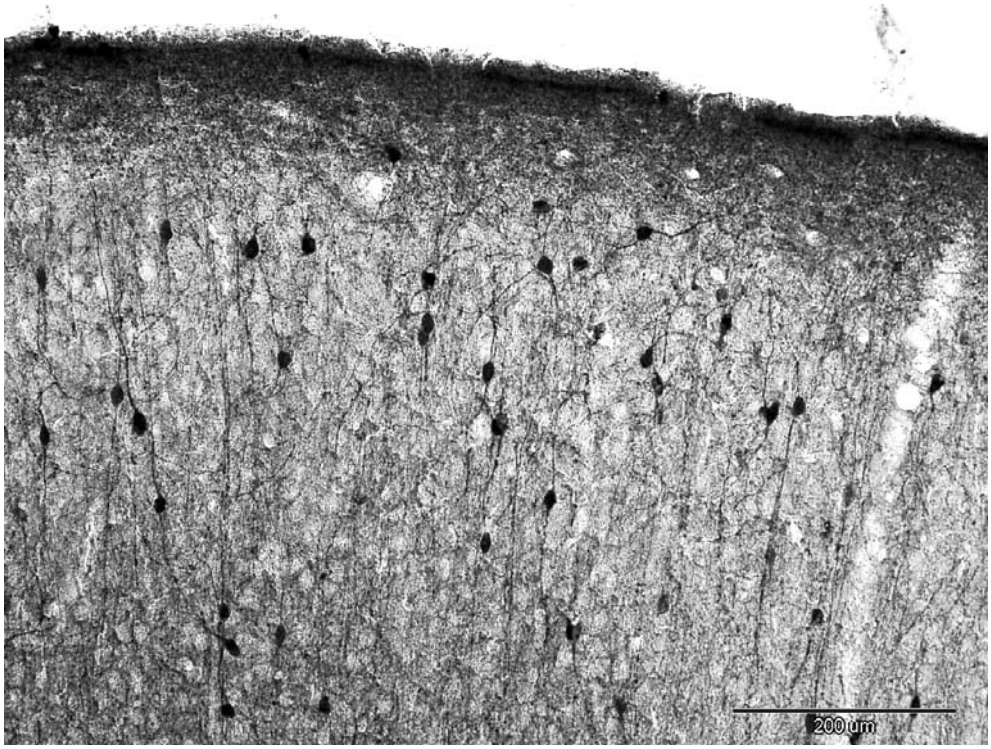

Fig. 5. CR-immunoreactive neurons in the supragranular layers of the guinea pig temporal cortex. Bar = 200 µm

al., 2008). They have small somata (8–12 µm) and showed a variety of shapes (round, fusiform, multipolar). Somatodendritic morphology of the CR-IR neurons corresponds to the bipolar, bi-tufted and double-bouquet cells and less frequently to the multipolar cells (ca 12 %). Dendritic arborization is in most CR-IR neurons vertically oriented and extends to several cortical layers (del Río and DeFelipe, 1997b; Gabbott et al., 1997a, b). Axons are highly branched near the cell body and often send descending collaterals extending to deeper cortical layers (layers V and VI), where they give off narrow pre-terminal plexuses (Gabbott and Bacon, 1996). CR-IR neurons did not co-localize PV, SOM, CB or NOS. The majority of CR-IR neurons (cca 80 %) co-express VIP (Gonchar and Burkhalter, 1997; Kawaguchi and Kubota, 1997; DeFelipe et al., 1999).

Two basic types of CR-IR neurons, bipolar and multipolar cells, differ in several aspects. Bipolar cells exhibit vertical axonal extensions and a bursting firing pattern. In multipolar cells, horizontal axonal extensions and regular firing pattern with pronounced adaptation and accommodation is evident. Somogyi et al. (1998) and Gonchar and Burkhalter (1999) demonstrated that CR-IR neurons within supragranular layers innervate preferentially GABAergic neurons. With regard that CR-positive boutons make symmetric as well as asymmetric synaptic contacts, it could be assumed that the population of CR-IR neurons is heterogeneous. CR-IR terminations were found on 20 % of pyramidal neurons, on 40 % of CB-IR neurons, but only on 3 % of PV-IR neurons (del Río and DeFelipe, 1997a). Later it was demonstrated that the most frequent target of CR-positive terminals were CR-positive dendrites (about 50 %) (Gonchar and Burkhalter, 1999; Caputti et al., 2008). Bipolar CR-IR cells form gap junctions (electrical synapses) with other cells of the same kind, while the multipolar CR-IR neurons do not form electrical synapses with other multipolar CR-IR cells. Concerning the vertical and bundled organization of bipolar and bi-tufted CR-IR axons and dendrites, the role of this subpopulation of the CR-IR neurons could be to provide intracolumnar inhibition. Due to preferential interaction of CR-IR neurons with other GABAergic neurons and specifically with CR-IR neurons suggests that these cells exert an inhibitory effect on other inhibitory neurons. This suppresses inhibition of pyramidal neurons. CR-IR neurons belong to the category of the non-fast-spiking neurons (Kawaguchi and Kubota, 1993, 1997; Zaitsev et al., 2005).

2. Cortical GABAergic cells are divided on the basis of neuropeptide and NOS expression

Neuropeptides differ from classical neurotransmitters in size, synthesis and mechanism of action. Neuropeptides are co-transmitters that modulate the state of the surrounding neurons. Their action is much slower than the classical neurotransmitters such as glutamate and GABA. Pharmacological studies indicate that neuropeptides expressed in the neocortex are involved in emotional and cognitive processes.

GABAergic neurons co-localized with one or more neuropeptides are specifically targeted by serotoninergic and catecholaminergic afferents. Serotoninergic af-

ferents preferentially innervate SOM-IR and NPY-IR neurons. Noradrenergic fibres contact perikarya and dendrites of SOM-IR, NPY-IR and VIP-IR interneurons (Paspalas and Papadopoulos, 2001). Analogously, dopaminergic fibres terminate on spines of pyramidal neurons as well as on cortical interneurons. Experiments based on recordings from cortical slides indicate that dopamine activates D 1 receptors of fast-spiking interneurons (BCs, ChCs) and thus produces an increase in GABAergic inhibition of pyramidal neurons (Kröner et al., 2006). Cholinergic fibres originating in the nucleus basalis (Meynert) innervate both pyramidal neurons and interneurons (Henny and Jones, 2008). Among interneurons, strongly influenced were MCs (SOM-IR) and CCK-IR cells (Lawrence, 2008).

2.1. VIP-IR neurons

VIP-immunopositive neurons comprise 1–3 % of cells in neocortex, prevail in the II–IV layers, mostly possess spindle cell bodies and radially mainly vertically oriented dendritic trees, spanning across several cortical layers. Axonal ramifications are vertically oriented and similarly as dendrites display restricted lateral expansion and thus may be evaluated as a main source of translaminar inhibition (Baykatar et al., 2000; Staiger et al., 2004). VIP-IR cells include several morphological types. Initial analysis suggested expression of VIP in DBCs, MCs and NgCs (Gabbott and Bacon, 1996). Later, SBCs were also included (Baykatar et al., 2000). All VIP-IR neurons co-localize GABA and about one third of VIP-IR neurons co-localize choline acetyltransferase in rat brain. According to several reports (Porter et al., 1998) about 50 % of the VIP-IR neurons in the superficial layers of the neocortex expressed CR. Axonal endings of the VIP-positive cells terminate on dendrites as well as on cell bodies of the CB-IR and PV-IR neurons. The majority of the CB-IR and PV-IR neurons are thus influenced by GABAergic cells co-expressing VIP, which exert strong inhibition on target cells (Staiger et al., 2004). On the other side, the majority of VIP-IR neurons are targets of the PV-IR neurons. VIP-IR neurons are under the direct influence of the cholinergic system (Dávid et al., 2007).

VIP induces a variety of different effects in the neocortex. It modulates the excitability of cortical neurons and is involved in the regulation of cerebral blood flow and metabolism.

2.2. Somatostatin-IR neurons

SOM-positive neurons comprise 1–3 % of neocortical cells and represent a heterogenous group exhibiting different expresion of CBPs and different electrophysiological characteristics. SOM-IR neurons are restricted to layers II–IV and V–VI. Their somatodendritic morphology is diverse. Multipolar (prevalent), fusiform and bipolar cells can be distinguished. Axon terminals mainly innervate somata and shafts of basal and apical dendrites and spines of pyramidal neurons. Some axon terminals were apposed to the axon initial segments of pyramidal neurons. SOM-IR neurons rarely contact aspiny dendrites and non-pyramidal somata.

GABA is expressed by 90 % of SOM neurons and in the subset of SOM neurons, nitric oxide synthase (NOS) was also demonstrated. NOS immunoreactivity was found mainly in a subpopulation of SOM-positive neurons that were strongly immunopositive for the selective substance P (SP) receptor, NK1. About 20 % of SOM-positive neurons co-express CB or CR (Gonchar and Burchhalter, 1997; Gonchar et al., 2002; Halabisky et al., 2006). SOM-IR neurons do not receive direct thalamocortical inputs and their excitatory drive probably derives from axon collaterals of pyramidal cells (Vruwink et al., 2001; Gonchar et al., 2002).

Somatostatin is a neuropeptide that acts as a co-released inhibitory neurotransmitter by opening different types of potassium channels. Somatostatin expression was found in MCs, in a small fraction of SBCs and NBCs and has also been detected in some bipolar, double-bouquet and bi-tufted cells (Toledo-Rodriguez et al., 2004; Wang et al., 2004; Ma et al., 2006).

SOM-IR cells are electrophysiologically diverse and include bursting and regular spiking phenotypes (Kawaguchi and Kubota, 1997; Kawaguchi and Kondo, 2002). Ma et al. (2006) described a novel type of SOM-IR neurons residing in and targeting the thalamo-recipient neocortical layers, namely layer IV. These neurons exhibited stuttering and quasi-fast-spiking firing.

2.3. Cholecystokinin-IR neurons

CCK-positive cells are distributed in layers II–III, V and VI. Cell bodies are medium sized, oval, spindle and less frequently multipolar.

CCK-positive neurons are positive for GABA and negative for PV and SOM. Half of the CCK-positive cells also demonstrate VIP immunoreactivity. Some of the CCK-IR neurons are basket cells with multiple boutons on other cortical cell bodies (Freund et al., 1986; Kawaguchi and Kubota, 1997). CCK-IR neurons belong to the non-fast-spiking neurons (Kawaguchi and Kondo, 2002).

2.4. NPY-IR neurons

NPY-IR neurons in the neocortex are GABAergic, medium sized, aspiny and exhibit ultrastructural characteristics typical for neurons producing and releasing peptides. Most of the NPY-IR neurons co-express SOM and exhibit nicotinamide adenine dinucleotide phosphate-diaphorase NADPH-d/NOS positivity (Aoki and Pickel, 1990; Kawaguchi and Kubota, 1997). NPY-IR neurons are distributed in cortical layers II, III, V and VI, but rarely in layers I and IV. Several types of NPY-IR neurons were classified by axonal morphology. Cells in layer VI exhibited horizontal and descending axons, cells in layer V descending axons, MCs in layers V and VI with ascending axons. In layers II and III bipolar cells were demonstrated with descending axons and SBCs (Obst and Wahle, 1995). Primary sensory and motor areas have a lesser density of NPY-containing ax-

ons than association and limbic areas (Kuljis and Rakic, 1989; Obst and Wahle 1995).

2.5. Substance P-IR neurons

These cells (SP-IR) are scattered throughout the cerebral cortex, concentrated in layers IV–V. Cell bodies are oval or spindle medium sized. The majority of SP-positive neurons (71 %) co-express PV. This co-localization indicates that SP-IR neurons belong to the fast-spiking functional class (Kawaguchi and Kubota, 1997). SP-positive neurons represent a subpopulation of PV-IR cells strongly immunopositive for the NO receptor, soluble guanylyl cyclase. Most of the synaptic contacts made by SP-positive neurons were onto pyramidal neurons. The functional significance of SP in the cortex has not been elucidated, but there are data indicating its excitatory role on cortical neurons (Vruwink et al., 2001).

2.6. Interneurons expressing NOS

Neuronal nitric oxide synthase (nNOS) is a complex protein generating nitric oxide (NO) from L-arginine. NO is a novel messenger molecule with a variety of roles in developing, adult and diseased brain (Iadecola, 1993). It has been established that neurons containing NADPH-d synthetize NO and can be detected using NADPH-d histochemistry (Hope et al., 1991). It has been demonstrated that NOS co-localizes NADPH-d and that only a small fraction of NADPH-d cells (< 2 %) are devoid of NOS immunoreactivity. NADPH-d/NOS-positive neurons were localized in layers II–VI with slight prevalence to infragranular layers (Ouda et al., 2003, Cruz-Rizzolo, 2006). In primate neocortex two types of NADPH-d/NOS positive neurons were identified. Type 1 neurons have large non-pyramidal soma (20–50 μm) with predominant stellate and bipolar morphology.

The majority of dendrites were poorly branched, smooth (in primates a small number of dendritic spines could be observed) and radiated in all directions. For each neuronal type a specific pattern of dendritic arborization was characteristic. Thin axons can be followed up to 100–200 μm. In monkeys, type 2 neurons had smaller (15–20 μm) round or oval soma with variable intensity of staining. Those neurons are about 20-fold more numerous than type 1 and are located exclusively in supragranular layers (Barone and Kennedy, 2000; Cruz-Rizzolo et al., 2006). The areal density of type 1 neurons increases in occipito-frontal direction. Compared with type 1 neurons, areal differences of type 2 neurons demonstrate the opposite tendency. Areal densities of type 2 neurons are higher in occipital areas and lower in frontal areas (Barone and Kennedy, 2000). In comparison with rodent brain, in primate hemispheres a significantly higher proportion of NADPH-d-positive neurons (type 1 neurons) are distributed in the subcortical white matter (Barone and Kennedy, 2000; Ouda et al., 2003; Cruz-Rizzolo et al., 2006). A major difference between rodents and primates concerns the laminar distribution of type 1 neurons. The predominance of type 1 neurons in upper cortical layers differs from that in rodents.

In rodents the majority of NADPH-d-positive cells corresponding to type 1 neurons are located in the infragranular layers (63.5 %) while the remaining 36.5 % are distributed in the supragranular layers (Barone and Kennedy, 2000; Ouda et al., 2003). Gabbott et al. (1997) described in the rat prefrontal cortex two types of NADPH-d-positive neurons strongly and weakly stained. It was suggested that type 2 cells may form a subpopulation of NADPH-d-positive neurons differentiated in higher mammals probably associated with higher cortical functions (Yan et al., 1996). Type 1 and 2 cells frequently co-express GABA, type 1 neurons in addition co-express NPY and SOM. The main difference between both types of NOS-positive neurons is in co-expression of CB. Whereas all neurons of type 2 in primates co-express this CBP, only 4 % of type 1 neurons are CB-positive. Neurons of type 2 in the primate neocortex thus co-localize GABA, CB and NADPH-d/NOS (Yan et al., 1996; Barone and Kennedy, 2000). In rodents co-localization of strong NADPH-d-positive neurons with CB, PV or CR is very infrequent (< 1 %) (Gabbott et al., 1997; Lee et al. 2005).

The distribution of nNOS-positive axonal terminals forming symmetric synapses matched the distribution of the NOS-positive cells. Their main postsynaptic targets are dendritic shafts of both spiny and aspiny dendrites. According to these data NOS-positive local circuit neurons form an axo-dendritic subpopulation of GABAergic neurons. In addition to axo-dendritic synaptic rela-

Table 1. Chemical and morphological characteristics of GABAergic neocortical neurons

Immunochemical marker	Morphological type	Co-expressed molecules
PV	Basket cells Chandelier cells	CB
CB	Double-bouquet cells Bipolar cells Bi-tufted cells Martinotti cells	PV, SOM, NPY
CR	Bipolar cells Bi-tufted cells Double-bouquet cells Multipolar cells	VIP
SOM	Bipolar cells Fusiform cells Martinotti cells	NPY, NOS, CB, CR
NOS	Bipolar cells Multipolar cells	NPY, SOM
CCK	Basket cells	VIP
VIP	Double-bouquet cells Martinotti cells Neurogliaform cells Small basket cells	CR, CCK
NPY	Bipolar cells Martinotti cells Small basket cells	SOM, NOS

tions, NADPH-d/NOS-positive neurons may regulate neighbouring neurons (interneurons as well as pyramidal cells) non-synaptically, because the freely diffusable NO may act as a transmembranous and transcellular messenger influencing intracellular enzymes. The coupling of neuronal activity with regional blood flow is another important feature of NO release (Seress et al., 2005). Morphological and neurochemical characteristics of GABAergic neocortical interneurons are summarized in Table l.

C. Differential origins of neocortical projection and local circuit neurons

Two basic types of neocortical neurons, glutamatergic projection neurons (neocortical pyramids) and GABAergic interneurons originate from different areas of the telencephalon. Cortical projection neurons derive from the dorsal telencephalon and migrate radially into cortical mantle, while the majority of cortical interneurons derive from the ventral telencephalon (anlage of the basal ganglia) and tangentially migrate into developing cortex. In the ventral telencephalon the medial, lateral and caudal ganglionic eminences (MGE, LGE, CGE) give rise to cortical interneurons at least in rodents (Marin and Rubenstein, 2001). The recent research indicates that distinct telencephalic domains (ganglionic eminences) give rise to phenotypically different subgroups of cortical interneurons.

It appears that MGE is the primary source of subcortically derived interneurons. Using tissue culture explant preparation it was demonstrated that the majority of cells within MGE express SOM, PV or NPY, whereas CR was expressed rarely (Wichterle et al., 2001; Xu et al., 2004). Although LGE is the main source of DARPP32-IR neurons, which are precursors of medium spiny striatal neurons, several lines of evidence indicate that some cortical interneurons may also originate in the LGE (Xu et al., 2004). In addition, distinct progenitor domains dorso-ventraly organized were demonstrated within LGE, giving rise to interneurons migrating to the lateral cortex, olfactory bulb and the striatum (Jimenez et al., 2002; Stenman et al., 2003).

The CGE is considered as a caudal extension of the fused MGE and LGE. The fate-mapping experiments using tissue explant preparation revealed that CGE at E 13 mouse embryos give rise to cortical interneurons expressing PV and SOM but not CR. The CR-IR interneurons were generated later at E 14.5 (Xu et al., 2004; Wonders and Anderson, 2005).

Some results indicate that an additional source of cortical interneurons is the septal region, specifically its ventro-lateral part. Explant experiments (using Dil staining) reveal cells migrating from the septum to the layer I of the rostral cortex. Molecular and other characteristics of these cells remain to be elucidated. Slice cultures of human embryonic forebrain revealed that some GABAergic cells migrate from the cortical ventricular and subventricular zones into the cortical plate. The additional production of interneurons in human dorsal telencephalon may be a primate-specific feature which is not present in the rodents (Wonders and Anderson, 2005).

D. Postnatal neurogenesis in the neocortex

It is widely accepted that adult mammalian neurogenesis is restricted to the hippocampal formation (dentate gyrus) and olfactory bulb. Recently, several studies have described new neurons also in the adult neocortex in both rodents and non-human primates. The number of cortical interneurons generated during adulthood appears to be very small. In the adult neocortex in macaques and rats only 1–2 cell per mm^3 have been observed (Gould and Gross, 2002; Dayer et al., 2005). All the new neurons are small with somatic diameters 8–14 μm. They express CBPs CB and CR. Positive immunostaining of new cortical neurons with GABA and GAD 67 suggests that they belong to the category of cortical, inhibitory interneurons and that their morphology resembles neurogliaform neurons (Cameron and Dayer, 2008). The source of new cortical interneurons was not definitively demonstrated; however, there is some evidence that they are generated locally from NG 2-positive multipotential progenitor cells that reside in the neocortex and differentiate into oligodendrocytes as well as interneurons (Cameron and Dayer, 2008).

E. Electrical coupling of GABAergic interneurons

Electrical synapses are specialized sites where gap-junction channels bridge the plasma membrane of two adjacent neurons. Gap junctions represent a low-resistance pathway for ions and small molecules. Functioning of the electrical synapse is conditioned by the expression of connexin 26 protein in an area of internodal contact. Six connexins are associated to form a macromolecule called a connexon (hemi-channel). Hemi-channels on apposed neuronal membranes form a single channel that bridges the cytoplasm of participating cells (Fukuda, 2007). Electrical synapses have been described in the nervous system of invertebrates and lower vertebrates. In the mammalian brain, where interneuronal communication is based mainly on chemical synapses, the electrical synapses between GABAergic interneurons have only been demonstrated in a limited number of regions of the adult brain (cerebral cortex, hippocampus, striatum, thalamus, cerebellum, inferior olive, dorsal cochlear nucleus). Most commonly, gap junctions have been found between two dendrites, between a dendrite and a soma, or between two somata. Several studies have shown that not all neurons use gap junctions for communication. Gap junctions have been observed only between inhibitory GABAergic neocortical interneurons.

Most often the coupling is bidirectional and occurs between GABAergic interneurons of the same type.

Electrical synapses were so far described between BCs (fast-spiking, PV-IR), MCs (SOM-IR, low-treshold spiking), NgCs and some classes of multipolar cells (Galaretta and Hestrin, 1999). Fukuda (2007) demonstrated that single PV neurons in the supragranular layer have approx. 60 gap junctions along their dendrites. The density of gap junctions gradually decreases along the dendrite. Gap junctions have been demonstrated along dendrites of all directions and these dendrites form dense interconnections. It appears that the dendritic network interconnected by gap junctions is oriented rather horizontaly, transcolumnary (within the framework of one layer) than vertically. This is consistent with the finding that the activity in neocortical inhibitory network (namely in supragranular layers) gap junctions gradually decreases along the dendrite. Gap junctions have been spread horizontally with using the synaptic as well as non-synaptic mechanism (DeFazio and Hablitz, 2005). Some neocortical GABAergic cells are connected simultaneously by electrical and chemical synapses (Fukuda and Kosaka, 2000). Electrical synapses between cells have been considered as an important factor of synchronous activity (Galarreta and Hestrin, 2001; Hestrin and Galarreta, 2005).

F. Interneurons and neurovascular regulation

The tight coupling between local perfusion of brain tissue and neuronal activity is central to normal brain function and at the basis of the signals used in functional neuroimaging (fMR). Innervation of cortical microvessels from neuronal populations expressing various neurotransmitters suggests that microvascular responses to perivascularly released neurotransmitters are important in locally regulated blood flow to changes in neuronal activity (Iadecola, 2002). In many studies it has been demonstrated that vascular changes (changes in local perfusion) reflect the local neuronal activities and the number of signals in a given area. In several recently published reports it was demonstrated that the regulation of vascular and capillary diameter and consecutive changes in blood flow is significantly influenced by gaseous neuromodulator NO and by several neuropeptides expressed in GABAergic neurons associated with cortical microvessels. There are differences in the distribution of GABAergic neuropeptide-expressing neurons in perivascular cortical areas (located within 50 μm of the blood vessel walls) and outside the perivascular zone. In perivascular areas NP-positive neurons (39 % vs. 16 %) and NOS-positive neurons (28 % vs. 7 %) significantly prevailed (Iadecola, 2002, 2004).

Vasodilatation was observed after stimulation of interneurons expressing and releasing NOS and VIP, while vasoconstriction resulted in the stimulation of SOM- and NPY-positive neurons (Fig. 6). CCK failed to elicit any vasomotor response and did not influence microvessel diameter (Cauli et al., 2004). Both vasoconstriction and vasodilatation have long-term character (up to 2 min) after stimulus duration of 120 s. GABAergic interneurons co-expressing neuropeptides and exhibiting vasomotor response are innervated by acetylcholine afferents, which originated in the basal forebrain (Meynert nucleus, Ch 4), and by serotoninergic projections originating in the brain stem. Cholinergic innervation influences the majority of the NOS-IR neurons (~70 %), followed by SOM-IR neurons (57 %) and finally by VIP-IR neurons (26 %).

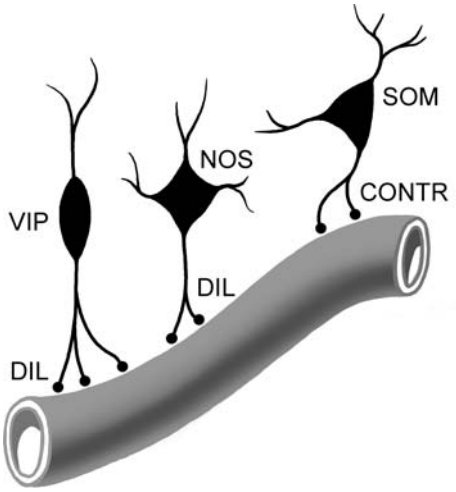

Fig. 6. Interneurons and regulation of cortical blood flow. Vasodilatation (DIL) was observed after stimulation of VIP-IR and NOS-IR neurons, while vasoconstriction resulted in the stimulation of SOM-IR neurons.

About 30 % of NOS-IR neurons also received both cholinergic and serotoninergic innervations and thus this subpopulation appears well-positioned to relay cholinergic and serotoninergic information to blood vessels (Estrada and DeFelipe, 1998; Cauli et al., 2004; Hamel, 2004; Iadecola, 2004). The subcortical vasoactive neurotransmitter systems (cholinergic, serotoninergic) are functionally complemented by activities of perivascular GABAergic interneurons that express neuropeptides.

G. Interneurons and neuropsychiatric diseases

Schizophrenia

The morphological substrate of schizophrenia involves a large number of changes including a decrease of brain volume, increase of ventricular system volume, as far as changes of neuronal populations. Changes of the GABAergic neurons of the frontal cortex have been analysed frequently. According to some concepts, the malfunction within the prefrontal cortex is a feature of schizophrenia. Therefore, specific attention is focused on functional and structural changes of prefrontal areas. Several studies analysed the changes of density of PV-IR neurons. Reduction of PV-IR neurons was found in prefrontal cortex (areas 9, 10, 46) and in the hippocampus in schizophrenia. Analogously were reported deficits in

CB-IR neurons. Laminar postmortem analysis demonstrated a decrease in the density of CB-IR neurons in layer II and decrease of PV-IR neurons in layer IV. In contrast, most of the studies have reported that CR-IR neurons are unaffected. It was suggested that GABAergic deficits in schizophrenia could represent a developmental disorder that results in consecutive neuronal dysfunction and development of the disease (Volk et al. 2000; Reynolds et al., 2001; Eyles et al. 2003; Reynolds and Harte, 2007; Sakai et al. 2008).

Schizophrenics have a significantly lower number of NADPH-d-positive neurons in the hippocampus and in the temporal and frontal neocortex, but a significantly greater number of NADPH-d-positive neurons in the white matter subcortically. Also, these differences are explained as developmental disturbances (Akbarian et al., 1993).

Major depression

Post mortem morphometric studies have demonstrated reductions in the density and size of CB-IR cortical neurons (prefrontal cortex) in major depressive disorder. In contrast, there was no difference in the density of PV-IR neurons (Rajkowska et al., 2007).

Epilepsy

Most hypotheses explaining the morphological substrate of the temporal lobe epilepsy (TLE) is based on alterations of glutamatergic (excitatory) and GABAergic (inhibitory) cortical neuronal system. In certain experimental animal models of epilepsy it has been shown that in the epileptogenic neocortex, there is preferential loss of GABAergic neurons, namely BCs and ChCs (Houser, 1991; Marco et al., 1996; Marco and DeFelipe, 1997). A diffuse decrease of PV, GAD 65 and GAT 1 immunoreactivity was observed in pilocarpine model of epilepsy in neocortical areas (Silva et al., 2002). A decrease in neocortical PV immunostaining was also described in non-colvulsive seizures (Kršek at al., 2004). Neuronal degeneration induced by status epilepticus in several neocortical areas was also demonstrated in immature animals. In shorter survival intervals (4–12 h), the majority of degenerated neurons exhibited nonpyramidal morphology, while in longer survival intervals (24–48 h, 1 week) pyramidal neurons were markedly represented (Druga et al., 2004).

A similar decrease in PV immunostaining was found in cortical tissue (temporal neocortex) removed from epileptic patients. Patches of decreased PV (BC, ChCs) and GAD immunostaining was repeatedly reported and a characteristic feature of epileptogenic temporal neocortex was also a decrease of synaptic terminals (candles) of ChCs. These findings indicate that the perisomatic inhibition (BCs) and axo-axonic inhibition exerted by ChCs might be seriously affected in the human epileptogenic neocortex (Marco et al., 1996; DeFelipe, 1999). Our data confirmed that the number of PV-IR neurons was significantly decreased in non-malformed temporal neocortex (Zámečník et al., 2006).

H. Interneurons and aging

The aging process has significant influences on sensory processing, including changes in cortical functioning. Human brain and experimental animals' studies demonstrate age-related regressive changes in cerebral cortex. While recent studies have shown that there is no significant cortical neuronal loss with age, in several studies a decrease in dendritic spine number and density has been reported. Because dendritic spines are the major postsynaptic sites of excitatory pyramidal neurons, changes in their number could reflect alterations of specific neocortical circuits. In addition to neuronal changes, the breakdown of myelin sheaths and structural changes of glial cells were reported as well (Morrison and Hof, 1997, 2007; Peters 2002). Age-related changes in the function of sensory cortical cells have been observed in several species and it was repeatedly demonstrated that sensory functions degrade with age (Hua et al., 2008). The functional degradation of cortical neurons in old animals was largely attributed to a decrease of intracortical GAGAergic inhibition. It was recently reported that GABA synthetic enzyme GAD in the rat primary auditory cortex is significantly reduced with age (Ling et al., 2005).

Aging is associated with a decrease in the numbers of GAD 65- and GAD 67-immunoreactive neurons and the optical density of their somas in the auditory cortex in Long-Evans as well as in Fischer 344 rats. Western blot analysis revealed a pronounced age-related decline in the levels of GAD 65 and GAD 67 proteins in the auditory cortex (Burianova et al., 2009). In visual cortex the decrease of both proteins was less pronounced (Burianova et al., 2009). Hua et al. (2008) reported a significantly reduced density and proportion of GABA-containing neurons in the primary visual cortex of old cats and a similar result was obtained in the auditory cortex of the same subject (Luo et al., 2006). The ultrastructural analysis of the sensorimotor cortex gave evidence of age-related decline in the numerical density of inhibitory synapses in layer II (Poe et al., 2001). Several papers also reported changes in the levels of CBPs, neuropeptides and NOS.

A quantitative analysis of GABAergic neocortical neurons expressing CBPs in dog and human brain indicate a specific vulnerability of CB-IR interneurons and resistance of PV-IR and CR-IR neurons during aging (Bu et al., 2003; Pugliese et al., 2004). However, a decreased number of PV-IR neurons in the somatosensory and motor cortex of aged rats was reported by Miettinen et al. (1993). Our results indicate that the changes in PV immunoreactivity are strain-dependent. In the auditory cortex of aged F 344 rats a pronounced decline in the number of PV-IR neurons was demonstrated, while in Long-Evans rats the auditory cortex exhibited a slight, non-significant increase (Ouda et al., 2008).

In the primate prefrontal cortex the density of PV-IR terminals increased more than 10-fold from newborn to adult. In contrast, the density of terminals labelled with

an antibody against a GABA membrane transporter (GAT-1) did not change throughout the development. These data indicate that the number of GABAergic terminals is stable over time, but that the level of PV protein within the terminals varies (Erickson and Lewis, 2002).

In aging a significant decrease in neocortical immunohistochemically detectable NPY and SOM was found (Unger and Schmidt, 1994). Similarly, a decreased number of SOM-IR in the sensorimotor cortex of aged rats was reported in a study of Miettinen et al. (1993).

Cortical neurons expressing NOS, which are also stained for NADPH-d, represent a subpopulation of GABAergic local interneurons that co-express SOM and NPY. Data about the density of neocortical NADPH-d-positive neurons in old animals vary. Huh et al. (1998) did not observe significant differences in the number of neocortical NADPH-d-positive neurons and NPY-IR/NADPH-d-positive neurons between young and old rats. However, the number of NPY-positive/NADPH-d--negative neurons was significantly decreased in the aged rats. These results indicate that NPY-IR neurons that do not contain NADPH-d are affected by aging. In contrast to this, Yamada et al. (1996) and Necchi et al. (2002) reported a significant decrease in the number of NADPH-d/NOS-positive neurons in the somatosensory, motor and auditory cortical areas of aged (20–29 months old) rats. In very old rats (36 months old), the total number of NADPH-d-positive neurons within the auditory cortex (fields Te 1 and Te 3) indicates a reduction of about 13 %. In addition, reduction in the thickness of the auditory cortex and changes in the shape and configuration of nerve cell bodies and their dendritic arborization were observed (Ouda et al., 2003).

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