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What types of neocortical GABAergic neurons do really exist?

Introduction

Neurons in the neocortex can be divided into a larger population of excitatory principal neurons (ca. 80–85 %), like pyramidal or spiny stellate cells, and a smaller but very diverse population of GABA (γ-aminobutyric acid)-ergic neurons (15–20 %). From their synaptic terminals the latter release GABA, which commonly inhibits the postsynaptic neuron. The effects of GABA are mediated by ionotropic $GABA_A$-receptors or metabotropic $GABA_B$-receptors (for more information see **Infobox 1**). Despite their small number, inhibitory neocortical neurons are likely to contribute to higher brain functions by several mechanisms. It is well established that GABAergic neurons (often called interneurons but see Chapter on GABAergic projection neurons) play a key role in (i) dynamically balancing cortical excitation with inhibition, (ii) coordinating oscillatory network activity, (iii) sharpening sensory receptive fields, (iv) providing gain control by changing the input–output relationship of excitatory cells, and (v) setting the window for temporal and/or spatial integration. Malfunctions of these neurons might play a role in a number of diseases ranging from epilepsy to schizophrenia, anxiety disorders, and autism. Therefore, in the past years cortical GABAergic neurons became the focus of many research groups, to investigate the types of (inter-)neurons existing in the neocortex, to reveal their connectivity and finally try to understand their functional integration into cortical microcircuits.

Cortical GABAergic neurons are located in all neocortical areas and layers. The proportion between excitatory and inhibitory neurons throughout the six layers of the neocortex varies from species to species and also between cortical areas. In layer (L) I, except for a few surviving Cajal–Retzius cells, almost all neurons are GABAergic. In L II–VI of, for example, the primary somatosensory (barrel) cortex of mice, the largest proportions of GABAergic neurons were found in L II/III (12–18 %) and L V (15–20 %), which strongly contribute to information processing within and between cortical areas (L II/III) or are responsible for most of the cortical output (L V). In L IV and VI, which are the main layers directly connected to the thalamus, roughly 8–9 % of the neurons are GABAergic. Assuming that most of the synaptic interactions with target cells are actually taking place in the layer where the respective somata of GABAergic neurons are located, they seem to prefer layers where they can locally suppress or modulate the activity of pyramidal neurons, which are determining the output of a cortical module.

GABAergic neurons of the cerebral cortex are a highly diverse cell population in terms of morphology, electrophysiology, and neurochemical features. This is a major drawback in attempts to fully understand their individual properties and impact onto cortical circuitry as well as information processing. Therefore, it was a major goal for the scientific community to define distinct subgroups in order to overcome this obstacle. Based on Golgi-impregnation studies, Fairen and colleagues [3] provided a first description of several different morphological subtypes of interneurons in the cerebral cortex. Over the years, cortical interneurons have been characterized in detail with anatomical, electrophysiological, and molecular biological methods. As a consequence, plenty of properties and different nomenclatures of at least partially the same GABAergic cell types have been described. Recently, a group of scientists presented an attempt towards a universally acceptable nomenclature and classification of cortical GABAergic interneurons [1]. This attempt to combine all available morphological (e.g., dendritic branching, subcellular axonal target specificity), electrophysiological (e.g., intrinsic membrane properties, action potential firing patterns), and neurochemical data (e.g., Ca^{2+}-binding proteins or neuropeptides) did not result in non-overlapping subgroups. Furthermore, it appears that since then only a few of the interneuron types reach a high degree of consensus, which are basically only axo-axonic and Martinotti cells identified by their unique axonal morphologies [2]. More recently some researchers re- focused on neurochemical markers. They found three major distinct and virtually non-overlapping subgroups of inhibitory interneurons, which account for nearly 100 % of GABAergic neurons in the neocortex [8]. These cells either express the Ca^{2+}-binding protein parvalbumin (PV), the neuropeptide somatostatin (SST), or the ionotropic serotonin receptor 3a (5HT3aR) (**Fig. 1a**).

By combining these two important advances in our knowledge, the three neurochemically defined subgroups (**Fig. 1a**) can be further subdivided according to the specific axonal morphology (**Figs. 1a and 3**). The ramification pattern and subcellular target specificity (axon initial segment, perisomatic area or peripheral dendrites; see **Fig. 2**) of the different interneuron (sub-) types are essential criteria for the functional impact of inhibition in neocortical informa-

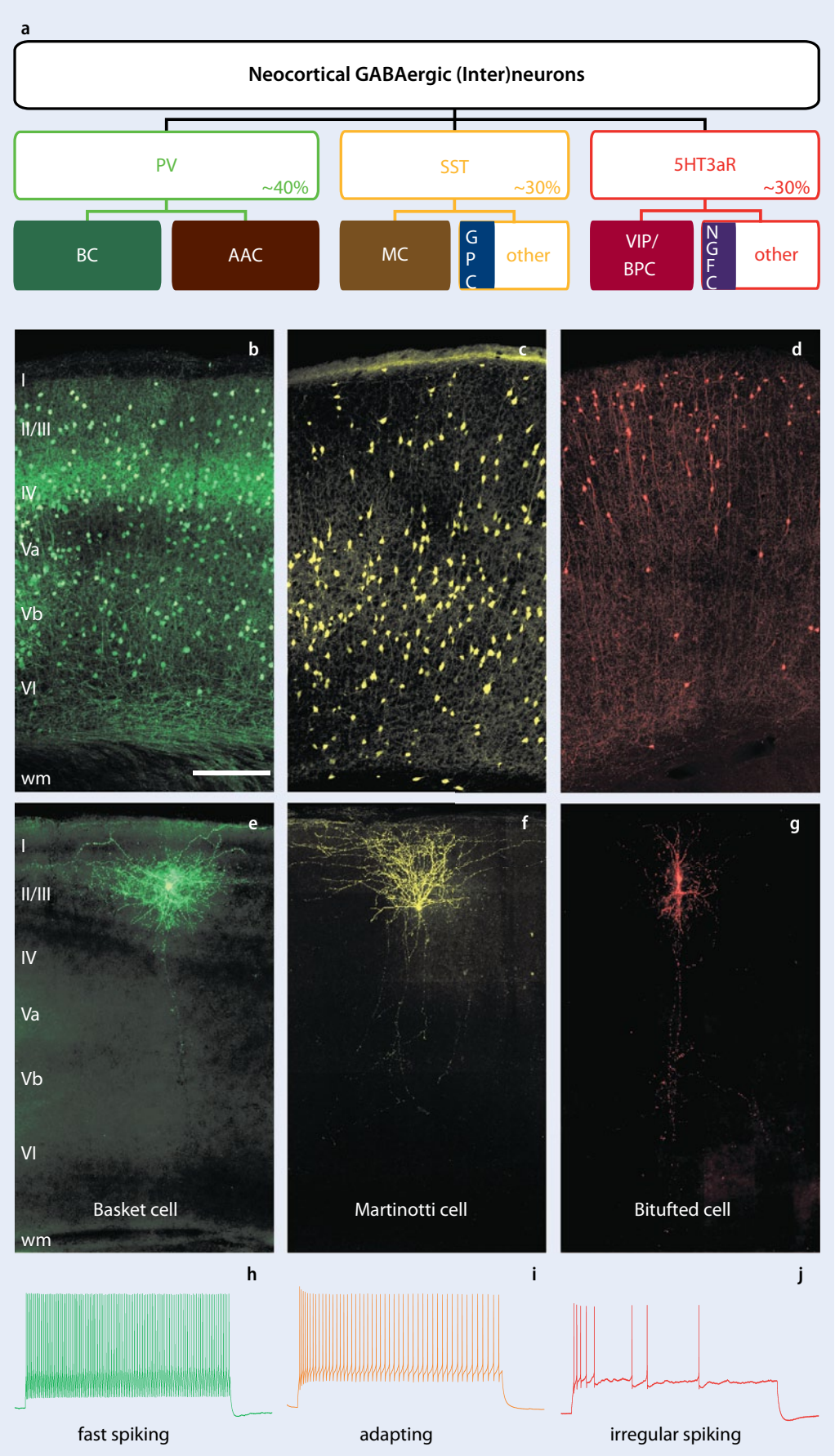

Fig. 1 ◀ Classification of three major neurochemically defined GABAergic neuronal populations and their basic morphological and electrophysiological properties in the barrel cortex. **a** Parcellation of neocortical GABAergic neurons based on molecular markers (*BC* basket cell, *AAC* axo-axonic cell, *MC* Martinotti cells, *GPC* GABAergic projection cell, *BPC* bipolar-bitufted cell, *NGFC* neurogliaform cell). **b** Low-magnification image of a PVcre section (*green*), which makes clear that PV cells (that should be mostly basket cells in this stain; see **e**) have a preferential location in layers IV and Vb. **c** Low-magnification image of a SSTcre section (*yellow*), showing that SST cells (that should be mostly Martinotti cells in this stain; see **f**) have a preferential location in layers V and VI. **d** Low-magnification image of a VIPcre section (*red*), demonstrating that VIP cells (that should be mostly bipolar/bitufted cells in this stain; see **g**) have a preferential location in layer II/III. *Roman numerals* indicate cortical layers; *scale bar*, 200 µm. **e–g** show photoreconstructions, all in layer II/III, of a parvalbumin-expressing, fast-spiking basket cell (**e**), a somatostatin-expressing, adapting Martinotti cell (**f**), and a VIP-expressing irregular-spiking bitufted cell (**g**). Please note the varicosities of the basket cell in L I, which are dendritic and not axonal. Please also note that the axonal arbor of the Martinotti cell in L I is truncated due to restrictions of figure size but extends over more than 2 mm tangentially below the pia. (**h–j**) show (one of) the typical action potential firing patterns upon strong depolarizing current injections via whole cell patch clamp electrodes (**h** continuous *fast-spiking*; **i** continuous *adapting*; **j** *irregular-spiking*)

tion processing. Based on this assumption and review of the available literature, we suggest that at least six cardinal types of GABAergic neurons do exist: (i) axo-axonic (or chandelier) cells; (ii) basket cells; (iii) Martinotti cells; (iv) bipolar/bitufted cells; (v) neurogliaform cells; and (vi) GABAergic projection neurons (**Fig. 3**). Chandelier and basket cells (**Fig. 1e**) express the Ca^{2+}-binding protein parvalbumin (**Fig. 1b**) and account for ca. 40 % of GABAergic neurons in the neocortex. They are mostly located in layers IV–VI (**Fig. 1b**) and exhibit a multipolar dendritic shape and show a fast-spiking action potential firing pattern. The SST group, which represents roughly 30 % of GABAergic neurons, includes Martinotti cells (**Fig. 1f**), GABAergic projecting neurons, and a subset of neurons that specifically target layer IV. SST-expressing neurons are preferentially found in layers V and VI (**Fig. 1c**). The 5HT3aR group, which also accounts for about 30 % of the total interneuron population, is the most heterogeneous subgroup. This subgroup includes bipolar/bitufted cells (**Fig. 1g**), neurogliaform cells and some yet unspecified interneurons. The bipolar/bitufted cells express vasoactive intestinal polypeptide (VIP) and are mainly located in supragranular layer II/III (**Fig. 1d**). In recent years, the distinct and nonoverlapping neurochemical markers PV, SST, and VIP attracted scientists. The main reason is that by using transgenic cre-driver mouse lines for these neurochemical markers, specific investigation of distinct GABAergic neurons is now much easier to perform under *in vitro* and especially *in vivo* conditions.

For the sake of completeness, it should be mentioned that a variety of other classification schemes have been proposed up to date. These were based on (i) embryonic origin, (ii) somatodendritic morphological archetypes, (iii) relationship to cortical layers and columns, (iv) action potential firing pattern and neurochemistry, (v) pathway control, (vi) synapse dynamics, (vii) contribution to circuit motifs (e.g., feedforward or feedback inhibition), or (viii) electrotonic coupling (for a detailed list of references, please contact the authors). Here, we strongly suggest that the axonal projection pattern and its subcellular target specificity on the postsynaptic neurons are the most fundamental criteria to understand the complex functionality of neocortical interneurons and therefore were the properties of choice for the present classification.

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Abstract

The neocortex is regarded as the brain structure responsible for mediating higher brain functions, like conscious perception of sensory signals, learning and memory or programming of goal-directed behavior. Cortical circuits that enable these functions are formed by, first, a larger population of excitatory so-called principal cells (i.e., glutamatergic pyramidal cells; ca. 80–85 %), which issue long-distance projections, in addition to local recurrent collaterals, which form the major part of local cortical excitatory circuits. A second, smaller population of inhibitory also called local or short-axoned interneurons (i.e., GABAergic neurons; ca. 15–20 %), however, contribute heavily to intracortical microcircuits too. They can be subdivided by their location in specific areas, layers, or columns, which possess specific input–output relationships, but also in terms of morphology, electrophysiology, molecular expression profiles, and subcellular target specificity. Here it is proposed that, at present, in the rodent neocortex this population of GABAergic neurons can be reasonably divided into six different types, mainly due to their unique axonal patterns and subcellular target specificity: (i) axo-axonic cells, (ii) basket cells, (iii) Martinotti cells, (iv) bipolar/bitufted cells, (v) neurogliaform cells, and (vi) projection neurons. These different types of GABAergic neurons strongly govern the working of cortical circuits for meaningful behavior by feed-forward and feedback inhibition as well as disinhibition. Thus, they keep excitation in check, perform gain modulation, and open temporal or spatial windows for input control or output generation.

Keywords

Action potential firing pattern · Calcium-binding proteins · Cortical circuits · Excitation–inhibition balance · Inhibitory interneurons · Neuropeptides

The best-defined types of cortical GABAergic neurons and their possible subtypes

We hold the opinion that at least six different types have been repeatedly and consistently described. The level of insight gained mainly with Golgi staining was conclusively summarized some 20 years ago [3]. Here we will mainly focus on work that was done thereafter, using biocytin-filled patch or conventional sharp electrodes *in vitro* or *in vivo*.

Axo-axonic (or chandelier) cells

The axo-axonic cell (AAC; **Fig. 2**) is the cell type with the highest subcellular target specificity, that is, it targets only the axon initial segment of pyramidal cells, which was firmly established by correlated light and electron microscopy. As such, it can be considered to contribute a different kind of inhibition than so-called perisomatic or dendritic inhibition (see below). Over a long period, only anecdotal reports appeared on the morphology or physiology of AAC. A series of recent reports then raised the big question whether these cells really are the strongest inhibitors of their target cells, effectively vetoing any attempt to generate an action potential, or, whether they are actually, under certain conditions, able to depolarize cortical pyramidal cells. It was further recognized that in the neocortex AAC are a rare cell type but with the advent of more refined genetic labeling techniques they can now be studied in a controlled manner [11].

Axo-axonic cells have been classically called chandelier cells, due to their recurrent axonal collaterals carrying cartridges of roughly 3–7 boutons. Thus, on average, the ca. 16 boutons found on the axon initial segment of a pyramidal cell should converge from four neighboring AAC. For still unknown functional reasons, there are two tiers of preferential occurrence of AAC-somata, upper L II/III and L Vb and VI. These somata mostly possess a bitufted or multipolar dendritic tree

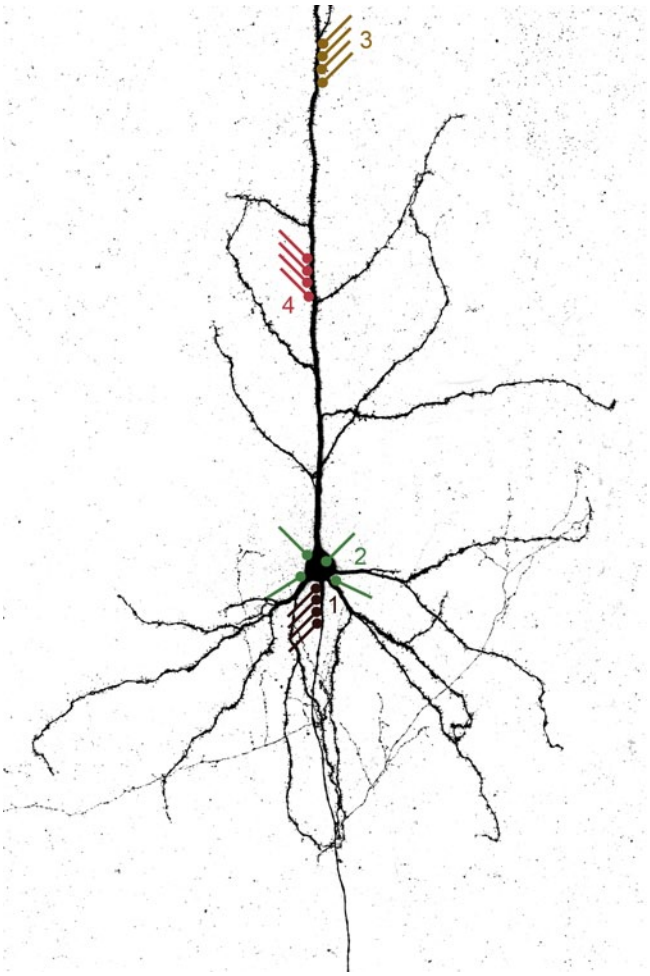

Fig. 2 ◀ Subcellular target specificity of GABAergic synapses formed with glutamatergic pyramidal cells. A part of a biocytin-filled layer Vb pyramidal cell is shown onto which schematically the position of synapses of (*1*) axo-axonic cells (*brown*) on the axon initial segment, (*2*) basket cells (*green*) on the soma, (*3*) bipolar-bitufted cells (*red*) on the "intermediate" dendrites, and (*4*) Martinotti cells (*yellow*) on the "distal" dendrites is indicated. This well-established concept formed the basis for the present segregation of GABAergic neurons into six cardinal types

and often express parvalbumin (in up to 50 % of the cases), in a cortical area-dependent manner. Interestingly, no matter whether they express parvalbumin or not, this landmark study [11] found that these neurons invariably show a fast-spiking action potential firing pattern. A recent notion suggests that AACs may play a dual role in cortical circuits: They could help to activate quiescent pyramidal neurons, while at the same time inhibiting (over-) active ones in an orchestrated and meaningful manner.

Basket cells

Basket cells (BC; ◘ **Fig. 3**) are the core-contributing cell type for perisomatic inhibition. In a broad sense, for this type 3 different subtypes were proposed: the classical (i) small and (ii) large basket cells that were recently complemented by the so called (iii) nest-basket cell. As a population, basket cells can be found in any cortical layer except L I. Maybe other subtypes exist as well (see below) and thus it is not too surprising that a recent classification attempt found little agreement on this cell type [2]. However, parvalbumin seems to be a suitable neurochemical marker, as is the fast-spiking firing pattern (◘ **Fig. 1h**) at the physiological level. Parvalbumin defines with a proportion of roughly 40 % the largest group of cortical GABAergic neurons [6, 8], with a preponderance in somatic and bouton staining in layers IV and Vb. It should, however, be noted that also non-parvalbumin, non-fast-spiking basket cells might exist.

Morphological features of small basket cells are a very local multipolar dendritic tree (aspiny and beaded) and a local (i.e., intralaminar and intracolumnar) as well as dense axonal plexus, whose boutons form symmetric synaptic contacts to a large degree on somata and perisomatic dendrites. At a single cell level, the formation of pericellular baskets is very subtle, which makes the morphological identification of these cells more difficult than expected. So, it is very helpful to have the postsynaptic target cells labeled, which can be excitatory as well as inhibitory neurons. However, a full quantitative analysis at the correlated light and electron microscopic level, to clearly show what proportion of the synapses target different subcellular compartments, is still missing. Thus, the reported range varies between 50 % somatic targeting for the cat visual cortex to just 12 % for rat motor cortex. Certainly, a "blanket of inhibition" caused by cortical basket cells can be imposed on principal neurons and other basket cells, but may be extended to more cell types, e.g., to VIP-expressing neurons as well as Martinotti cells (own unpublished observations).

The main features of large basket cells are their extensive layer- and column-spanning dendritic and axonal arbors. However, these are less dense (than those of small basket cells) and therefore even more difficult to classify correctly at a single cell-level. In an elegant study in the cat visual cortex, many different modes of operation became apparent that do make the previously assumed single mode of lateral inhibition for large basket cells unlikely.

The nest basket cells stand in-between many features and thus it is difficult to judge whether they are a cell type in its own right or just the middle portion of a feature continuum. Such a continuum could represent the layer-specific needs of instantaneous excitation–inhibition balance by means of feed-forward and feedback inhibition that are considered to be the main functions of basket cells.

Martinotti cells

Because of their unique axonal ramification in layer I, Martinotti cells (MC; ◘ **Fig. 3**) are the second best-recognizable cell type (in terms of morphology; [2]). Due to recently established specific mouse models, also a lot has been learned about other properties, especially functional ones. From the present data, it can be stated that Martinotti cells with a mainly bitufted to multipolar dendritic tree (often being not so sparsely spiny) can be found in all cortical layers (again except L I), and no matter where the somata are located, always send a significant

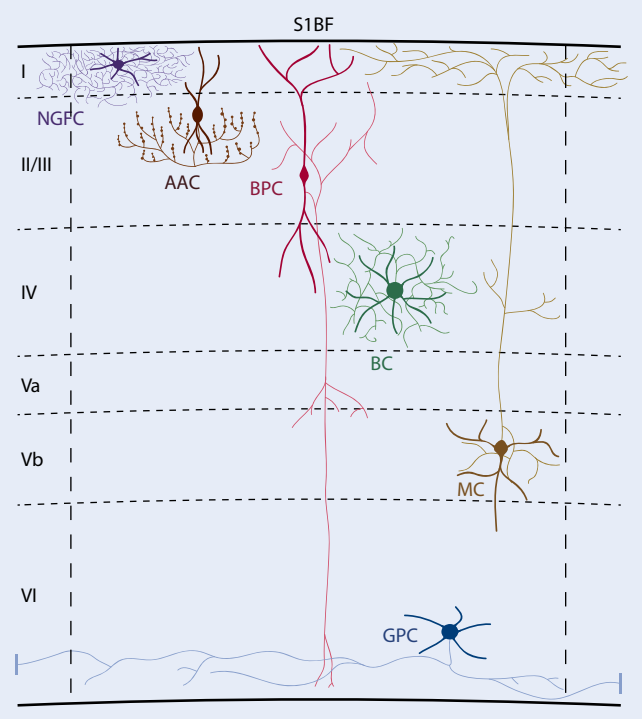

Fig. 3 ▲ Diagrammatic representation of the major somatodendritic and axonal morphological features of the proposed six types of GABAergic neurons. The neurons are exemplified for the primary somatosensory (barrel) cortex (*S1BF*). *Vertical stippled lines* delineate areal borders (to the *left* being secondary somatosensory cortex and to the right the hind limb area) whereas *horizontalstippled lines* indicate layer borders (*Roman numerals* indicate cortical layers; *wm* white matter). The different cell types are color coded and placed into a "most typical" layer (which in no instance is intended to be the only one), *darker hue* is used for the somatodendritic domain, *lighter hue* for the axon (AAC, *brown*; BC, *green*; MC, *yellowish*; BPC, *red*; NGFC, *mauve*; GPC, *blue*). Please note the much higher complexity of the axonal arbors in the photoreconstructions shown in **Fig. 1g–i**

portion of their axon into L I (**Fig. 1f**). However, although conceptually ignored up to now, the largest portion of the axon stays outside of L I and has not been studied functionally so far. Unfortunately, no detailed synaptology for Martinotti cells does exist in the neocortex. Interestingly, data from hippocampus strongly suggest that dendritic shafts and spines of pyramidal cells as well as many types of interneurons are postsynaptic to Martinotti cell boutons. This agrees well with morphologically more indirect but functionally conclusive results from neocortical studies [6, 9]. These cells express somatostatin as a marker molecule and are nearly exclusively of the adapting (regular or low-threshold spiking; **Fig. 1i**) action potential firing type. It was estimated that ca. 30 % of all GABAergic neurons are somatostatin-expressing [6, 8] and are mostly found in layers V and VI.

The best studied circuit motif until recently was disynaptic inhibition of neighboring pyramidal cells in layers II/III and V, which very likely represents a form of lateral inhibition. Recently, a large number of studies covering many different cortical areas, have revealed that Martinotti cells are a primary target for a disinhibitory action by which VIP-expressing bipolar/bitufted neurons (see below) can boost the activity in principal cell ensembles, a mechanisms which seems to improve sensory processing and sensory-dependent learning [4, 6].

Bipolar/bitufted cells

Until very recently, bipolar/bitufted cells (BPC; **Fig. 3**) were considered to be enigmatic and bear a lot of further names that are often more ornamental than scientifically helpful, like for instance double-bouquet, arcade, or horsetail cells [2]. In the primate neocortex, however, the term double-bouquet cell is reasonably used for a very fascinating calbindin D28k-expressing cell type that possesses a unique axonal feature, namely a microcolumnar organization. In rodents, the term bipolar/bitufted cell is strictly used to describe the vertically oriented somatodendritic organization; the axon, however, also being vertically extended, is often spanning many or even all cortical layers in a narrow, column-restricted manner. This suggests that this cell type not only can integrate many different inputs across all cortical layers, but also feeds back/forward inhibition to all these same layers. A useful molecular marker is either 5HT3aR, or more specifically VIP (**Fig. 2c**). Also calretinin and choline acetyl-transferase have been detected in many bipolar/bitufted cells. The electrophysiological "markers" are a very high input resistance and a predominantly adapting (previously: regular spiking non-pyramidal, RSNP) or bursting (burst spiking, BS), but very often also irregular spiking pattern (IS; **Fig. 2j**).

We are focusing here on the VIP-expressing bipolar/bitufted neurons that, as a relatively small subpopulation, have been neglected for a long time but are now in focus of many research groups, due to the availability of specific mouse models. It was estimated that roughly 12–17 % of all cortical GABAergic neurons express VIP [6, 8], which are highly concentrated in layer II/III. In the early days, a detailed ultrastructural examination already suggested that medium-sized dendrites (very likely originating from other inhibitory interneurons) are their main subcellular target structure. Indeed, VIP neurons are considered to be of the dendrite-targeting type, although at least small basket neurons in rat also do seem to express this neuropeptide. From the beginning, it had been obvious that bipolar/bitufted VIP neurons are predominantly vertically organized, so it was self-evident to imply them in columnar or even "microcolumnar" function. A net excitation is likely to be one of the outcomes of a behaviorally specific activation of VIP neurons by (cholinergic) arousal or attention mechanisms because that leads to inhibition of Martinotti cells as a preferred target, which—in turn—results in disinhibition of principal neurons.

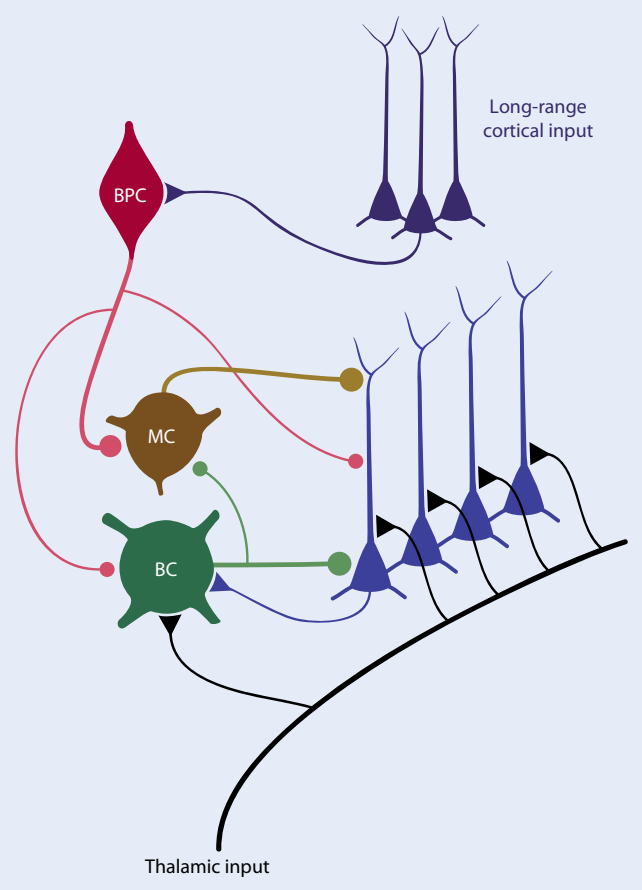

Fig. 4 ▲ Connectional motifs of basket, Martinotti and bipolar/bitufted cells. The diagram shows thalamus-generated feedforward inhibition by basket cells (*BC*) on local principal neurons (*lighter blue*). At the same time, this neuron type is involved in feedback inhibition, possibly of the very same neurons that excite them, thus, forming reciprocal connections. This diagram further shows that bipolar/bitufted cells (*BPC*) can be activated by (amongst others) distant pyramidal cells (*darker blue*) and subsequently inhibit Martinotti cells (*MC*), but also BC and glutamatergic principal neurons (*blue*) to cause a complex inhibition–disinhibition configuration of the local circuit. The thickness of the axons depicts the relative strength of the connections

Neurogliaform cells

The neurogliaform cells (NGFC; ◘ **Fig. 2**) actually are still very enigmatic. Since the landmark papers published by Gabor Tamas' group, the notion is up that they may be an unconventional type of volume transmission-utilizing interneuron. These act at a slower time scale via $GABA_B$-receptors [10] but also activate $GABA_A$-receptors and are promiscuously connected to other interneurons by gap junctions. There are few specific markers to easily and reliably identify NGFC. They are characterized by a late-spiking action potential firing pattern, alpha-actinin 2, reelin, NPY, or nNOS expression, and also their unique morphology with many short primary dendrites and amongst the thinnest and most densely arborizing axonal arbor of all neurons. This morphology also led to the names "dwarf cell" or "spider-web cell". In contrast to all previous cell types described in this chapter, it has a frequent occurrence in layer I, which is, however, not an exclusive location since it can be found in any layer.

Very little is known about the precise connectivity of this neuronal type. The available studies suggest that they are connected by chemical synapses to local pyramidal cells in a reciprocal manner whereas electrical synapses are made with different interneurons. Non-synaptic communication might take place with all somatodendritic structures located within the axonal cloud. There was, however, a recent publication that questioned the universality of this indiscriminate volume transmission scheme. It was reported that neurogliaform cells at an unusual location (i.e., layer IV of the primary somatosensory cortex) specifically modulate thalamic feedforward inhibition on spiny stellate cells but not thalamic feedforward excitation on nearby synapses. This might be only one example of a more general scheme of how $GABA_B$-receptor mediated inhibition is able to shape the activity of larger networks of principal neurons by diverse mechanisms.

GABAergic projection neurons

This GABAergic projection neuron-cell type (GPC; ◘ **Fig. 3**) is the single reason why the whole article is not called "What types of neocortical GABAergic *inter*neurons do really exist?" So we should finally shortly discuss what an *inter*neuron is supposed to be. In our opinion, there is no strict definition for this term, but intuitively "local interneurons" should not project out of the cortex and probably also not out of their home area. If this is the criterion to be met, only the neurons described in this paragraph are GABAergic projection neurons. If it is a more conservative criterion (not outside the home column or even layer), most of the GABAergic neurons would be projection and not local neurons, especially Martinotti cells or so called elongated neurogliaform cells of layer I, which possess horizontal collaterals that can span more than 2 mm. Here, a projection neuron is defined as one projecting into a neighboring area, no matter whether the route taken by the axon leads through the gray or white matter.

Interestingly, one of the few studies demonstrating this type of GABAergic neuron also established somatostatin (together with NPY and nNOS) as their main neurochemical marker [12], which is similar to Martinotti cells. But although Martinotti cells may extend their axon for more than 2 mm, these projection neurons were found at distances up to 8 mm remote from the injection site of the retrograde tracer and thus cannot be simply back-labeled classical Martinotti cells. Basically, several other groups have obtained similar results, both for ipsilateral and even callosal cortico-cortical projections. Very often, these cells are located deep in layer VI or even in the white matter [12]. To

Infobox 1 GABA actions in the neocortex

Neocortical GABAergic transmission is highly diverse. GABAergic neurons mostly form true junctional contacts with their postsynaptic targets, but some cell types can form *en passant* boutons too. On the other side, they act on their partners by way of ionotropic ($GABA_A$) and metabotropic ($GABA_B$) receptors. These two receptor types are not only distributed unevenly—$GABA_A$ receptors are found mostly at the postsynapse and only to a much smaller degree at extrasynaptic locations, whereas the primary locations of $GABA_B$ receptors are extrasynaptic sides and presynaptic terminals—they also utilize different conductance. $GABA_A$ receptors directly gate a large-conductance ion channel permeable to Cl^- and, to a lesser degree, HCO_3^-. In contrast, $GABA_B$ receptors, via G proteins, increase a small-conductance, inwardly rectifying K^+ conductance and decrease voltage-dependent Ca^{2+} conductances.

Hyperpolarization of the postsynaptic membrane, and thereby inhibiting the postsynaptic neuron, is traditionally regarded as the effect of GABAergic transmission. Indeed, hyperpolarizing postsynaptic potentials are often observed because the reversal potentials of $GABA_A$ (around −70 mV) and $GABA_B$ receptors (around −90 mV) are slightly or much more negative than the membrane potentials in many cases. However, the situation is actually more complicated.

In the case of junctional $GABA_A$ receptors the postsynaptic membrane potential is often very close to the reversal potential and, therefore, only a very small or no hyperpolarization as well as small depolarizing postsynaptic potentials might occur. However, still in these cases, the net effect will be inhibitory, i.e., counteracting spike generation, due to a decrease in input resistance and shunting of excitatory inputs. If the presynaptic GABAergic neuron fires extended spike trains, the Cl^- gradient will collapse more than the HCO_3^- gradient eventually shifting the reversal potential to even less negative but clearly subthreshold values. A second mechanism able to shift the reversal potential of $GABA_A$ receptors to less negative values is the lack of Cl^- transporters. This has been originally described for the developing brain but was recently also found at the axon initial segment. While it has been clearly shown that during development GABAergic transmission via $GABA_A$ receptors is indeed excitatory, i.e., elicits action potentials, there is no evidence that this is also true at the axon initial segment in elder animals. Surely, postsynaptic responses can be depolarizing if the postsynaptic neuron is at rest but the measured reversal potentials are well below firing threshold.

$GABA_B$ receptor-mediated postsynaptic responses are much slower and longer than $GABA_A$ receptor-mediated responses. In addition, their amplitudes are small in any case. Their main postsynaptic effect is to decrease excitability by increasing K^+ conductance. In parallel to lowered excitability, $GABA_B$ receptors also decrease synaptic inputs onto the postsynaptic neuron. This might be simply explained by a decrease in input resistance, however, the presynaptic location of $GABA_B$ receptors point to a second possibility. Indeed, they decrease the influx of Ca^{2+} at the presynaptic terminal and as a consequence decrease the release of transmitter.

the best of our knowledge, they have not been characterized electrophysiologically or morphologically at an identified single cell level so far. Nothing is known about their function to date. A look into the archicortex offers the interesting possibility that GABAergic projection neurons targeting local GABAergic interneurons can synchronize distant areas to behaviorally relevant rhythmic discharges.

Circuit motifs of GABAergic neurons

The existence of distinct types of GABAergic neurons suggests that they might participate in different kinds of microcircuits (**Fig. 4**) and not only target different subcellular parts of postsynaptic neurons (**Fig. 2**). Simultaneous intracellular recordings of two or more neurons *in vitro* and *in vivo* have widely been used to detect di- or oligosynaptic connectivity patterns involving GABAergic neurons. The advent of specific cre-driver lines, which help to directly target specific cell types by means of specific molecular markers, substantially improved this approach. Based on such experiments a number of circuit motifs have been described across different cortical areas, which are: feedforward inhibition, feedback inhibition, lateral inhibition, recurrent inhibition, and most recently (disynaptic) disinhibition (see also **Fig. 1** of Roux and Buzsaki [7]).

The best studied small-scale circuit motif is feedforward inhibition. Here, a common source passes over excitation to an excitatory principal neuron as well as to a GABAergic interneuron. The latter then directly inhibits the principal neuron which receives the shared excitatory drive. Such a mechanism effectively controls the time window for integration. This pattern is the hallmark of thalamocortical inputs to layer IV of primary sensory cortices but it also exists in information transfer from layer IV principal neurons to layer II/III pyramidal neurons as well as within layer II/III. In all examples strong feedforward inhibition is mediated via fast spiking basket cells (**Fig. 4**). However, this is possibly true only for low-frequency stimuli because excitatory inputs onto fast spiking neurons strongly depress at frequencies of about 10 Hz and above. Basket cells are, therefore, suited to provide phasic feedforward inhibition but not sustained one. This gap might be filled by SST-expressing Martinotti cells in layer V, which also receive thalamocortical input and target thalamorecipient principal neurons. These cells require repetitive stimulation for both, their activation as well as effective inhibitory output.

Feedback inhibition, i.e., reciprocally connected excitatory and inhibitory neurons, effectively controls excitation levels in principal neurons. It was not the central topic of studies so far, but occurs between different GABAergic cell types and principal neurons (again, see basket cell in **Fig. 4**). Connections between pyramidal neurons and fast spiking basket cells in layer II/III have been frequently described. Pyramidal neurons in layer II/III are also reciprocally connected with SST-expressing, calretinin-expressing bipolar and calretinin-negative multipolar neurons, or VIP-expressing bipolar cells, all of these inhibitory neurons being located in layer II/III, too. Interestingly, all of these reciprocal pairs show either synaptic depression or facilitation opening a wide dynamic range of activation patterns. Finally, reciprocal connections also exist between principal neurons and either fast spiking or regular spiking nonpyramidal cells in layer IV.

Lateral inhibition in the classical sense sharpens contrast between parallel channels of information transfer. Accordingly, we assume that this motif should involve transcolumnar inhibition rather than intracolumnar inhibition. Disynaptic inhibition between neighboring principal neurons has been shown frequently but reports on long-range disynaptic inhibition between principal neurons, based on simultaneous intracellular recordings, are rare. Disynaptic inhibition was found between layer V pyramidal neurons up to

about 525 µm away from each other. The connectivity rates drop substantially at distances >150 µm but this does not necessarily represent the true rates. In *in vitro* preparations long-range connections are much more severed than local ones. Based on the study by Silberberg and Markram [9] it is argued that Martinotti cells are the most likely candidate for lateral inhibition.

Recurrent inhibition is a generic term, to our opinion not very precisely defined. Therefore, all of the abovementioned motifs could be types of recurrent inhibition, too. Besides them, a number of other disynaptic inhibitory circuits including almost all GABAergic neuron types are usually listed under this category.

The most recently discovered inhibitory circuit motif is disynaptic disinhibition (exemplified best by the bipolar/bitufted cell in **Fig. 4**). Here, a GABAergic neuron inhibits a second GABAergic neuron which in turn targets an excitatory principal neuron. As a result, the principal neuron is released from inhibition. So far this motif has been described in supra- and infragranular layers of visual as well as in layers IV and II/III of somatosensory cortex. Different combinations of GABAergic neurons contribute to this motif, primarily studied in primary visual and somatosensory cortex: SST-expressing cells target PV-expressing cells, VIP-expressing cells target SST-expressing cells, and PV-expressing cells target other PV-expressing cells. The only known source of excitatory drive to these disinhibiting neurons is pyramidal neurons in primary motor cortex, which target VIP-expressing cells in supragranular layers of primary somatosensory cortex. This type of GABAergic neurons, however, can also be driven by local pyramidal neurons and cholinergic afferents from basal forebrain.

Based on these considerations, it is obvious that probing of local unitary connectivity is likely not the key to an understanding of larger circuit motifs. Moreover, individual GABAergic neurons may participate in different circuit motifs or even execute also "unspecific" inhibition. For example, SST and PV cells as well as chandelier cells and neurogliaform cells densely innervate most of the pyramidal neurons within their axonal sphere. Therefore, it was recently proposed that the most prominent types of GABAergic neurons provide a general "blanket of inhibition" across an entire column. This blanket, however, seems to be dynamically regulated by more specific circuit patterns, amongst which VIP neurons play an important but probably not exclusive role [5].

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