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# INTERNEURONS OF THE NEOCORTICAL INHIBITORY SYSTEM

*Henry Markram\*, Maria Toledo-Rodriguez\*, Yun Wang[‡], Anirudh Gupta\*, Gilad Silberberg\* and Caizhi Wu[§]*

Abstract | Mammals adapt to a rapidly changing world because of the sophisticated cognitive functions that are supported by the neocortex. The neocortex, which forms almost 80% of the human brain, seems to have arisen from repeated duplication of a stereotypical microcircuit template with subtle specializations for different brain regions and species. The quest to unravel the blueprint of this template started more than a century ago and has revealed an immensely intricate design. The largest obstacle is the daunting variety of inhibitory interneurons that are found in the circuit. This review focuses on the organizing principles that govern the diversity of inhibitory interneurons and their circuits.

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Neocortical neurons are not randomly distributed in the cortical sheet, but are arranged in layers (layers I–VI) that connect to different cortical and subcortical regions[1–3]. In rodents, a neocortical column of about 0.3 mm in diameter contains roughly 7,500 neurons[4,5] (100 neurons in layer I; 2,150 in layer II/III; 1,500 in layer IV; 1,250 in layer V and 2,500 in layer VI). Most neocortical neurons (70–80%) are excitatory pyramidal neurons[1,3,6,7], which have relatively stereotyped anatomical, physiological and molecular properties[1,8]. The remaining 20–30% of neocortical neurons are interneurons, mostly inhibitory interneurons, which have diverse morphological, physiological, molecular and synaptic characteristics[3,6,8–16].

Despite this diversity, inhibitory interneurons have many common features, some of which distinguish them from pyramidal neurons. First, most mature inhibitory interneurons have aspiny dendrites[3,6,17]. Second, interneurons can receive both excitatory and inhibitory synapses onto their somata[3,17,18]. Third, the axons of inhibitory neurons usually arborize within a cortical column and can project laterally across columns, but do not typically project down into the white matter to contact distant brain regions[19]. Indeed, neocortical interneurons in general are also called 'local circuit neurons' to reflect the restriction of their axonal and dendritic arbours to the neocortex[20]. Fourth, different

types of inhibitory neuron seem to be especially capable of targeting different subdomains of neurons (dendritic regions, soma or axon)[14,21].

Interneurons can also be excitatory. The spiny stellate cell (SSC) is an important type of excitatory (glutamatergic) interneuron that has a star-like dendritic arborization with a high density of spines around its soma. These cells are found only in layer IV of primary sensory areas[22,23]; receive excitatory inputs from specific thalamic nuclei[3]; and relay this information to layer II/III[24–26], indicating that they are specialized to process thalamic input. SSCs share many characteristics with pyramidal neurons, but lack a prominent apical dendrite[27]; instead, they have only a partial apical-like dendrite that can reach layer III. There are also excitatory peptidergic interneurons, including a subgroup of bipolar interneurons — small, oval cells with axons and dendrites that stretch vertically in a narrow band across all layers.

Inhibitory interneurons, which use GABA (γ-aminobutyric acid) as their transmitter, vary greatly in their somatic, dendritic and axonal morphologies (FIG. 1). Dendritic morphology is the most variable feature and cannot reliably define the type of interneuron. However, the axonal aborization can reveal the anatomical identity of an interneuron because interneurons seem to be particularly specialized to target different domains of neurons, different layers of a column and different columns.
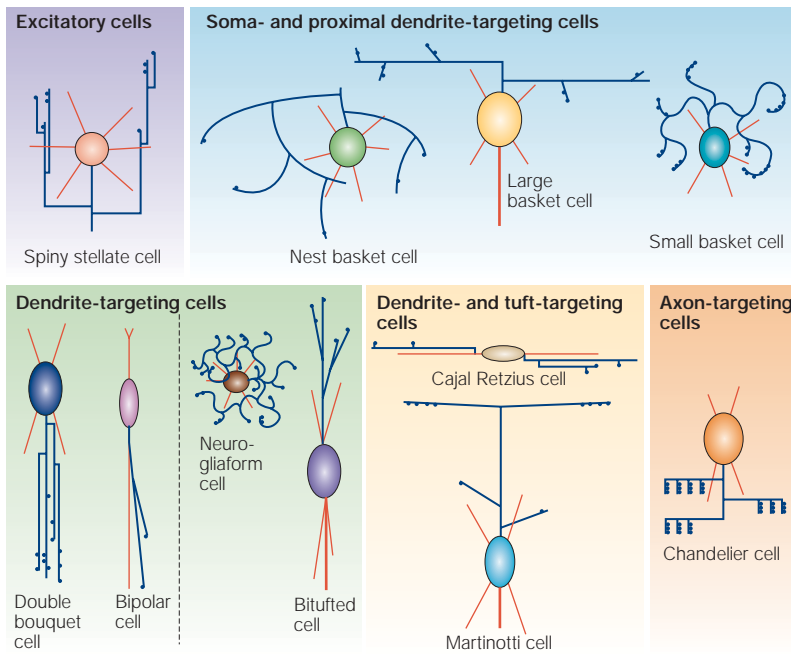
Figure 1 | **Anatomical diversity of neocortical neurons.** Scheme summarizing the main anatomical properties of neocortical inhibitory interneurons. Each neuron type has a different coloured soma; dendrites, red; axons, blue lines; axonal boutons, blue dots. Spines are omitted for clarity. Neurons are orientated with the pia facing upwards and white matter downwards. Some interneurons have a prominent, vertical dendrite directed towards the white matter. Inhibitory interneurons are mainly distinguished by the structure of their axonal arbour (see text) and typically innervate selective domains ((peri-) somatic, dendritic or axonal) of their target cells. Modified, with permission, from REF. 167 © (2002) MIT Press.

This is a crucial issue as many studies, especially recent studies that lack a thorough anatomical background, have confused cell types by relying only on dendritic features. In other words, the multipolar, bipolar and bitufted classifications of interneurons cannot be used alone to identify a cell type (see section on morphological properties). However, on the basis of their axon targeting, interneurons can be functionally divided into axon-targeting, soma- and proximal dendrite-targeting, dendrite-targeting, and dendrite- and tuft-targeting interneurons[14,21], and also into intralaminar–intracolumnar, interlaminar–intracolumnar, intralaminar–intercolumnar and interlaminar–intercolumnar interneurons. The relative percentages of each interneuron type vary in different species, brain regions and layers[3,12,28–37]. Here, we review the characteristic features and connections of the most common types of inhibitory interneuron, with a particular emphasis on quantification obtained in the rat somatosensory cortex.

### Morphological properties of interneurons

*Basket cells.* About 50% of all inhibitory interneurons are basket cells (FIG. 2). Basket cells specialize in targeting the somata and proximal dendrites of pyramidal neurons and interneurons[2,3,31,38–40] (FIG. 1), which places them in a unique position to adjust the gain of the integrated synaptic response. The term 'basket cell' comes from the basket-like appearance around pyramidal cell somata

that results from convergent innervation by many basket cells. On the basis of differences in their axonal and dendritic morphologies, basket cells can be divided into three main subclasses: large basket cells (LBCs), small basket cells (SBCs) and nest basket cells (NBCs). Basket cells typically express many neuropeptides and the two calcium-binding proteins, PARVALBUMIN (PV) and CALBINDIN (CB) (FIG. 3).

*Large basket cells.* LBCs are the classic basket cells. They have large, aspiny, multipolar dendrites and expansive axonal arborizations that can inhibit neurons in upper and lower layers and in neighbouring and distant columns[2,3,17,28,38,39,41]. LBCs are therefore the primary source of lateral inhibition across columns within the layer that contains their somata. The local axonal arborization of LBCs is sparse, has a low bouton density and, uniquely, tends to branch sharply (FIG. 1; online supplementary information S1 (table)), often giving it a stick-like appearance. The somato–dendritic morphology is often multipolar, but can be bitufted, pyramidal or bipolar. LBCs can express CB, PV, neuropeptide Y (NPY), cholecystokinin (CCK) and occasionally somatostatin (SOM) and CALRETININ (CR). They never express vasoactive intestinal peptide (VIP) (FIG. 3).

*Small basket cells.* SBCs are aspiny, soma-targeting interneurons with local, dense and highly varicose axonal arborizations that seldom send axons beyond a cortical layer or column[2,28,38,39,42,43] (see online supplementary information S1 (table)). Their somato–dendritic morphology can be multipolar, bitufted or bipolar, with different tendencies depending on the layer; SBCs in layer IV are often multipolar and in layer II/III are more often bitufted or bipolar. SBCs can also be readily distinguished from LBCs by their frequently branching and 'curvy' axons (FIG. 1; online supplementary information S1 (table)). Occasionally, a few collaterals extend out of the local axonal cluster. SBCs form the highest number of synapses on pyramidal neurons (FIG. 4; online supplementary information S2 (table)). They differ from other basket cells in that they express VIP (FIG. 3). A special subtype of SBC, the clutch cell, is found in layer IV of the visual cortices of cats and monkeys[43]. These are medium sized, multipolar cells that typically produce curvy axonal collaterals with large bulbous terminals that appear to 'clutch' the somata of their target cells.

*Nest basket cells.* NBCs were frequently reported in the past and sometimes referred to as interneurons with 'irregular arborizations'[30,44–46], but were only recently shown to be a distinct class of soma-targeting cell[15,38]. The name 'nest' arises because of their birds'-nest-like appearance. NBCs seem to be a hybrid of LBCs and SBCs, but have a local axonal cluster more like SBCs and less frequent branching and longer axonal collaterals with a lower density of boutons, more like LBCs (FIG. 1; online supplementary information S1 (table)). NBCs do not typically express CR and never express VIP (FIG. 3).

PARVALBUMIN
(PV). A calcium-binding protein that can act as an endogenous buffer in certain neurons.

CALBINDIN
(CB). A calcium-binding protein that might function as a calcium buffer.

CALRETININ
(CR). A calcium-binding protein that can be used as a marker of preplate neurons.
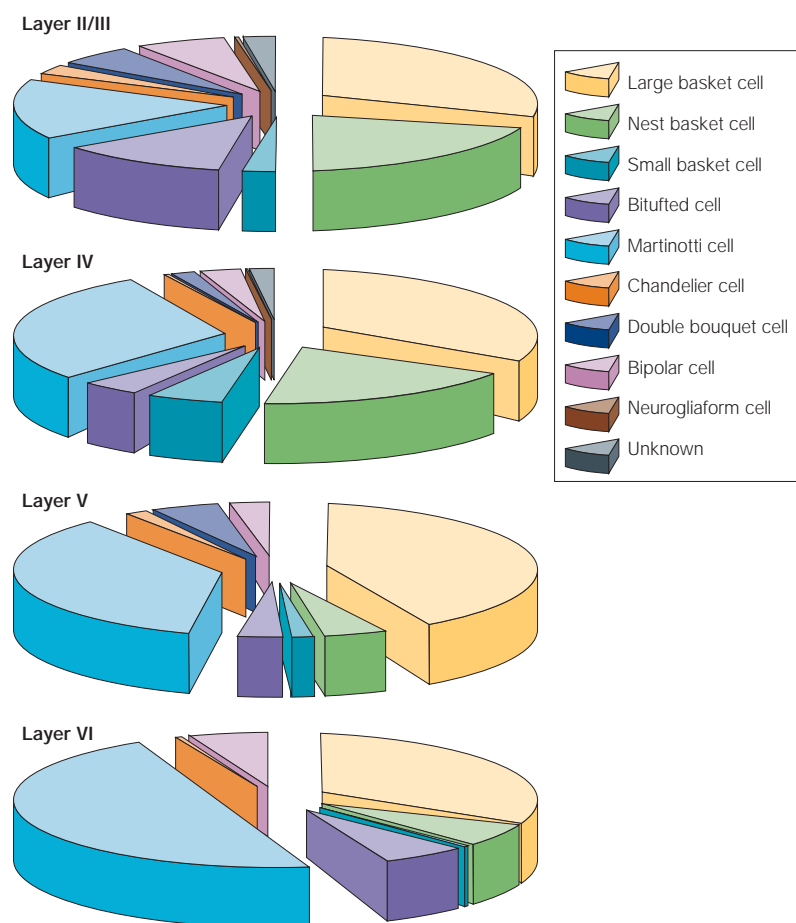
Figure 2 | **Different types of interneuron in the layers of somatosensory cortex of juvenile rats.** Note that the percentage of neurogliaform cells might be artificially small because very small somata were often overlooked during recording.

*Chandelier cells.* Chandelier cells (ChCs) are axon-targeting interneurons[14,21,47,48]. This targeting could place ChCs in a powerful position to override all the complex dendritic integration and somatic gain settings by 'editing' the action potential output[49–51]. They can be multipolar or bitufted. Their local axonal clusters are formed by high-frequency branching at shallow angles, often ramifying around, above or below their somata with a high bouton density. The characteristic terminal portions of the axon form short vertical rows of boutons, resembling a chandelier[47,52] (FIG. 1). The chandelier-like appearance seems to become progressively more refined in 'higher' species, with the clearest form (and perhaps greater abundance) in primates[12,53]. ChCs have been found in layers II–VI. They typically express one or both of the calcium-binding proteins PV[54] and CB (FIG. 3). Similar axon-targeting neurons have been described in the hippocampus[55].

*Martinotti cells.* Martinotti cells (MCs) are found in layers II–VI. They specialize in projecting their axons towards layer I, where they inhibit the tuft dendrites of pyramidal neurons (FIG. 1; online supplementary information S1 (table)). Their axons can also project

horizontally in layer I for millimetres[3,8,12,19,56,57] to inhibit tuft dendrites in neighbouring and distant columns, providing the only source for cross-columnar inhibition via layer I from layers II–VI. MCs target not only the most distal dendrites, but also proximal dendrites, peri-somatic dendrites and somata[56]. Infragranular MCs can also selectively target layer IV[56]. MCs are therefore unusual in that they target multiple domains and multiple layers. They most often have bitufted morphology with a more elaborate dendritic tree than most interneurons. The axons of MCs are also unusual in that they form spiny boutons. MCs always express SOM and never express PV or VIP (FIG. 3).

*Bipolar cells.* Bipolar cells (BPCs) are small cells with spindle or ovoid somata and narrow bipolar (most often) or bitufted dendrites that extend vertically towards layer I and down to layer VI[3,58–60] (FIG. 1). Their axon commonly emerges from one of the primary dendrites and forms a narrow (<50 µm) band that crosses all layers (see online supplementary information S1 (table)). Bipolar neurons can be excitatory by releasing only VIP, or inhibitory by releasing mainly GABA (inhibitory BPCs also express VIP). Their bouton density is low compared with other interneurons, and they therefore contact only a few cells, mainly on the basal dendrites of pyramidal neurons. BPCs occur in layers II–VI, and typically express CR and VIP (FIG. 3).

*Double bouquet cells.* Double bouquet cells (DBCs) usually have a bitufted dendritic morphology. Their special feature is a tight fascicular axonal cylinder[3,61–63] that resembles a 'horse tail' (FIG. 1). The highly varicose collaterals that form these columnar bundles are unusually thicker than the axonal main stem and can extend across all layers. In primates, DBCs seem to be interleaved with pyramidal cells to inhibit their basal dendrites[63]. The axons of DBCs branch frequently to form higher-order branches and are densely studded with boutons. DBCs mainly innervate dendrites (spines and shafts) and are therefore dendritic-targeting cells (see online supplementary information S1 (table)). DBCs occur in layers II–V, although they seem to be preferentially located in the supragranular layers. They express CB, have the unique tendency to express CR and CB together and can also express VIP or CCK, but not PV, SOM or NPY (FIG. 3).

*Bitufted cells.* Bitufted cells (BTCs) are similar to BPCs and DBCs in that they usually have ovoid somata and give rise to primary dendrites from opposite poles to form a bitufted morphology (FIG. 1). However, unlike the narrow vertical axonal projection of BPCs, and the 'horse-tail' axonal cluster of DBCs, BTC axons have wider horizontal axonal spans, even across a cortical column (see online supplementary information S1 (table)). The vertical projection is also less extensive and crosses mostly to neighboring layers. BTCs are dendritic-targeting cells[14] that are found in layers II–VI. They can express CB, CR, NPY, VIP, SOM and CCK, but not PV (FIG. 3).
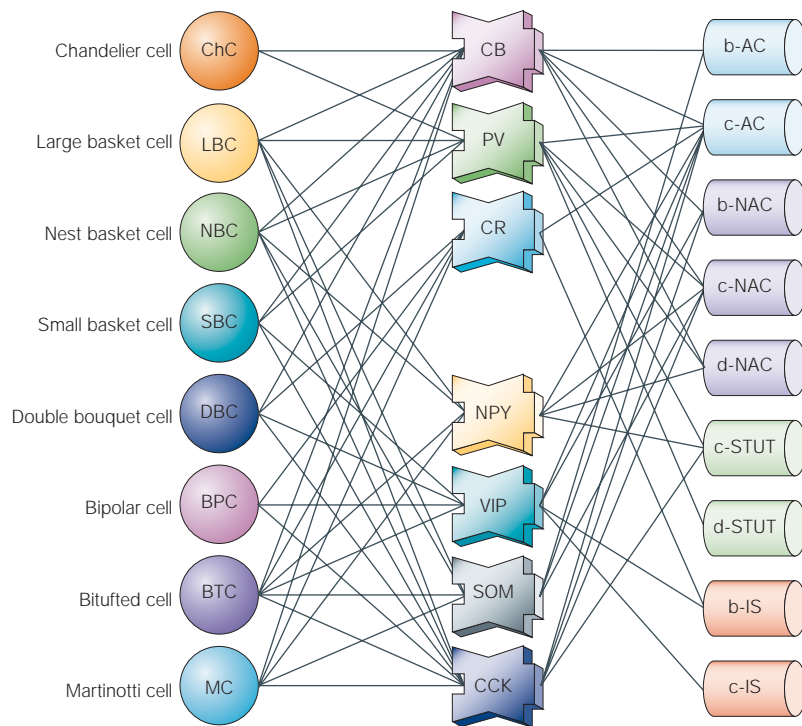
Figure 3 | **Expression of calcium-binding proteins (CBPs) and neuropeptides in interneurons.** Expression profiles of the CBPs calbindin (CB), parvalbumin (PV) and calretinin (CR) and the neuropeptides neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), somatostatin (SOM) and cholecystokinin (CCK) by different morphological and electrophysiological classes of interneuron. AC, accommodating; b, burst subtype; c, classic subtype; d, delay subtype; IS, irregular spiking; NAC, non-accomodating; STUT, stuttering.

*Neurogliaform cells.* Neurogliaform cells (NGCs) are small, 'button-type' cells with many fine, radiating dendrites that are short, aspiny, finely beaded and rarely branched[3,39,64]. They form a highly symmetrical and spherical dendritic field. The axon can arise from any part of the soma or from the base of a dendrite, and shortly after its origin, it breaks up into a dense, intertwined arborization of ultra-thin axons with as many as ten orders of branching (FIG. 1). Fine boutons are distributed on the axonal collaterals to form GABA synapses onto the dendrites of target cells[39]. The molecular characteristics of NGCs are not well studied.

*Layer I interneurons.* Virtually all layer I neurons are inhibitory[65,66], and they fall into two categories, which might reflect different origins[65–68]. The first comprises large neurons with horizontal processes, known as Cajal Retzius cells, which seem to be present mostly during development, and are unique to layer I. These multipolar cells can have various soma shapes, which probably arise from adaptations to different locations in layer I. Their axons, which are confined to layer I, can be extensive and typically have a horizontal trajectory from which extend many short ascending and some descending terminal fibrils that are believed to target the terminal tufts of pyramidal cells (FIG. 1). The second category is a heterogeneous group of small, multipolar interneurons with varying axonal arborizations (poor and rich axonal plexus cells).

## Electrical properties of interneurons

Neocortical neurons have various active and passive properties, so they respond differently when excited with a depolarizing step current pulse[69–72]. The classification of these responses has been refined over the past decade. Originally, all inhibitory interneurons were described as fast spiking (FS)[69,71], but subsequent recordings revealed other discharge patterns[11,73], such as those of low-threshold-spiking (LTS) cells, also known as burst-spiking non-pyramidal (BSNP) cells[11,73,74]. These show typical burst-like discharges after a hyperpolarizing pre-pulse and are found in layer V (which also contains many bursting pyramidal cells). Some of these interneurons have been anatomically identified as MCs and DBCs. Regular-spiking non-pyramidal (RSNP) cells discharge in a manner that resembles regular-spiking pyramidal cells[11,75,76]. These cells have been recorded in layers II/III and V, and some have been identified as MCs, DBCs and BPCs. Late-spiking (LS) cells, which discharge with a considerable delay after a depolarizing step, have also been reported. These cells were found in layers II/III and V, and some were identified as NGCs[11,75]. Irregular-spiking (IS) cells fire an initial burst of action potentials followed by irregularly spaced action potentials, and form a small fraction of interneurons with bipolar morphology in layers II/III and V[9,77]. IS cells have been further divided, according to the duration of the initial burst, into IS1 and IS2 cells[77]. The characteristics of these bursts differ from those of intrinsically bursting pyramidal cells (TABLE 1). An attempt to map synapse types between pairs of different types of neuron led to the conclusion that the resolution of these classification schemes was not sufficient to uniquely identify inhibitory interneurons. A classification scheme was therefore recently developed that is based on both the onset and the steady-state response to a step current injection into the soma[15].

*Steady-state response types.* When divided according to their steady-state response, interneurons fall into five groups (TABLE 1; FIG. 5): non-accommodating (NAC); accommodating (AC); stuttering (STUT); irregular spiking (IS); and bursting (BST). NACs fire repetitively without frequency adaptation in response to a wide range of sustained somatic current injections. The interspike intervals of consecutive action potentials during the steady state either do not change or change minimally, and the discharge frequency increases steeply as a function of the injected current amplitude. Their single action potentials are very brief and characteristically have a deep fast afterhyperpolarization (fAHP). ACs fire repetitively with frequency adaptation and therefore do not reach such high firing rates as NACs, but some could be classified as fast spiking (TABLE 1). STUT cells fire high-frequency clusters of action potentials intermingled with unpredictable periods of silence ('Morse-code'-like discharges) for a wide range of sustained somatic current injections. The action potentials in a cluster show hardly any accommodation, and the silent periods between clusters vary unpredictably in duration.
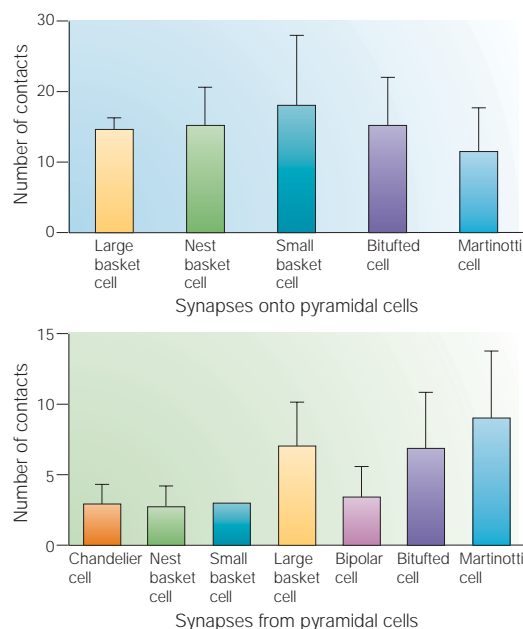
Figure 4 | **Contact numbers of interneurons onto and from pyramidal cells.** Top, the number of synapses onto pyramidal cells made by the various interneuron types; bottom, the number of synapses each type of interneuron receives from pyramidal cells. Error bars correspond to the standard deviation.

IS cells discharge single action potentials randomly throughout the 'steady-state' phase of sustained current injections and have been found only occasionally in layers II–V[77]. IS cells tend to show marked accommodation. BST cells characteristically fire a cluster of 3 to 5 action potentials riding on a slow depolarizing wave, followed by a strong slow afterhyperpolarization (sAHP). This burst is similar to the classic burst found in pyramidal cells and not like the transient bursts found in other interneurons (see below). ACs and NACs are the most common response types encountered in the juvenile somatosensory cortex. Stuttering behaviour is less common, but has been observed in all layers (II–VI). BST cells have been observed in layers II–V, but are most commonly found in layer V.

*Onset response types.* Neurons can be divided into three subclasses according to the type of onset that their response to a step depolarization shows. b-NAC cells initially discharge a cluster of three or more action potentials, d-NAC cells show a delay before discharge onset and c-NAC cells have neither bursts nor delays at the onset (the 'onset' phase is indistinguishable from the 'steady-state' phase). These responses are referred to as classical responses (TABLE 1; FIG. 5). All three of these initial response subclasses are also seen in AC and STUT cells, and the b- and c- subclasses have also been found for IS cells. b-IS cells have been further subdivided into those with brief bursts and those with prolonged bursts (IS1 and IS2 subtypes)[77]. The BST subclasses are named differently: r-BST cells repetitively burst in a manner similar to the 'chattering' response of some pyramidal

cells in supragranular layers; t-BST cells produce a powerful burst only once at the onset of the depolarization followed by complete cessation of spiking after the initial burst due to a powerful sAHP; and i-BST cells initially burst and then switch to slow accommodation after the initial burst response, like some pyramidal neurons (FIG. 5; TABLE 1).

*Electrophysiological–anatomical relationship.* Originally, it was assumed that a given firing pattern reflected a certain anatomical type of interneuron. However, detailed examination of many of the neocortical interneuron types has shown that a morphologically identified interneuron can have many discharge behaviours[8,11,15,38,73,78]. TABLE 1 shows the mapping between the different classification schemes, and FIG. 6 shows the mapping between the electrical and anatomical types. A principal component analysis using many specific electrical parameters can generate clusters that map differently onto pyramidal cells, MCs and basket cells (M.T.-R., B. Blumenfeld and H.M., unpublished observations), indicating that higher-order statistics of the electrical properties of interneurons might, however, correspond to specific morphologies.

*Classes or a continuum?* Electrical classifications provide a useful means to refer to different types of response. The proof that these responses represent distinct classes and that each class maps onto anatomically and molecularly distinct types of interneuron is still lacking. Extrapolating these types to the *in vivo* situation or assigning them greater significance than a 'marker' might also be misleading, because the different responses are characteristic for highly standardized experimental conditions — that is, brain slices perfused with artificial cerebrospinal fluid. *In vivo* neurons also receive greater synaptic bombardment and are subject to neuromodulatory control, which could profoundly alter their discharge properties[79]. Nevertheless, under controlled conditions the response types are useful markers for describing the microcircuit and understanding the relationships between electrical behaviour and the morphological, molecular and synaptic properties of the microcircuit, regardless of whether these are distinct classes or specific ranges of responses.

### Ion-channel composition

Electrophysiological diversity results from the combined activity of different combinations of ion channels on a neuron's membrane (active properties)[80] and from the morphology of the neuron (passive properties)[81]. Electrophysiology, pharmacology, immunohistochemistry, *in situ* hybridization and gene-expression studies in neocortical regions or single cells have shown that many ion channels are involved in generating electrical behaviour in neocortical neurons. Each type of neuron expresses a specific combination of ion channels, produces certain amounts of each channel, uniquely modifies each channel and distributes them in a characteristic pattern across the membrane surface to generate a specific type of electrical behaviour.

Table 1 | **Electrophysiological classes of neocortical inhibitory neurons**

| Main classes | Subclasses | Other classification schemes |
|---|---|---|
| NAC (layers I–VI) | b-NAC | FS |
| | d-NAC | FS; LS |
| | c-NAC | FS |
| AC (layers II–VI) | b-AC | BSNP |
| | d-AC | FS; LS |
| | c-AC | RSNP |
| STUT (layers II–VI) | b-STUT | BSNP |
| | d-STUT | FS; LS |
| | c-STUT | FS |
| IS (layers II–V) | b-IS | IS1, IS2 |
| | d-IS | – |
| | c-IS | – |
| BST (layers II–V) | r-BST | – |
| | t-BST | BSNP |
| | i-BST | BSNP |

AC, accommodating; b, burst subtype; BSNP, burst spiking non-pyramidal; BST, bursting; c, classical subtype; d, delay subtype; FS, fast spiking; i, initial; IS, irregular spiking; LS, late spiking; NAC, non-accommodating; r, repetitive; RSNP, regular spiking non-pyramidal; STUT, stuttering; t, transient.

The gene-expression rules that govern such 'forward engineering' of electrical behaviour are becoming clear. The powerful delayed rectifying, voltage-gated potassium channels Kv3.1 and Kv3.2 are typically expressed in PV-containing, fast-spiking neocortical interneurons[82–84], although Kv3.1 is also expressed in PV-negative interneurons and pyramidal neurons. In a recent study, the mRNA expression of 26 ion channels and 3 calcium-binding proteins was tested in single neurons[85].

***The correlation map.*** This recent study found a significant correlation between the ion-channel genes expressed in an interneuron and its electrical phenotype. FIGURE 7 shows the correlation map from this study, which relates different ion-channel genes to specific electrical properties (see online supplementary information S3 (table) for electrophysiological parameters). The correlation map provides a coefficient of correlation for each gene with respect to the value of each electrophysiological parameter. In other words, red predicts high electrophysiological parameter values if the gene is expressed, and blue predicts low values. The sum of the colours for all those genes expressed in a neuron predicts the value of any of the measured electrophysiological parameters (such as the amplitude or duration of the action potential or the rate of accommodation). The accuracy with which electrophysiological parameters can be predicted is surprisingly high, given the false negatives and the lack of knowledge about the quantities of mRNA or protein produced by each gene, the extent to which each channel is modified and the distribution patterns of ion channels. The correlation coefficients are also independent of morphology as they were derived using a training data set that included neurons from multiple morphological types. So, merely knowing whether a gene is switched on is highly informative, and knowing the profile of expression for only a few genes allows the electrical phenotype of an interneuron to be predicted.

***Revealing candidate genes.*** The correlation map revealed many candidate genes that underlie different electrical properties. For example, in addition to Kv3.1, Kv3.2 and PV, the expression of another delayed rectifier, Kv1.6, and of the high-threshold $Ca^{2+}$ channel gene Caα1G and its auxiliary subunit, Caβ4, is also highly correlated with fast spiking, whereas the expression of CR, Caα1I, Kv2.2, HCN4 and SK2 is negatively correlated with fast spiking. The precise pacing of spiking to minimize accommodation in fast-spiking neurons is also mostly correlated with the expression of the hyperpolarization-activated channels HCN1 and HCN2 and of Kvβ1 — an auxiliary subunit of the Kv1 gene family that transforms these delayed rectifiers into transient A-type channels with important pacemaker properties.

***Three gene clusters.*** A cluster analysis of co-expression also revealed three main classes of ion-channel expression, which surprisingly mapped around the three calcium-binding proteins (PV, CB and CR) that are expressed in neocortical neurons[8,10,11] (FIG. 3). The ion-channel genes that are co-expressed with CR (the 'CR cluster') include SK2, Kv3.4, CR and Caα1B; the 'CB cluster' includes CB, Caβ4, HCN3, Kv1.4, Caα1G, Caβ1, HCN4, Kv3.3 and Caβ3; and the 'PV cluster' includes HCN2, Kv3.1, Kv1.2, Kv1.6, Kv1.1, PV, Kv3.2, HCN1, Kvβ1 and Caα1A. The biophysical properties of the ion channels in each cluster are consistent with and might complement each other to generate the three broad classes of discharge behaviour: ion channels in the CR cluster are associated with accommodation[86]; those in the CB cluster are associated with bursting[87]; and those in the PV cluster are associated with high-frequency discharge[82,83,88].

***Four co-expression principles.*** These clusters seem to arise because of specific constraints on the types of gene that can be co-expressed. The constraints seem to be governed by four principles: a synergizing principle, whereby certain gene pairs, such as SK2–CR, that predict the same electrical phenotype are expressed in the same cells; an antagonizing principle, whereby certain gene pairs, such as Kv1.2–Kv3.1 and Kv1.2–Kv3.2, that predict opposite phenotypes are co-expressed; a homogenizing principle, whereby certain gene pairs, such as Kv1.1–Kv1.4 and PV–Kv1.4, that predict the same phenotype are expressed in different cells; and a heterogenizing principle, whereby certain gene pairs, such as HCN4–PV, Kcn4–Kvβ1, Caβ4–SK2 and Caβ4–CR, that predict opposite phenotypes are expressed in different cells. These four ion-channel co-expression constraints could govern the generation of electrical diversity in interneurons.

***Inversion of expression.*** Specific gene-expression profiles map onto different discharge response types. The most striking example is a near-perfect inversion of the expression profile between cells that discharge initially with a burst onset (b-subtype) and those with a delayed onset (d-subtype)[85]. So, even cells that are normally
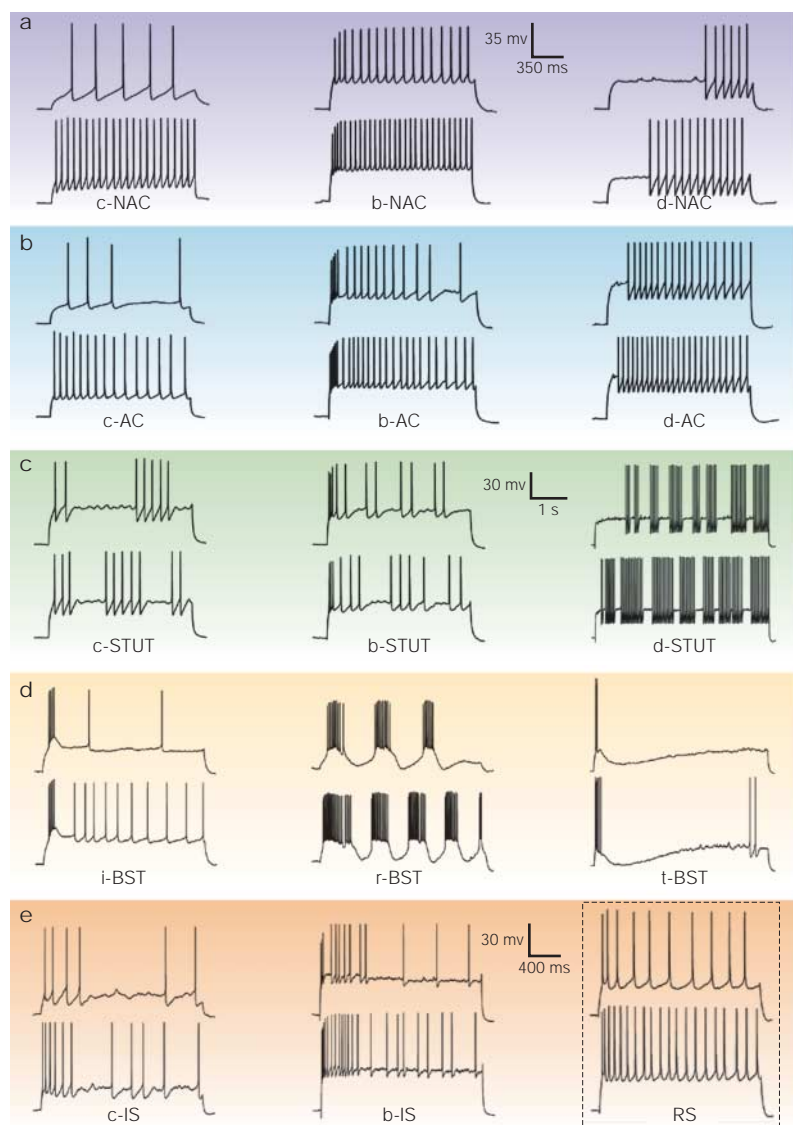
Figure 5 | **Different electrophysiological classes of inhibitory interneurons.** Five classes have been observed, based on the steady-state response to a sustained current injection in the soma: non-accommodating (NAC); accommodating (AC); stuttering (STUT); bursting (BST); and irregular spiking (IS). Most classes contain three subclasses: delay (d); classic (c) and burst (b). For bursting interneurons, the three types are repetitive (r), initial (i) and transient (t). RS (regular spiking) is an example of a classic discharge of a pyramidal cell. See also main text.

*Calcium-binding proteins.* The three CBPs (CB, PV and CR) tend to be expressed in three separate populations of interneurons[90,91] (see also REFS 8–11,85,89,92), which indicates that an exclusion principle might operate in the expression of CBPs. However, there is some overlap in expression, especially between CB and PV[11,38] and to a lesser extent between CB and CR[11,89]. These three populations correspond approximately to the three broad discharge response classes: CR in accommodating or irregular-spiking interneurons[77]; CB in bursting interneurons[74,85]; and PV in fast-spiking interneurons[74,85,88]. As there are more than three anatomical, electrical and combined anatomical–electrical types, it is of course not possible for any one CBP alone to map onto any one type of interneuron (FIG. 3). Even the most commonly accepted notion — that PV is a marker for basket cells — is not strictly correct, as PV is expressed in only about half of basket cells and can also be expressed in ChCs[53]. The common use of PV to isolate fast-spiking cells is also not entirely correct because not all PV-expressing cells are fast spiking and not all fast-spiking cells express PV (FIG. 3). Ultimately, the combined expression of other markers is required to identify any one of the anatomical–electrophysiological types of interneuron.

*Neuropeptides.* Other common interneuronal markers include the neuropeptides SOM, VIP, CCK and NPY[93–99]. As with CBPs, no single neuropeptide correlates with a single anatomical or electrophysiological type of interneuron[11,38,76]. However, some expression patterns are striking, such as SOM expression in MCs[100] and VIP expression in SBCs[34], DBCs[11] and BPCs[96]. Although some combinations of neuropeptides map better onto specific subtypes, they still do not map perfectly[9,11,38]. For example, MCs always express SOM and never VIP[11,95] (see also REF. 56), but this pattern can also be seen in some BTCs[101]. Expression patterns, used with caution, are nevertheless important general indicators of anatomical and electrophysiological types of interneuron (FIG. 3).

*Combined CBP–neuropeptide expression.* An interneuron can co-express up to five of the seven different neuropeptides and CBPs[38]. FIGURE 3 shows different types of neuron that express neuropeptides and CBPs. At the protein level, PV, SOM and VIP are found in separate populations of neurons[11], illustrating another exclusion principle. However, this exclusion is not perfect at the mRNA level[9,38,85]. There might also be an inclusion principle, as SOM–NPY[11], VIP–CCK[102], VIP–CR[77] and CR–CCK[102] co-expression has been detected. Lastly, many of the markers seem to be expressed independently of each other, providing evidence for an independence principle. For example, CB is promiscously co-expressed with many neuropeptides and even other CBPs (see above).

*Other markers.* Neocortical cells also express distinct cell-surface molecules (membrane proteins or lipids with characteristic carbohydrate moieties that can be identified using antibodies or lectins)[10]. Futhermore, pyramidal cells and different types of interneuron differ

classified in the same broad class, such as fast spiking, can have diametrically opposite expression profiles depending on the onset response. This finding also indicates that only a few transcription factors might control the expression of entire sets of ion-channel genes, in which case it is probable that different combinations of transcription factors would give rise to a finite number of distinct electrical classes.

**Molecular properties**

Whereas excitatory cells (co-) express only a limited set of the commonly probed CBPs and neuropeptides, inhibitory interneurons have more diverse (co-) expression profiles[89] (see also REFS 8–11).
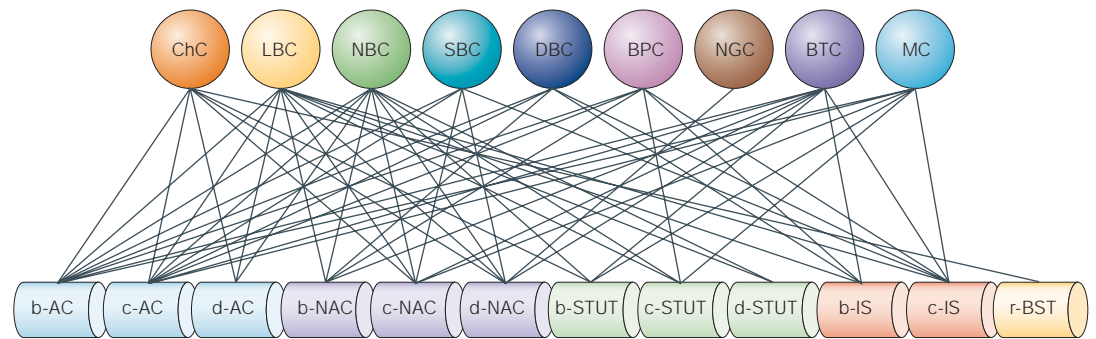
Figure 6 | **Anatomical–electrophysiological diversity of neocortical inhibitory neurons.** Electrophysiological classification of interneurons: main classes and subclasses are defined according to discharge responses at steady-state and onset phase to somatic current injections, respectively. Any given anatomically defined interneuron in general has several distinct discharge behaviours, and, conversely, a given discharge behaviour can be found in several anatomically defined interneuron types. AC, accommodating; b, burst subtype; BPC, bipolar cell; BTC, bitufted cell; BST, bursting; c, classic subtype; ChC, chandelier cell; d, delay subtype; DBC, double bouquet cell; IS, irregular spiking; LBC, large basket cell; MC, Martinotti cell; NAC, non-accommodating; NBC, nest basket cell; NGC, neurogliaform cell; r, repetitive; SBC, small basket cell; STUT, stuttering.

in their expression of neurotransmitter receptors[103–106] (see also REF. 107). Interneurons are therefore diverse in terms of their molecular properties, and the molecular profile is a tangential dimension that spans interneurons with different classification rules.

### Excitatory synapses on interneurons

*Identifying synapses.* The first recordings of synaptically connected neurons in the neocortex were performed by Thomson[108]. Physiological recordings are the most direct method for isolating synaptic connections, but it is important to obtain estimates of the numbers and distributions of synapses at the anatomical level. Most estimates are based on light microscopic analyses and should be considered estimates of putative synapses until verified at the electron microscope level. Nevertheless, comparisons between light and electron microscope estimates[109–114] and between light microscope and BINOMIAL ESTIMATES[15] show a reasonable correspondence. As it is impossible to verify all synapses at the electron microscope level, light microscopy will remain central in the study of neuronal microcircuits.

*Anatomical properties.* Glutamatergic neurons form multiple synapses onto interneurons. These synapses typically form in clusters on a small fraction of dendrites[114,115], in contrast to the highly distributed innervation of glutamatergic synapses on excitatory cells. Most synapses are formed on dendrites, but glutamatergic synapses can also form on the somata of interneurons[3,116]. For example, pyramidal neurons form about six synapses onto a basket cell[38,117] (FIG. 4; online supplementary information S2 (table)), with around 60% on 'basal' dendrites, 30% on the main dendrite and 10% on the soma. There is a correlation between synapse numbers and interneuron types in the cat, ferret[114,118] and rat neocortices (FIG. 4; online supplementary information S2 (table)).

*Physiological properties.* Unlike glutamatergic synapses on excitatory neurons, glutamatergic synapses on inhibitory cells use different AMPA ($\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor subunits[119], lack a significant NMDA (*N*-methyl-D-aspartate) receptor component[120] and often have frequency-dependent facilitation[120–122]. These features are found even in some interneurons with different types of electrophysiological behaviour (classic FS and LTS/BSNP cells)[121–123] and indicate that the glutamatergic system might have a functional dichotomy, with different modes for recruiting pyramidal neurons and interneurons[25,120,123,124]. Differential synaptic transmission was confirmed by simultaneous recordings from a pyramidal neuron that formed depressing synapses onto other pyramidal neurons and facilitating synapses onto interneurons[125]. Interestingly, the static (quantal) and dynamic (depression and facilitation) properties of facilitating synapses from single pyramidal neurons onto interneurons vary across layers[126], which might cause targeted inhibitory cells in deep cortical layers to discharge before those in supragranular layers. Such layer-specific differences in the recruitment of interneurons could influence the direction of information flow in the cortical column.

*Differential synaptic transmission.* Although many connections from pyramidal cells onto interneurons in neocortical layers II–V show frequency-dependent facilitation[123,125,126], some interneurons receive depressing synapses from pyramidal neurons[121,123,126]. Simultaneous recordings from the same presynaptic pyramidal neuron and different types of interneuron showed that there was differential glutamatergic transmission onto interneurons[126,127]; accommodating interneurons with bitufted dendritic morphologies received facilitating synapses, whereas non-accommodating interneurons with multipolar dendritic morphologies (presumably basket cells) received depressing synapses. Depressing synapses have also been observed for connections from pyramidal neurons onto DBCs in layers II/III[114] (see also REF. 128), onto fast-spiking basket cells in layer V[129] and onto irregular-spiking BPCs in layers II/III and V[77], indicating that these synapses are more common than was previously thought.

BINOMIAL ESTIMATES
The number of functional release sites is referred to as binomial *n* because it is estimated in a quantal analysis using binomial statistics.
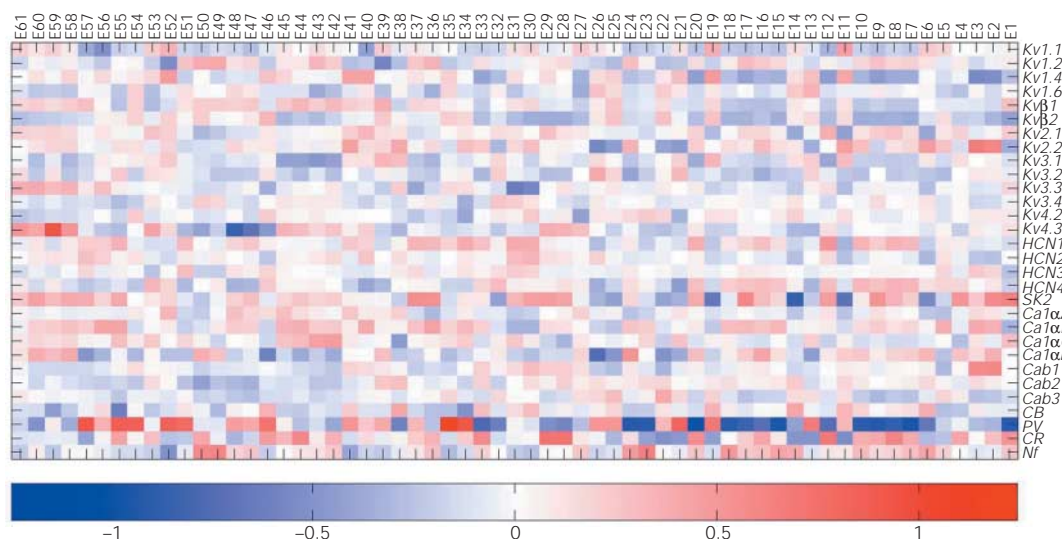
Figure 7 | **Correlation map relating the different ion-channel genes with specific electrical parameters.** Ion-channel genes are indicated on the right, electrical parameters along the top. See online supplementary information S3 (table) for identities of electrical parameters. The colour indicates the value of the coefficient for each gene, which represents the sign and magnitude of the correlation between the gene and the value of each electrical parameter. Red indicates that if the gene is expressed, the value of the electrical parameter will be towards the maximal value recorded in the 203 cells, and vice versa for blue. The value of the electrical parameter can be calculated by summing the 'colours' (coefficients) horizontally for all those genes that were detected in a neuron. Modified, with permission, from REF. 85 © (2004) Oxford University Press.

*Determinants of synaptic dynamics.* Although it is tempting to assume that the physiological properties of glutamatergic synapses are determined purely by the postsynaptic target[127,128,130], not all multipolar inter-neurons or FS cells receive depressing synapses, and not all BTCs, including classic BTCs, receive facilitating synapses[38,123,126]. The multipolar–bitufted distinction has led to many confusing reports and the idea that dendritic-targeting interneurons receive facilitating synapses, whereas soma-targeting interneurons receive depressing synapses, is also mistaken (FIG. 8a). Further-more, a single neocortical neuron can receive both depressing and facilitating GABA synapses[15], indicating that the target cell does not determine the dynamics of all incoming synapses. The type of glutamatergic synapse formed also depends on the electrophysiology of the target cell; if the interneuron has a delayed onset response, synapses tend to be depressing, and if the onset response is classic or bursting, synapses tend to be facilitating (A.G., G.S. and H.M., unpublished observations). FIG. 8a shows the mapping of glutamatergic synapse types onto different interneurons.

**Inhibitory synapses on pyramidal neurons**
*Strategic innervation.* Each type of interneuron inner-vates its target cell by preferentially distributing multiple synapses in a characteristic manner onto selected mem-brane domains (axon initial segments, somata, proximal and distal dendritic shafts and spines, and dendritic tufts)[3,14,21]. Neurons that preferentially target axon initial segments are optimally positioned to 'edit' a neuron's output by affecting the generation and timing of action potentials. The preferential innervation of the (peri-) somatic domain allows presynaptic neurons to control

the gain of summated potentials and thereby the action potential discharge of target cells[38,50,131]. These interneurons are involved in phasing and synchroniz-ing neuronal activity[132–134]. Neurons that preferentially innervate the dendritic domain are positioned to influence the dendritic processing and integration of synaptic inputs[135,136]; to influence synaptic plasticity either locally or by interacting with back-propagating action potentials[137]; and to affect the generation and propagation of dendritic calcium spikes[138,139]. Finally, the preferential innervation of distal dendritic and tuft regions could allow neurons to affect local dendritic integration.

*Anatomical properties.* In general, inhibitory neurons form more synapses onto their target cells than excita-tory neurons do (as many as 30 synapses per target, with an average of around 15)[14,15,38]. Inhibitory synapses are highly distributed across the dendritic surface of target cells and are mainly formed onto dendritic shafts. Occasionally, synapses cluster on target cell dendrites[112]. The most commonly studied inhibitory neocortical con-nections are synapses from basket cells onto pyramidal neurons. Basket cells in layers II–IV form many putative synapses onto neighbouring pyramidal neurons (LBC: 14.5 ± 1.7 synapses; SBC: 20.5 ± 10.5 synapses; NBC: 15.8 ± 4.1; REF. 38; FIG. 4; online supplementary informa-tion S2 (table)). NBCs in layers II/III and IV did not show layer-specific differences[38], which indicates that the innervation rules remain consistent across layers. Synaptic innervation differs for other types of interneu-ron, as well as in different cortical areas, species and developmental ages[112] (FIG. 4; online supplementary information S2 (table)).
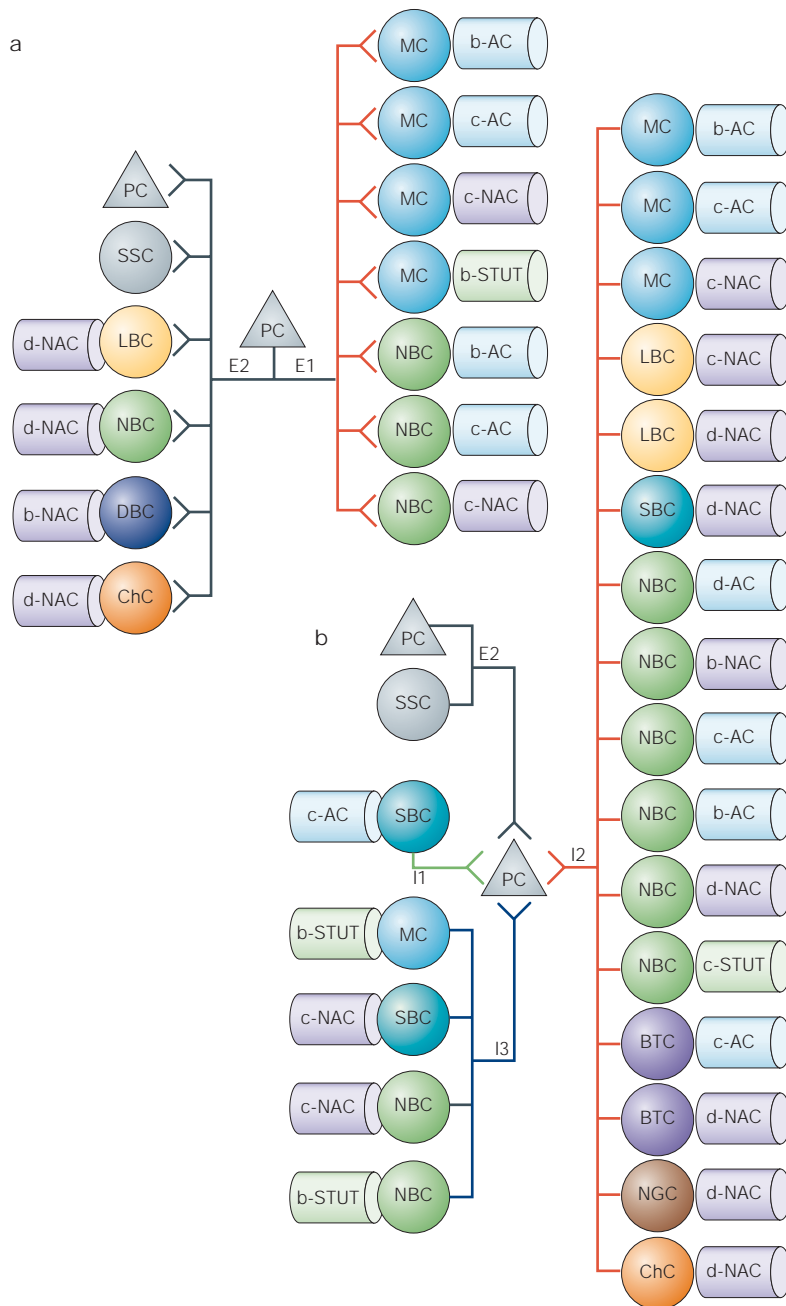
Figure 8 | **Mapping of synaptic dynamics.** Mapping of glutamate (E1 and E2) synapses from neocortical pyramidal neurons (PCs) onto interneurons (**a**), and GABA (γ-aminobutyric acid) (I1, I2, I3) and glutamate (E1and E2) synapses from interneurons onto PCs (**b**), according to the anatomical and electrophysiological identity of the different interneurons. Only connections in which the anatomical and electrical properties were conclusively defined are included. For example, some b-AC inteneurons generate facilitating input onto PCs (I1) but their anatomical identity has not been established. In addition, some c-AC interneurons whose morphology was not determined were also shown to generate facilitating input onto PCs (I1). AC, accommodating; b, burst subtype; BTC, bitufted cell; c, classic subtype; ChC, chandelier cell; d, delay subtype; DBC, double bouquet cell; LBC, large basket cell; MC, Martinotti cell; NAC, non-accommodating; NBC, nest basket cell; NGC, neurogliaform cell; SBC, small basket cell; SSC, spiny stellate cell; STUT, stuttering.

*Physiological properties.* An important advance in understanding the physiological properties of neocortical inhibitory circuits came with the systematic study of connections formed by many anatomically (SBCs, NBCs, LBCs, MCs and BTCs) and electrophysiologically

(NACs, ACs and STUTs, and b-, c- and d-subclasses) defined interneurons onto pyramidal cells in layers II–IV[15]. This study showed that synaptic transmission mediated by $GABA_A$ (GABA type A) receptors was physiologically much more diverse than previously reported: inhibitory synapses showed synaptic depression as well as facilitation to varying degrees, yielding three distinct classes of GABA synapse (types I1, I2 and I3, defined according to the ratio of time constants of recovery from synaptic facilitation compared with depression; FIG. 8b). This study also showed that each type of interneuron deploys one of these three types of inhibitory synapse, depending on the anatomical and physiological properties of both pre- and postsynaptic cells (FIG. 8b). This indicates that a 'pre–post handshake principle', the molecular basis of which is unknown, underlies the formation of a specific type of synapse.

*Homogeneous transmission.* Remarkably, all the synapses formed by one interneuron onto multiple pyramidal neurons show identical synaptic dynamics[15]. All the synapses from an interneuron onto all targets of the same type (pyramidal neurons in this case) seem to have identical release probabilities and time constants for recovery from synaptic depression and facilitation. This homogeneity principle contrasts sharply with the heterogeneity of glutamatergic synapses formed by a pyramidal neuron onto other pyramidal neurons and also has implications for the forms of learning that might shape these synapses. The absolute strength of these synaptic connections is heterogeneous (probably due to different numbers of synapses and/or postsynaptic receptors), indicating that they could be modified by the relative timing of activity in only one pair of neurons (presynaptic and postsynaptic), but their dynamics are homogeneous, suggesting that these parameters must be modified by the activity patterns of the entire population of postsynaptic pyramidal neurons relative to the single presynaptic interneuron.

*$GABA_B$ transmission.* As well as fast $GABA_A$-receptor-mediated inhibition, neocortical neurons also show slow inhibitory synaptic responses mediated by metabotropic $GABA_B$ (GABA type B) receptors. Such responses have mainly been detected after strong extracellular stimulation or repetitive, high-frequency stimulation of interneurons[140,141]. This has led to the idea that the $GABA_B$ receptors are located extrasynaptically and are activated by GABA 'spillover' from the synaptic cleft. Alternatively, neocortical microcircuits might comprise two separate populations of interneurons, each responsible for either $GABA_A$- or $GABA_B$-receptor-mediated responses[142]. Evidence for the segregation of interneuron populations has recently been obtained for a few connections from FS and RSNP cells onto pyramidal neurons in layer V.

**Inhibitory synapses on interneurons**
*Anatomical properties.* With around 50 anatomical–electrical types of interneuron and a handshake principle for setting synapse types, the number of potential types of connection between inhibitory neurons is enormous.

Nevertheless, there are some general principles. First, it is rare to record connections between interneurons, indicating that they are sparsely connected[15,38,143]. Second, connections between interneurons are also mediated by multiple synapses (an average of around ten). Third, interneurons can target specific domains of other interneurons. Last, inhibitory synapses onto interneurons are highly distributed on the dendrites, as for pyramidal neurons. Only a few of the possible combinations of interneuron–interneuron synaptic connection have been studied, possibly because specific types of interneuron are preferentially interconnected. Basket cells innervate neighbouring basket cells, dendritic-targeting cells and DBCs[113]. These synapses are located somatically and peri-somatically, but whereas both dendritic-targeting cells and DBCs receive only a few synapses, postsynaptic basket cells receive more (FIG. 4; online supplementary information S2 (table)). Such differences have been interpreted to indicate both selectivity and preference in inhibitory neocortical microcircuits[14]. The large numbers of synapses between basket cells[113] might underlie extensive basket-cell networks, which have been implicated in long-range lateral disinhibition[144]. Activity in such networks might also be coordinated by electrical synapses[145–149].

*Physiological properties.* Only a few types of interneuron–interneuron connection have been studied physiologically. Fast-spiking basket cells in layers II–IV form depressing synapses onto other basket cells, DBCs and dendritic-targeting cells[113]. Divergent connections onto non-accommodating multipolar interneurons (possibly basket cells) and accommodating interneurons with bitufted dendrites show different degrees of depression[127]. Interneurons can deploy any of the three types of synapse[15] (FIG. 8b). Although these studies seem to imply that each type of interneuron can innervate, and be innervated by, all other types, this is not likely. Inter-interneuron connections would need to be orders of magnitude greater for 'all-types to all-types' connectivity. Indeed, some interneurons, such as ChCs, do not contact certain other types of interneuron at all[21]. Finally, there is anatomical and physiological evidence that some interneuron types, such as LBCs, are highly interconnected, whereas other types, such as DBCs, are much less interconnected[41,134,150].

## Function of interneuron diversity

Sensory stimulation results in a coordinated increase in excitatory and inhibitory conductances[151,152]. Surprisingly, a balance between these two opposing conductances is maintained over a large dynamic range and for many stimuli[152,153]. This means that regardless of the level of excitation, the inhibitory system can automatically scale its output to provide matching opposition across a large dynamic range, analogous to a Yin-like inhibition opposing a Yang-like excitation[154]. Excitatory and inhibitory inputs are, however, distributed in various temporal combinations, and large imbalances can occur transiently during the response to stimuli[151–153,155–157]. Interneuron diversity might be crucial for providing

sufficient sensitivity, complexity and dynamic range for the inhibitory system to match excitation regardless of the intensity and complexity of the stimulus, and synaptic diversity might be crucial to secure the dynamic range and to choreograph moments of imbalance between excitation and inhibition in the context of any background[158].

*Balancing regions of neurons.* The first challenge is to balance excitation in different regions of a single neuron. This might require a range of interneurons that are specialized to target different regions and to collect, integrate and respond to different types of input. Balancing excitation in dendrites might require various interneurons to 'monitor' excitatory input to many dendritic regions, balancing excitation in the cell body might require interneurons that sense global excitatory input, and balancing excitation in terms of the spike output might require an interneuron that can collect sufficient information to veto spiking after integration. So it is perhaps not so surprising that there is greatest anatomical variety of dendritic-targeting interneurons (DBCs, BTCs, BPs, MCs and NGCs), with a smaller variety of soma-targeting interneurons (LBCs, NBCs and SBCs) and only one type of axon-taregeting interneuron (ChCs). Diversity in general, and of electrical subtypes in particular, could also be driven by the need for interneurons to monitor and respond to many sources of excitatory input (same layer, cross layer, neighbouring columns, many neocortical regions, opposite hemisphere and subcortical input). Further experiments are required to prove that processing input diversity requires interneuron diversity.

*Recruiting balanced inhibition.* The second challenge is for the inhibitory system to sense the appropriate level of excitation across a wide dynamic range and under various stimulus conditions. A broad spectrum of action potential thresholds is found in different types of interneuron (thresholds can vary by up to 20 mV; A.G., M.T.-R. and H.M., unpublished observations), and different discharge rates and patterns might make this dynamic range possible. The use of glutamatergic synapses with varying dynamics could also support the dynamic range and the sensitivity to specific stimulus conditions. For example, pyramidal neurons recruit MCs through facilitating synapses, meaning that during transient activation of the microcircuit, hardly any MCs will be recruited. However, many LBCs receive depressing synapses, so they would be instantly recruited; prolonged excitation of the microcircuit would have the opposite effect[38]. Each anatomical–electrophysiological subtype of interneuron therefore has its own conditions for recruitment.

*Applying balanced inhibition.* Applying the right amount of inhibition is not a simple process, as only 16% of all synapses on a pyramidal neuron are inhibitory. The first parameter that needs to be adjusted to deliver more inhibitory current is the duration of GABA-receptor activation. The time course of inhibitory

synaptic currents is about twofold longer than for excitatory currents[15,159]. This is still not enough to deliver matching inhibition across a large dynamic range, so interneurons can discharge 2–3 times faster than pyramidal neurons. To make use of these properties, inhibitory synapses have greater synaptic facilitation than excitatory synapses, which allows transmission at higher frequencies[38,129,160].

*Balancing the circuit.* The fourth challenge in the balancing act is to apply inhibition at the right moment in each neuron of the microcircuit. In particular, the timing and amount of inhibition to each pyramidal neuron — subsets of which receive input from and transmit output to different locations — must be orchestrated in a stimulus-dependent manner. Dynamic synapses choreograph precise millisecond timing of synaptic activity in different interneurons relative to pyramidal neurons in a manner that depends on the structure of the stimulus[158].

*Why balance Yang with Yin?* Why does excitation need to be balanced with inhibition and why do transient moments of imbalance occur? This is a vast area, which will not be dealt with in this review, except to speculate on two potential reasons. At the level of individual neurons, matching inhibition as a function of stimulus intensity could allow information to be processed and encoded at a higher or lower temporal resolution, depending on the baseline firing rates. This can be achieved by changing the membrane time constant, which changes the time window for temporal integration[161] (see also REF. 162) and by changing the temporal precision of spike generation by adding high-frequency membrane 'noise'[163–165]. At this level, balance might be required to normalize the baseline for synaptic integration as a function of activity (to normalize the mutual information between the input channels) and spiking might reflect moments of imbalance (high mutual information between the input channels). At the microcircuit level, a sliding scale between integration and coincidence detection as a function of activity in each neuron could be important to control which neurons synchronize at which frequencies[162]. Balance might be required to keep all neurons independent (to normalize mutual information across neurons) and oscillations might reflect orchestrated momentary imbalances of groups of neurons (high mutual information between neurons). Needless to say, considerable work is required to test and turn theory into fact.

### Classes or a continuum?

At the anatomical level, neocortical interneurons are generally accepted as being in distinct classes, not because of any objective analyses, but because of more obvious functional specializations indicated by their different domain-targeting tendencies. At the molecular level, the issue is, on the one hand, simpler because some markers are expressed only by certain interneuron types, but, on the other hand, more complex because no one marker points unambiguously towards only one anatomical or electrical type of interneuron; the expression pattern of four or five markers might be required (M.T.-R., M. Ilic, P. Goodman

and H. M., unpublished observations; see also REF. 92). At the electrical level, the diversity might seem arbitrary, but this is probably due to the lack of defined functions for the different behaviours. The class issue at all levels will probably only be resolved objectively at the level of gene expression. The correlation between expression profiles and electrical phenotypes, the constraints in co-expression profiles and the 'flip' of entire expression profiles to form opposite electrical phenotypes[85] all indicate that only a few transcription factors, expressed in different combinations, might give rise to a finite number of distinct classes of interneuron. So, most interneurons probably lie in distinct electrical, morphological and molecular classes. The observed diversity is several orders of magnitude smaller than expected for a continuum of electrical types using more than 100 ion-channel genes, indicating powerful constraints on diversity. Understanding these constraints is also key to resolving the class-versus-continuum debate.

### Stereotypical GABA innervation

The neocortical microcircuitry is stereotypical in many respects[166], but subtle variations become apparent as the microcircuit is studied at higher resolution. For example, inhibitory input is stereotypical in that all pyramidal cells receive inputs from the three broad types of interneuron (dendritic, somatic and axon-targeting), but does each pyramidal neuron receive inputs from the same subtypes of interneuron? About 16% of the synapses on a pyramidal neuron in layers II/III are inhibitory[1,18], and these come from about 70 interneurons (half being basket cells). Roughly 50 of these interneurons arise from the same layer and column as the pyramidal neuron, 10 from the same column but a different layer, and 10 from different columns (A.G. and H.M., unpublished observations). Each of the 70 interneurons places around 15 synapses on the pyramidal neuron, together making around 1,000 inhibitory synapses. In principle, therefore, each pyramidal cell could receive input from at least one of each anatomical type of interneuron needed to innervate all parts of the neuron. However, it is unlikely that all the different electrical subtypes could be represented in their correct proportions on every pyramidal neuron. If anatomical–electrical–molecular variants are also considered, then it is certainly not possible for each pyramidal neuron to receive inputs from an identical set of interneurons. The next question is whether there is such high-resolution stereotypy for small 'sets' of pyramidal neurons, where sets receive inputs from the same complement of anatomical–electrical–molecular subtypes of interneuron. This might be possible within a layer, but as there are layer-specific differences in molecular expression profiles and electrical subtypes of interneuron, such high-resolution stereotypy will not hold across layers. The fundamental question now is how microcircuits in different species, different brain regions of the same species, different layers and even different neurons in the same layer are driven to diversify to form countless variations of the microcircuit template — in particular, whether stimulus diversity is the ultimate driving force behind interneuron diversity.

## Conclusion

A template neocortical microcircuit seems to have been duplicated repeatedly to construct the neocortex. In terms of its general architecture, this template is highly stereotypical — a 'generic' microcircuit — with subtle specializations that presumably optimize processing in different brain regions and species to form a 'task-specific' microcircuit. In terms of its function, this generic microcircuit at any one location seems to support simultaneous processing of multiple features, and has immense flexibility to dynamically re-configure, forming a transient task-specific microcircuit. The diversity of inhibitory interneurons and synapses could be crucial to impart this 'omnipotence' to a microcircuit, which is required to support optimal information processing of any stimulus in any species and brain region in a rapidly changing and unpredictable environment.

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