# New Insights in the Classification and Nomenclature of Cortical GABAergic Interneurons

Javier DeFelipe\*, Pedro L. López-Cruz, Ruth Benavides-Piccione, Concha Bielza, Pedro Larrañaga\*, Stewart Anderson, Andreas Burkhalter, Bruno Cauli, Alfonso Fairén, Dirk Feldmeyer, Gord Fishell, David Fitzpatrick, Tamás F. Freund, Guillermo González-Burgos, Shaul Hestrin, Sean Hill, Patrick R. Hof, Josh Huang, Edward G. Jones, Yasuo Kawaguchi, Zoltán Kisvárday, Yoshiyuki Kubota, David A. Lewis, Oscar Marín, Henry Markram, Chris J. McBain, Hanno S. Meyer, Hannah Monyer, Sacha B. Nelson, Kathleen Rockland, Jean Rossier, John L.R. Rubenstein, Bernardo Rudy, Massimo Scanziani, Gordon M. Shepherd, Chet C. Sherwood, Jochen F. Staiger, Gábor Tamás, Alex Thomson, Yun Wang, Rafael Yuste, Giorgio A. Ascoli\*

\*Corresponding authors:

Javier DeFelipe (defelipe@cajal.csic.es)
Pedro Larrañaga (pedro.larranaga@fi.upm.es)

Giorgio Ascoli (ascoli@gmu.edu)

#### Abstract

Systematic classification and accepted nomenclature of neuron types are still lacking and much needed. This article describes a possible taxonomical solution, focusing on GABAergic interneurons of the cerebral cortex. We developed a novel, web-based interactive system allowing experts to classify neurons with pre-determined criteria. Using Bayesian analysis and clustering algorithms on the resulting data, we investigated the suitability of several anatomical terms and neuron names. Moreover, we show that supervised classification models could automatically categorize interneurons in agreement with experts' assignments. These results demonstrate a practical and objective approach to advance neuron naming, characterization, and classification based on community consensus.

## Introduction

The problem of classifying and naming neurons has been a topic of debate for over a hundred years. Nevertheless, a satisfactory consensus remains to be reached, even for restricted neuronal populations such as the GABAergic interneurons of the cerebral cortex. Over the last two decades, the amount of morphological, molecular, physiological and developmental data has grown rapidly, making classification harder rather than easier. A consistent neuronal classification and terminology will help researchers manage this multidisciplinary knowledge, and is needed for specialists in neuroscience subfields to establish and maintain effective communication and data sharing (Petilla Terminology; PING, 2008). As in other domains of science, taxonomies can be empirical or scientific. This distinction was well described by John Hughlings Jackson in 1874 in his chapter entitled "On classification and on methods of investigation" (Selected Writings; London, Hodder and Stoughton, 1931):

"There are two ways of investigating diseases, and two kinds of classification corresponding thereto, the empirical and the scientific. The former is to be illustrated by the way in which a gardener classifies plants, the latter by the way in which a botanist classifies them. The former is, strictly speaking, only an arrangement. The gardener arranges his plants as they are fit for food, for ornament, etc. One of his classifications of ornamental plants is into trees, shrubs, and flowers. His object is the direct application of knowledge to utilitarian purposes. It is, so to speak, practical. The other kind of classification (the classification properly so-called) is rather for the better organization of existing knowledge, and for discovering the relations of new facts; its principles are methodical guides to further investigation. It is of great utilitarian value, but not directly."

In spite of the many studies performed since the original findings of Santiago Ramón y Cajal, it is surprising that we still lack a catalog of neuron types and names that is accepted by the general scientific community. Recognizing this problem, the International Neuroinformatics Coordinating Facility (INCF) has recently established a Neuron Registry within the Program on Ontologies of Neural Structures (PONS), with the aim to identify known neuron types on the basis of their defining properties (Hamilton et al., 2012; http://incf.org/core/programs/pons). A collation of terms referring to neuron types is available as part of the Neuroscience Information Framework (NIF) from NeuroLex (Larson and Martone, 2009; http://neurolex.org/wiki/Category:Neuron).

A milestone towards a future classification of the GABAergic interneurons in the cerebral cortex (neocortex, hippocampus, and related areas) was the standardization of the nomenclature of their properties (PING, 2008). However, at that time it was not possible to identify a set of anatomical traits that unambiguously define an interneuron class. In this article, we describe a

new, community-based strategy for defining a morphological taxonomy. Our goal was to establish a list of terms that could be used by all researchers to distinguish neuronal morphologies. Because the developmental and evolutionary processes that gave rise to these morphologies are incompletely understood, we sought a utilitarian rather than a scientific classification: a 'gardener's approach'. To this end, we selected a limited number of neuron types and morphological properties, based on studies performed over the years in many laboratories. These neuron types and morphological properties are not meant to be imposed but rather are proposed, with the goals of incorporating community feedback and reaching consensus.

In this article, we first provide an overview of historical and current issues involved in classifying cortical neurons, and interneurons in particular. We then describe a novel, webbased interactive system (**Fig. 1**) that collects data about the terminological choices for a set of 320 cortical interneurons by 48 experts in the field. We used several analysis methods to empirically test the consistency, clarity, and emerging agreement on these terminological choices. This article deals primarily with neocortical GABAergic aspiny or sparsely spiny non-pyramidal neurons with non-projecting axons. Unless otherwise specified, these are the neurons we refer to, for simplicity, as 'cortical interneurons'.

#### **Historical Overview**

#### Two major classes of cortical neurons: Principal cells and interneurons

Before the discovery of the Golgi method, the existence of different morphological types of cortical neurons was already recognized (Kölliker, 1852). Since then, researchers have tried to deduce the functional role of neurons from their morphological characteristics. Using preparations stained with a carmine dye, a technique introduced by Joseph von Gerlach (1820-1896), Rudolf Berlin (1833-1897) was the first to suggest a classification into three main cell types (Berlin 1858; quoted in Clarke and O'Malley, 1968): pyramidal cells (based on the triangular shape of their somata); granular cells (small and irregular somata); and spindleshaped cells (fusiform somata). This was the beginning of cytoarchitectural studies that were based mainly on the density and laminar distribution of different neuronal shapes. However, carmine staining and other methods available at that time only allowed visualization of neuronal cell bodies and a small portion of their proximal processes, making further characterization of cortical neurons difficult. In contrast, Golgi-stained preparations allowed much more complete staining of the neuron, including most of its parts (soma, dendrites and the unmyelinated axon), enabling visualization of their finer morphological details in young animals (Golgi, 1873). This led to a fuller characterization of neurons, allowing for the first time the exploration of their possible interconnections.

According to Cajal (1892), Golgi suggested that, in general, there were two morphologically and physiologically different types of neurons: motor (type I) neurons and sensory (type II) neurons. Motor neurons had long axons that gave rise to collaterals but also projected outside of the gray matter. Sensory neurons had short axons that arborized near the parent cell and did not leave the gray matter. The former cells were thought to have a motor function because their axons were considered to be continuous with the motor roots, whereas the second type was thought to be sensory because their axonal branches were linked with afferent fibers. Cajal argued that it was not possible to maintain such a physiological distinction and designated Golgi's two types as cells with a long axon (projection neurons) and cells with a short axon (intrinsic neurons or interneurons), avoiding any consideration of their possible physiological roles. Since then, the term 'interneuron' has commonly been used as synonymous with short-axon cell (Rakic, 1976; DeFelipe, 2002). Notably, some neurons are axonless, such as retina amacrine cells and olfactory granule cells.

Researchers soon realized that in the cerebral cortex the majority of neurons were pyramidal cells whose axons were seen to enter or be directed towards the white matter (e.g., Meynert, 1871). Therefore, pyramidal cells started being generally considered as both 'principal cells' and projection neurons (that is, cells with long axons).

Furthermore, using the Golgi method, it was obvious that neurons showed a great diversity of morphologies. Thus, in addition to the terms based on the shape of the soma, neuroanatomists described neurons with names that were somewhat descriptive of their dendritic morphology and axonal arborization, alone or in combination. However, with few exceptions, no general consensus has emerged for naming cortical neurons. For example, at present most neuroscientists agree on the usage of terms such as pyramidal neuron, non-pyramidal neuron, interneuron, and chandelier (or axo-axonic) cell. These cell types are readily distinguished by their clear morphological attributes. However, other common names, such as double bouquet cell, Martinotti cell, neurogliaform cell, and basket cell, seem to lack a consensual definition. In these cases, the same name is often assigned to neurons of varying morphologies by different authors, and a variety of terms are inconsistently adopted in different laboratories to represent the same cell classification. As a consequence, virtually every author has his/her own classification scheme and neuron terms, making the comparison and exchange of information among laboratories rather difficult, if not impossible.

#### What is a cortical interneuron?

By our definition, a cortical interneuron is a short-axon cell — i.e., a neuron whose axon does not leave the neocortex — and has a soma that is located in the cerebral cortex. Most cortical interneurons lack the typical somatodendritic morphological characteristics used to identify

projection neurons, namely a pyramidal-shaped cell body and an apical dendritic tree that is distinct from and lies opposite to the basal dendritic arbor. However, the absence of these features should not be used to define interneurons, as they are neither necessary nor sufficient for distinguishing interneurons from projection neurons. Indeed, there are interneurons that have a somatodendritic morphology resembling that of pyramidal cells (e.g., the so-called 'pyramidal basket cells' (Amaral and Lavenex, 2006)), and projection neurons that have a non-pyramidal appearance in their somata and dendrites (Peters and Jones, 1984).

Traditionally, interneurons have been subdivided into two main groups (Peters and Jones, 1984): spiny non-pyramidal cells and aspiny or sparsely spiny non-pyramidal cells. Spiny non-pyramidal cells are located in the middle cortical layers, especially in layer IV of primary sensory cortices. They comprise a morphologically heterogeneous group of interneurons with ovoid, fusiform, and triangular somata. Most spiny non-pyramidal cells are excitatory (glutamatergic (Feldmeyer et al., 1999)), and their axons are distributed within layer IV or in the adjacent layers above or below the somatic location (Staiger et al, 2004). Aspiny or sparsely spiny non-pyramidal cells usually have axons that remain near the parent cell, although some run prominent collaterals in the horizontal (parallel to the cortical surface) or vertical dimension (ascending and/or descending, reaching other cortical layers). These interneurons appear to be mostly GABAergic and constitute ~10-30% of the total neuron population, the percentage varying substantially between cortical layers, areas and species (DeFelipe et al., 2002; Meyer et al., 2011). They are the main component of inhibitory cortical circuits.

Following the approach of the Petilla Terminology (PING, 2008), we concentrate our effort on GABAergic cortical interneurons, thus excluding the majority of spiny non-pyramidal cells from the classification attempt. This choice is motivated by functional considerations, in that the neurotransmitter released by a neuron is intimately linked to the role played by this neuron in the circuitry. Moreover, restricting the scope of this classification to GABAergic interneurons has also a practical utility, given the availability of reliable methods to identify GABA and related chemicals, such as its synthesizing enzymes (glutamate decarboxylase (GAD) 65 and GAD67). Despite this relatively narrow definition, GABAergic cortical interneurons are located in all cortical layers and show a great variety of morphological, biochemical, and physiological characteristics. Thus, rather than attempting a comprehensive classification of cortical interneurons, we focus on a group of less controversial cell types for which relatively more abundant experimental evidence converges on a limited number of defining properties within the anatomical domain.

# Clarifications and remarks

In light of the above definitions, and before classifying specific interneuron types, it is useful to consider from the collective work of many investigators a number of points regarding the morphology and naming of cortical neurons.

First, over the years, the term interneuron has been most commonly used when referring to aspiny or sparsely spiny GABAergic non-pyramidal cells. These cells represent the majority of interneurons, and have come to epitomize the 'typical' cortical interneuron. As noted above, however, a minority of GABAergic interneurons are spiny (Kubota et al., 2011). Moreover, many interneurons that will become aspiny as they develop are spiny in the neonate (Peters and Jones, 1984). For clarity, we propose to add the term 'spiny' to their name.

Second, some GABAergic non-pyramidal cortical cells (spiny and aspiny alike) project to other cortical areas (Tomioka and Rockland, 2007; Melzer et al., 2012) and might not, therefore, be strictly considered as interneurons. We propose to add the term 'projecting' to their name.

Third, there are glutamatergic spiny non-pyramidal and pyramidal cells (mostly in layer IV of sensory cortices) with locally confined axons that are distributed near the parent cell soma and do not leave the cortical gray matter. Therefore, they might be considered to be short-axon neurons. However, because these cells are both morphologically and neurochemically rather distinct, we prefer to avoid the term 'interneuron' for glutamatergic spiny cells, and propose instead to call them 'intrinsic (or local) glutamatergic spiny cells'.

Fourth, although most GABArgic interneurons have a non-pyramidal somatodendritic phenotype, some display a pyramidal (triangular) somatic shape. To minimize confusion, we propose to use the term 'triangular' to describe the somatic morphology of these interneurons.

Fifth, interneurons are highly diverse with regard to the morphology of their somata and of their dendritic and axonal arbors. For instance, interneurons displaying the same somatodendritic morphology may have different patterns of axonal arborization. Importantly, the axonal geometry is pivotal in establishing circuit connectivity. In several cases, axonal morphology is very distinct, facilitating comparisons of different interneurons. We therefore recommend, whenever historically tenable, using terms such as fusiform, stellate, multipolar, bitufted (neurons with two main dendrites running in opposite directions which, after a relatively short trajectory, resolve into two dendritic tufts), and bipolar (neurons with two principal long dendrites running in opposite directions and showing few dendritic collaterals) only to describe the somatic and/or dendritic morphology and not to name a particular interneuron type. Although these terms are useful descriptors of interneuron somatodendritic morphologies revealed by immunohistochemical staining against calcium-binding proteins and neuropeptides, such staining does not label the full extent of the axonal arbor and therefore does not allow one to unambiguously identify interneuron types. A good example is the double bouquet cell, a term adopted inconsistently in the literature. Some authors used this name for

neurons with a bitufted dendritic morphology, regardless of the pattern of axonal arborization. Other authors use the term double bouquet cells for neurons whose descending axons form tightly intertwined bundles of long descending vertical collaterals resembling a horse tail (DeFelipe, 2002). Although these cells may have bitufted dendrites, interneurons with the same axonal patterns but with different somatodendritic morphologies also exist (Peters and Jones, 1984). We propose that cortical interneurons identified by these characteristic axonal bundles be called horse-tail cells.

Sixth, numerous neurons exist whose axon collaterals do not exhibit any orientation preferences. That is, they present more or less equal numbers of horizontal, oblique or vertical branches. In fact, most interneurons visualized in Golgi preparations or following intracellular labeling could match this description. We propose to introduce the term 'common type' to describe cells without strikingly recognizable shape.

Seventh, an important morphological feature of cortical interneurons is the laminar and columnar reach of their axonal arbors. Following the Petilla Terminology (PING, 2008), we propose to describe neurons whose axonal arbor is confined to a single layer as intralaminar; and neurons whose axonal arbor is not confined to a single layer as translaminar. Similarly, we refer to neurons whose axonal arbor is confined to a single column as intracolumnar, whereas neurons whose axonal arbor is not confined to a single column are referred to as transcolumnar (**Fig. 2**).

Finally, a relevant morphological feature of interneurons is the relative location of dendritic and axonal arbors. We propose to use the term 'centered' for neurons whose dendritic and axonal arbors are largely colocalized, and to use the term 'displaced' otherwise (**Fig. 2**). In this latter case, axons of translaminar interneurons can be 'ascending' and/or 'descending' depending on whether, relative to the dendritic trees, they are distributed mostly towards the cortical surface, the white matter, or approximately equally towards both.

# **Classification attempts**

The Petilla Terminology (PING, 2008) considered the characteristics that are suitable for describing GABAergic cortical interneurons and organized them into morphological, physiological, and molecular properties. Although the identity of a neuron is characterized by all of its properties, a typical experimental identification of a given neuron is commonly limited to a subset of properties. Indeed, most studies primarily (if not exclusively) rely on detailed anatomical, physiological or molecular evidence, and few studies use a balanced combination of these characteristics. Consequently, on the basis of existing data, neurons could in principle be classified using any of these groups of criteria. Several initial attempts at neuronal classification formulated from the Petilla Terminology effort are briefly summarized below.

#### Anatomical

The anatomical classification established in the Petilla Terminology (PING, 2008) divided GABAergic cortical interneurons into those targeting pyramidal cells or displaying no target specificity and, at least in the hippocampus, those specifically targeting other interneurons. Interneurons targeting pyramidal cells were further subdivided on the basis of the target location and included interneurons targeting the axonal initial segment (axo-axonic or chandelier cells), interneurons targeting the perisomatic region (basket cells), and interneurons targeting the dendrites. Basket cells were further distinguished, on the basis of their axonal morphology, into interneurons with tangential (horizontal) axons, interneurons with radial (vertical) axons, interneurons with both tangential and radial axons, and interneurons with axons that are too local to discern a tangential/radial orientation. Dendrite-targeting interneurons were subclassified on an even finer scale as having either a shaft bias or a spine bias, with both of these categories finally separated based on their axonal morphology: shaft-biased interneurons have radial axons that either descend towards the white matter (willow cells) or ascend towards the pia (Martinotti cells); spine-biased interneurons were further divided on the basis of their axonal patterns and include horse-tail and neurogliaform cells.

#### Molecular

The molecular classification of the Petilla Terminology (PING, 2008) divided cortical interneurons based on the expression of specific molecular markers. In particular, five main groups of interneurons can be distinguished: those expressing parvalbumin (PV), including chandelier and basket cells; those expressing somatostatin (SOM), such as Martinotti cells; those expressing neuropeptide Y (NPY) but not SOM; those expressing vasoactive intestinal peptide (VIP); and those expressing cholecystokinin (CCK), but neither SOM nor VIP. These five groups can be further subdivided in multiple subtypes based on several molecular categories: transcription factors, neurotransmitters or their synthesizing enzymes, neuropeptides, calcium-binding proteins, neurotransmitter receptors, structural proteins, ion channels, connexins, pannexins, and membrane transporters. For example, SOM-expressing interneurons can be subdivided depending on whether they also express NPY or calretinin (CR). Similarly, NPY-expressing interneurons and VIP-expressing interneurons can be subdivided depending on whether they also express CR (Karagiannis et al., 2009; Porter et al., 1998). A parallel effort to characterize interneurons based on transcription factors is also gaining traction (Welagen and Anderson, 2011). This developmental classification separates cortical interneurons with an origin in the medial ganglionic eminence (MGE), lateral/dorsocaudal ganglionic eminence (CGE), and preoptic area (POA). The former group encompasses neocortical interneurons

identified based on their molecular markers, including those expressing PV, SOM, and, early in development, NPY. The CGE group includes the interneurons expressing both CR and VIP (horse-tail cells) and those expressing NPY later in development. The POA group expresses NPY. This mapping does not apply exactly to the hippocampus as some differences have been reported (Tricoire et al., 2011).

# **Physiological**

The physiological classification of the Petilla Terminology (PING, 2008) identified six main types of interneurons. Fast-spiking (FS) neurons show non-adapting spiking at steady-state, brief spikes and large fast afterhyperpolarizations, and include continuous FS cells, delayed FS cells, stuttering FS cells, and continuous stuttering FS cells. Non-adapting, non-fast spiking (NA-NFS) neurons display no apparent increase in the interspike interval at steady-state, and include continuous NA-NFS cells and burst-firing NA-NFS cells. Adapting (AD) neurons display a visually obvious increase in the interspike interval at steady-state, and include continuous AD cells, bursting AD cells, and delayed AD cells. Accelerating (AC) neurons display a decrease in the interspike interval at steady-state, and include continuous AC cells and delayed AC cells. Irregular spiking (IS) neurons display an irregular interspike interval and include continuous IS cells and bursting IS cells. Lastly, intrinsic bursting (IB) neurons produce a stereotypical burst of two or more spikes riding on a depolarization envelope followed by a slow afterhyperpolarization potential, and include rhythmic IB cells and initial IB cells.

# Limitations of the Petilla Terminology

Each of these classification schemes has limitations. For many cell types, the anatomical approach requires the identification of the subcellular postsynaptic target(s) in addition to the interneuron of interest. The molecular approach does not provide functional insight, as the functional roles of the most useful and commonly used markers are largely unknown. The physiological approach is greatly dependent on the experimental conditions, and requires a complete specification and possibly standardization of experimental conditions to be widely acceptable. Thus, each of these complementary classifications provide only partial knowledge when taken individually, yet a more comprehensive scheme involving multiple anatomical and functional criteria imposes considerable practical burdens.

#### Feature-based nomenclature proposal

As a pragmatic alternative and update to the anatomical characterization, we propose a taxonomic solution that is based mainly on axonal arborization patterns. We think that

identification of these patterns may be among the most powerful tools available for the subclassification of interneurons.

Our classification design is based on six axonal features numbered 1-6 (**Fig. 2**). These six features were selected as a representative subset of axonal morphological properties that may provide to be suitable for interneuron classification. After introducing all relevant definitions, we describe a web-based interactive system (**Fig. 1**) designed to evaluate this solution empirically, to test its potential for fostering consensus, and to explore preliminary statistical patterns among the generated data. Several statistical and pattern recognition techniques were employed to achieve this goal, including the computation of agreement indices and the use of clustering and supervised classification algorithms.

## First axonal feature

The first axonal feature refers to the distribution of the interneuron axonal arborization relative to cortical layers (**Fig. 2**). Within this feature, we propose two categories: intralaminar, which refers to interneurons with axonal arbors distributed predominantly in the layer of the parent soma; and translaminar, which refers to interneurons with axonal arbors distributed mainly above and/or below the cortical layer of the parent soma.

#### Second axonal feature

The second axonal feature refers to the distribution of the axonal arborization relative to the size of cortical 'columns', from a broad anatomical point of view. Certainly, the term column is vague (Rakic, 2008; Rockland, 2010), since it can refer to small-scale minicolumns (diameter >50 μm), to larger-scale macrocolumns (diameter >300-500 μm), and to multiple different structures within both categories (including barrel columns and ocular dominance columns, extent of arborization of single thalamic afferent fibers, cytochrome oxidase blobs, individual dendritic arbors of pyramidal cells, and tangential widths of axonal patches originated from pyramidal cells). Thus, we have arbitrarily set the size of a cortical column at a diameter of 300 μm, a value that remains rather similar across several species and cortical areas for many of these structures (Malach, 1994; Mountcastle, 1998). Within this feature, we propose two categories: intracolumnar, which refers to interneurons with axonal arbors primarily distributed at a distance from the parent soma that does not exceed 300 μm in the horizontal dimension (Fig. 2); and transcolumnar, which refers to interneurons with horizontal axonal collaterals exceeding a distance of 300 μm from the parent soma in the horizontal dimension.

#### Third axonal feature

The third axonal feature refers to the relative location of the axonal and dendritic arbors (**Fig. 2**). Within this feature, we propose the following categories: centered, which refers to interneurons whose dendritic arbor is located mostly in the center of the axonal arborization; and displaced, which refers to interneurons whose dendritic arbor is shifted with respect to the axonal arborization (**Fig. 2**).

# Fourth axonal feature

If a neuron is categorized as being both translaminar (for the first axonal feature) and displaced (for the third axonal feature), it can be further distinguished into the following categories (PING, 2008): ascending, which refers to interneurons whose axonal arborization is distributed mostly towards the cortical surface; descending, which refers to interneurons whose axonal arborization is distributed mostly towards the white matter; or ascending and descending, which refers to interneurons whose axonal arborization is distributed towards both the cortical surface and the white matter (**Fig. 2**).

## Fifth axonal feature: Interneuron type

defined a limited of cell classification number types for this (http://cajalbbp.cesvima.upm.es/gardenerclassification) on the basis of recognizable morphological characteristics (Fig. 2) and the common usage of their name in the literature (Peters and Jones, 1984). The first cell type, arcade or willow cells, denotes neurons with somata in layers II-VI, multipolar or bitufted dendrites, and axons that give rise to axonal arcades, with predominantly vertical arbors and relatively long descending collaterals. The second cell type, common basket cells, denotes neurons with somata in layers II-VI, multipolar or bitufted dendritic arbors and axon collaterals that have numerous curved pre-terminal axon branches. The third cell type, large basket cells, denotes neurons with somata in layers II-VI, multipolar or bitufted dendrites, and horizontally oriented axon collaterals that can reach a length of several hundred micrometers. These collaterals show numerous curved pre-terminal axon branches that innervate the somata and proximal dendrites of neurons. Frequently, these cells display one or several long descending axonal branches. The fourth cell type, Cajal-Retzius cells, denotes neurons with an axon plexus that is restricted to layer I and long dendrites with ascending branchlets to the pia. These neurons are not present in adult neocortex and in rodents persist only during the two first postnatal weeks (Chowdhury et al., 2010, but see Marín-Padilla, 1998). Cajal-Retzius cells proper do not contain GABA or express GABAsynthesizing enzymes GAD65/GAD67 (Meyer et al., 1999; Hevner et al., 2003). There are also GABAergic neurons with somata in layer I and prominent long horizontal axon collaterals and/or dendrites (Meyer et al., 1999), and these are often also named Cajal-Retzius neurons in the developing neocortex, in spite of their different molecular characteristics from Cajal-Retzius neurons proper (Hevner et al., 2003). Given the purely morphological nature of the present study, most of the authors practically considered any GABAergic neuron in layer I with horizontally oriented axonal arborization as a putative Cajal-Retzius cells. The fifth cell type, chandelier cells, denotes neurons with somata in layers II-VI, multipolar or bitufted dendritic arbors, and terminal axon branches that form short vertical rows of boutons resembling candlesticks. These interneurons are also referred to as axo-axonic cells as they synapse on the axonal initial segment of their pyramidal targets. The sixth cell type, horse-tail cells, denotes neurons with somata mostly in layers II-III, multipolar, bitufted or bipolar dendrites, and axons forming tightly intertwined bundles of long descending vertical collaterals. The seventh cell type, Martinotti cells, denotes neurons with somata in layers II-VI, multipolar, bitufted or bipolar dendrites, and ascending axons that give rise to two axonal arbors, one near the soma and another at a variable distance above. This second plexus may be dense (axonal tuft) or diffuse, and it can be either in the same layer as the soma of origin or in the layers above (ascending axons can travel from layer VI to layer I). The eighth cell type, neurogliaform cells, denotes neurons with somata in layers I-VI, with multipolar dendritic arbors, and are characterized by very small and dense local axonal arborization around the parent cell body. Finally, we included in the web-based interactive system the option common type to denote neurons with somata in layers I-VI, multipolar, bipolar or bitufted dendritic arbors, and axon collaterals without any apparent target or orientation preference (not shown in Fig. 2). Also, we added the option other to label any given neuron with an alternative name in case the expert considered another term more appropriate.

#### Sixth axonal feature: Uncharacterized versus characterized neurons.

Interneurons that are uniquely characterized by peculiar morphological features can often be easily recognized, even when their axon is rather incompletely labeled. However, in many other cases the axon needs to be fully labeled and reconstructed in order to distinguish the neuronal identity unequivocally. Thus, although it is not always necessary to visualize the full axonal and dendritic arborization to distinguish a given neuron, this is the preferred situation. Pragmatically, 'sufficiently complete' labeling simply means 'clear enough' to allow for the identification of a given morphological type. When an insufficient number of morphological axonal features are visualized for a given interneuron (because of incomplete staining, tissue slicing, etc.), we propose that the cell should be deemed an anatomically uncharacterized interneuron.

# Proposal validation and inter-neuroscientist agreement

We designed and deployed interactive web-based an system (http://cajalbbp.cesvima.upm.es/gardenerclassification) to test empirically the level of agreement among a representative sample of 48 experts in assigning the six features to individual cortical interneurons. The approach takes advantage of a common digital format to display, analyze, and manipulate three-dimensional neuromorphological tracings reconstructed from light microscopy (Ascoli, 2006). Images of the 320 interneurons included in the experiment were obtained either from NeuroMorpho.Org (Ascoli et al., 2007) or by scanning two-dimensional drawings from previous publications. Altogether, this pool includes interneurons from different areas and layers of the cerebral cortex of the mouse, rat, rabbit, cat, monkey, and human (Supplementary Online Information S1). The database does not necessarily constitute a representative sample from the neuron population in different areas, layers and species. Furthermore, most of the anatomy recovered from electrophysiological work in vitro is conditioned both by slice thickness and plane of cut, which may vary across laboratories. Nonetheless, these conditions reflect the typical experimental variability confronting researchers in the field. Experienced neuroscientists who are knowledgeable in this field were asked to ascribe the category (each possible value of every feature) they considered most appropriate (6 features, 21 categories; **Fig. 2**):

- Feature 1 (F1): Intralaminar vs Translaminar
- Feature 2 (F2): Intracolumnar vs Transcolumnar
- Feature 3 (F3): Centered vs Displaced
- Feature 4 (F4): Ascending vs Descending vs Both
- Feature 5 (F5): Arcade vs Common basket vs Large basket vs Cajal-Retzius vs Chandelier vs Horse-tail vs Martinotti vs Neurogliaform vs Common type vs Other
- Feature 6 (F6): Characterized vs Uncharacterized

To study the agreement regarding the assignment of the features between neuroscientists, we computed typical statistical measures of inter-expert concordance for each feature and for each category (a possible value for a feature). We also identified sets of neurons using clustering algorithms. Furthermore, we induced from the data a Bayesian network model for each expert, to allow analysis of their choices by comparing the network structures of different neuroscientists (**Supplementary Online Information S1**). With this approach, the possible reasoning of the experts can be inferred from the probabilistic models. Finally, we built automatic classifiers to assign each neuron to one category for each of the six features (**Supplementary Online Information S1**).

# Analysis of the raw data

First, we performed a preliminary exploratory analysis of the raw data to study how the votes of the experts were distributed for the different features. We assessed the relative frequency of each category in the experiment, i.e. of each possible value for each feature. Less than 10% of neurons were rated as anatomically uncharacterized; as described above, this pertains to neurons with an insufficient number of morphological axonal features to allow classification. Thus, the vast majority of the neurons in the experiment were considered as 'characterized'. The most frequently assigned categories of descriptive axonal features proposed in this study were translaminar, intracolumnar, and displaced. The categories 'ascending' and 'descending' received a similar percentage of the ratings, whereas fewer neurons were assigned to the category both.

We then assessed the frequency with which interneurons were assigned to specific interneuron types. The most commonly assigned interneuron types were 'Common type', 'Common basket', and 'Large basket'. The interneuron types Martinotti, Neurogliaform, and horse-tail received an intermediate percentage of ratings, whereas chandelier and arcade received the lowest percentage of ratings. Only three cells were classified as Cajal-Retzius by six experts; the remaining experts classified these neurons as 'uncharacterized', Common type, Common basket, Large basket, Martinotti or 'other'.

Finally, we checked whether the names given to the 79 neurons that were scanned from original publications were maintained in the present experiment by the authors of those publications. Interestingly, the authors were frequently inconsistent for certain neurons. For example, some neurons named 'neurogliaform cells' in the publication were classified as 'uncharacterized' in the current experiment by the same author.

# Experts' agreement analysis

We computed statistical measures of inter-expert agreement to analyze the degree of concordance between the ratings given by the experts (see **Supplementary Online Information S1**). Here, the goal is to quantify the agreement among experts for each feature independently. We studied the agreement for both features and categories using the two most studied agreement indices: Fleiss' pi and Cohen's kappa indices. Agreement indices correct the observed agreement values to take into account chance agreement. When the inter-expert coincidence was not at random levels, the chance-corrected agreement indices yielded values above 0.

We first analyzed feature agreement. We found high level of observed agreement between experts in the classification of neurons according to Feature 1-4 and 6 (observed agreement values exceeding 0.7; **Fig. 3B**). The lowest level of inter-expert agreement (below 0.5) was found for Feature 5.

After correcting for chance agreement (i.e., correcting the observed agreement to erase the influence of chance agreements; see **Supplementary Online Information S1**), the highest

chance-corrected Fleiss' pi inter-expert agreement was found for Feature 4 (**Fig. 3B**). Features 1, 2, and 3 yielded intermediate chance-corrected agreement values, whereas Features 5 and 6 had low agreement. The difference between 'observed agreement' and Fleiss' pi index was particularly high for Feature 6, that is, for the decision on whether or not a neuron could be characterized; this feature had the highest observed agreement and the lowest Fleiss' pi value. This was due to the fact that the category prevalence of this feature was very unbalanced, such that characterized neurons were much more frequent than uncharacterized ones, reducing the values of the agreement measures (see **Supplementary Online Information S2**).

We then calculated the chance-corrected agreement achieved for each category of every feature (see **Supplementary Online Information S2**). Ascending and Descending were the two categories with the highest inter-expert agreement as indicated by the high values obtained for the chance-corrected Fleiss' pi index (**Fig. 3C** and **Supplementary Online Information S2**; **Fig. S7**). Medium-high agreement levels were found for categories Intralaminar, Translaminar, Intracolumnar, Transcolumnar, Centered, and Displaced.

Regarding Feature 5, we found that the Chandelier category yielded the highest agreement (that is, there was little disagreement between all experts over whether a given neuron should be classified as a Chandelier cell). The level of agreement was also high or medium for Martinotti, Horse-tail and Neurogliaform cells, whereas it was lower for the rest of the proposed interneuron types (Large basket, Common basket, Common type, Cajal-Retzius, Arcade and Other). As in the above agreement analysis for Feature 6, Characterized and Uncharacterized were the categories with the lowest level of chance-corrected inter-expert agreement (Supplementary Online Information S2). Moreover, a specific analysis of chance-corrected Fleiss' pi index excluding one or three experts showed similar results, further revealing those experts who contributed to the low agreement for some features (Supplementary Online Information S2; Fig. S8).

Additionally, we assessed whether Fleiss' pi values changed if two categories of Feature 5 were merged into one category. The rationale for this was that certain pairs of categories seemed to overlap in terms of the neurons that were assigned to them. In fact, Fleiss' pi values increased when categories Common type, Common basket, and Large basket were merged with each other (Supplementary Online Information S2; Table S1), revealing ill-defined neuron types. In contrast, when the Martinotti and/or Chandelier categories were combined with other categories, a lower chance-corrected agreement value was obtained, suggesting that these neuron types are well defined. Furthermore, the above results were confirmed in a separate analysis using Cohen's kappa index (Fig. 4C, G-I). This index is defined for scenarios with two experts and two categories. Thus, we assessed the level of agreement between all possible pairs of experts resulting in a comparison of each expert with all the other experts (Supplementary Online

**Information S2; Figs. S9-S11**). For example, the first blue box in **Fig. 4C** summarizes the agreement between the first expert and the other 41 experts regarding the categorization of a neuron as Martinotti. Thus, this high-valued box means that this expert categorized as Martinotti the same neurons as the majority of the remaining experts. Also, we can conclude that there was a high agreement between experts for category Martinotti, since all boxplots (excluding expert #41) showed high Cohen's kappa index values. In contrast, a low level of agreement was found for Common type, Common basket, and Large basket cells, as reflected by the low values of the boxplots (**Fig. 4G-I**). See **Supplementary Online Information S2** for further analyses regarding Cohen's kappa index.

### Neuron clustering

We used clustering algorithms on the classification data from the experts to find groups of interneurons (clusters) with similar morphological properties. The rationale for this analysis was not to define interneuron types. Instead, the goal was to check whether or not the experts' votes for a given feature could separate neurons into clear groups matching the categories of the features. We performed the clustering analysis at two levels: neuron clustering for each feature and neuron clustering for all the features (Supplementary Online Information S1).

First, we grouped the 320 neurons considering each feature independently. Thus, the clustering algorithm takes into account, for a given feature, which category was selected for each neuron by each individual expert. For Features 1-3 and 6 (Supplementary Online Information Figs. S12-14 and S17), clear clusters of neurons could be identified for each category, whereas the clusters for Feature 4 (Supplementary Online Information Fig. S15) showed confusion about the category 'Both'. With regard to Feature 5, we run the algorithm to divide the set of 320 neurons into eight clusters (Supplementary Online Information S1). Fig. 4D shows one cluster of neurons clearly corresponding to Martinotti cells. On the contrary, panels J-L of Fig. 4 show clusters that did not identify neurons corresponding to a single category, mainly corresponding to those categories for which no agreement was achieved by the experts. Results for the remaining clusters of Feature 5 are reported in Supplementary Online Information S2; Figs. S16. These results indicate that, while some concepts were clear for the scientific community (Features 1-3 and 6), other categories (in Feature 4 and 5) were controversial.

Next, we used another clustering algorithm to analyze the neurons taking into account all the features at the same time. In this case, for a given neuron, the algorithm analyzes the number of experts who selected each category of each feature, without distinguishing between individual experts. Thus, we can study possible relationships between the features. **Fig. 5** 

represents the clusters obtained in the analysis. We found some clusters containing neurons with clearly identified categories. For example, **Fig. 5A** shows a cluster of neurons that were clearly categorized as Intralaminar, Intracolumnar, Centered, and Characterized. Furthermore, some of these neurons were mainly categorized as either Common type, Chandelier, Common basket or Neurogliaform. Similarly, **Fig. 5B** shows neurons that were mainly categorized as Translaminar, Transcolumnar, Displaced, Ascending, Martinotti, and Characterized. On the contrary, **Fig. 5C** shows a cluster of neurons mainly categorized as Translaminar and Intracolumnar, but were not clearly categorized for the rest of the features. Finally, **Fig. 5F** shows a cluster of neurons showing no clearly identified categories, corresponding mainly to Uncharacterized neurons.

## Bayesian networks for modeling experts' opinions

Bayesian network models can capture the way by which an expert understands the (probabilistic) relationships among all the features (see Pearl, 1988; and López-Cruz et al., 2011 for an application to neuroanatomy). As opposed to previous analyses, which focused on studying each feature independently, Bayesian networks allow us to analyze the associations between a set of features. The graphical representation of a Bayesian network allows one to visualize and inspect the relationships between the features. Here, we trained a probabilistic graphical model for each expert and used these models to analyze the experts' choice behaviors. In general, some Bayesian networks presented similar structures whereas others showed different relationships between the features. For example, Fig. 6 shows the Bayesian networks learned for experts 16 and 27 when they selected 'Martinotti' or 'Common basket'. The two models had a different structure as shown by the variations in the relationships between the features (Fig. 6 and Supplementary Online Information S2, Fig. S18). Additionally, Bayesian networks allow us to draw conclusions about the categories from a probabilistic point of view. Based on Bayes' rule, we can also infer the likely reasoning of each expert and compare the behaviors of the different experts. Here, we select some of the categories of Feature 5 as evidence, and infer the most probable values for the rest of the features. This enabled the identification of the main properties for each interneuron type, allowing us to study the different conceptual thinking of the experts. In general, when we studied categories with high level of agreement, the propagated probabilities were similar in all the Bayesian networks. For example, when the category Martinotti was analyzed, Bayesian networks yielded similar propagated probabilities (e.g., Fig. 6A and B and Supplementary Online Information S2, Fig. S18). On the contrary, when we analyzed a category with low level of agreement, the propagated probabilities were clearly different (e.g., Common basket in Fig. 6C and D and Supplementary Online Information S2, Fig. S19). That is, experts had a similar concept for Martinotti cells

whereas for Common basket cells they rather differed in their reasoning for assigning this interneuron type (see **Supplementary Online Information S2** for further details).

## Supervised classification of neurons: automatic classification

The ultimate goal of our experiment was to build a model that could automatically classify a neuron on the basis of its morphological characteristics and more specifically in terms of the six features defined in the present study. A supervised classifier is a model which can assign a category to a neuron based on its characteristics. Such a classifier must be trained with a dataset of neurons for which the true category is known. For this purpose, we used those neurons from the experiment (241) that had been reconstructed in 3D. We first measured 2886 morphological parameters using Neurolucida Explorer. Then, we built mathematical models that could automatically classify these 241 neurons according to the values of their morphological parameters (Duda et al., 2001; Jain et al., 2000). Because supervised classification tools require a single class value for each neuron, we used a naive approach of assigning to each neuron the category that received the highest number of votes (Supplementary Online Information S1; Raykar et al., 2010). As a first approach, we built six classifiers, one per feature (Feature 1 up to Feature 6). Moreover, we tested multiple different supervised classifier algorithms (Supplementary Online Information S1). The easiest problem was classifying the neurons as either characterized or uncharacterized, and was solved with the highest accuracy, with only two misclassified neurons for this feature; that is, for this feature the result of the classifier matched that of the (majority of the) experts. For Features 1, 2 and 3, the classifiers also yielded fairly high accuracies (Supplementary Online Information S2; Table S3). By contrast, the accuracy for Feature 4 was much lower. This could be explained by two main reasons. First, the Both category was confusing for the experts, so the assigned category using the majority of votes might not capture the true morphological properties of the neurons for this feature. Second, none of the used morphological variables (Supplementary Online Information S1) might adequately capture the vertical orientation of the axon with respect to the soma. Considering additional variables specifically referring to the orientation of the axon might help improve the accuracy of the classifiers for this feature. The classifiers also yielded low accuracies when distinguishing the categories in Feature 5.(Supplementary Online Information S2). These results were not surprising because distinguishing the 9 proposed neuronal types proved to be difficult for the experts.

Additionally, we further analyzed Feature 5 by training binary classifiers which distinguished one category against all the other categories considered together. We drew similar conclusions as those obtained in previous analyses (**Supplementary Online Information S2**). Finally, since throughout the analyses of the supervised classification experiment we observed a

frequent disagreement between Common type, Common basket, and Large basket categories, we merged these three neuronal types and repeated the automatic classification experiment. Higher accuracy was obtained when merging Common type, Common basket, and Large basket categories into one single category (**Supplementary Online Information S2**).

#### Discussion and future directions

The present study empirically and quantitatively demonstrates that the 'gardener's' approach to neuron classification is untenable at this time, confirming the impression that different investigators use their own, mutually inconsistent schemes for classifying neurons based on morphological criteria. Many ambiguities are independent of the relative reconstruction quality and completeness of the tested neurons. A striking indication of the problem is that in several cases experts assigned a different name to a neuron than the term they had chosen in their own original publication from which that same neuron was taken. This takes us back to the time of Cajal, who also inconsistently named various morphological types of interneurons. For example, Cajal termed neurons that show different dendritic and axonal morphologies 'double bouquet cells' (células bipenachadas in Spanish; bitufted cells in English) (DeFelipe, 2002). In the present study, however, statistical analyses of inter-expert agreement, application of Bayesian networks, and different clustering and supervised classification algorithms clearly separated readily distinguishable interneuron types from apparently confusing interneuron names. High-consensus terms included chandelier and Martinotti cells, indicating that these are more easily identifiable interneuron types. Low-consensus terms included arcade, basket cells, and Cajal-Retzius cells, suggesting that these are potentially less useful names. Researchers generally agreed on specific morphological features, such as ascending vs. descending and intracolumnar vs. transcolumnar axonal arbor.

# A solution: the Neuroclassifier

How might the situation be improved? Based on the supervised classification models described here, we envision a future computer tool for automatic classification of neurons, a Neuroclassifier. This machine will initially use probabilistic labels — based on the categories provided by experts — as neuron names and will evolve by combining supervised (known labels) and unsupervised (new labels) classification techniques. This may foster naming unification, robust classification, and education of new students in the field through online learning techniques. As the scientific community uses the tool, more data will be incorporated into the Neuroclassifier, allowing model updates, and increasing classification robustness and accuracy. Furthermore, other morphometric measurements, encoding different aspects of neuronal anatomy, could be considered. Eventually, multiple correlative criteria including

molecular, physiological, and synaptic connectivity attributes would enable a more complete neuronal classification, a critical step towards better understanding of neuronal circuits.

Finally, it should be kept in mind that the present analysis is limited to neurons from a small number of species, representing mammals commonly used in brain research. These include one lagomorph, two rodents, one felid, and two primates. Although the results from our analysis may be consistent among these mammalian orders, the level of inter-expert agreement was not compared between species. Furthermore, the selection of interneurons from these species does not cover the likely variability of interneuronal morphologies among all mammalian families. In fact, except for the cat, the species in our study all belong to only one mammalian superorder – the Euarchontoglires. Although several 'canonical' neuronal morphologies are doubtlessly common to all placental mammals, some species (such as cetartiodactyls and xenarthrans) depart from the commonly observed neuron types (Hof et al., 1999; Sherwood et al., 2009). Future inclusion of other species in the Neuroclassifier will allow detailed analysis of evolutionary conservation and species-specific neuron types.

Acknowledgements. Test participants (in addition to the authors of the article) in alphabetical order: Lidia Alonso-Nanclares, Csaba Dávid, Hélène Geoffroy, Melis Inan, Virginia Garcia-Marín, Ángel Merchán-Pérez, Laura McGarry, Alberto Muñoz, Cecilia Palazzetti, Nadya Povysheva, Diana Rotaru, Ricardo Scott, Robin Tremblay, Aleksey Zaitsev.

#### References

Amaral D, Lavenex P (2006) *Hippocampal Neuroanatomy*. In Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J (Eds): The Hippocampus Book, New York: Oxford University Press, pp 37-114.

Ascoli GA (2006) Mobilizing the base of neuroscience data: The case of neuronal morphologies. Nat Rev Neurosci 7:318-324.

Ascoli GA, Donohue DE, Halavi M (2007) NeuroMorpho.Org: A central resource for neuronal morphologies. J Neurosci 27:9247-9251.

Berlin, R. (1858) Beitrag zur Strukturlehre der Grosshirnwindungen. Junge, Erlangen.

Cajal, SR (1892) El nuevo concepto de la histología de los centros nerviosos. Rev. Cienc. Méd. Barcelona, 18:361-376. Translated in DeFelipe J and Jones EG: *Cajal on the cerebral cortex* (Oxford University Press, New York, 1988).

Chowdhury TG, Jimenez JC, Bomar JM, Cruz-Martin A, Cantle JP and Portera-Cailliau C (2010) Fate of Cajal–Retzius neurons in the postnatal mouse neocortex. Front. Neuroanat. 4:10.

Clarke E, O'Malley CD (1968) *The Human Brain and Spinal Cord. A Historical Study Illustrated by Writings from Antiquity to the Twentieth Century.* University of California Press, Berkeley.

DeFelipe J (2002) Cortical interneurons: From Cajal to 2001. In Azmitia E, DeFelipe J, Jones EG, Rakic P, Ribak C (Eds): Changing Views of Cajal's Neuron. Prog Brain Res 136:215-238.

DeFelipe J, Alonso-Nanclares L, Arellano JI (2002) Microstructure of the neocortex: Comparative aspects. J Neurocytol 31: 299-316.

Duda RO, Hart PE, Stork DG (2001) Pattern Classification, 2nd edition. Wiley.

Feldmeyer D, Egger V, Lubke J, Sakmann B (1999) Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single 'barrel' of developing rat somatosensory cortex. J Physiol 521:169-190.

Golgi C (1873) Sulla struttura della sostanza grigia del cervello (Comunicazione preventiva). Gaz. Med. Ital. Lombardia 33:244-246.

Hamilton D, Shepherd GM, Martone ME, Ascoli GA (2012) An ontological approach to describing neurons and their relationships. *In Press*, Front Neuroinf.

Hevner RF, Neogi T, Englund C, Daza RA, Fink A (2003) Cajal-Retzius cells in the mouse: transcription factors, neurotransmitters, and birthdays suggest a pallial origin. Dev Brain Res 141:39-53.

Hof PR, Glezer II, Condé F, Flagg RA, Rubin MB, Nimchinsky EA, Vogt Weisenhorn DM (1999) Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. J Chem Neuroanat 16:77-116.

Jain AK, Duin RPW, Mao J (2000) Statistical pattern recognition: A review. IEEE Trans Pattern Anal Mach Intell 22: 4-37.

Karagiannis A, Gallopin T, Dávid C, Battaglia D, Geoffroy H, Rossier J, Hillman EM, Staiger JF, Cauli B (2009) Classification of NPY-expressing neocortical interneurons. J Neurosci 29:3642-3659.

von Kölliker A (1852) Handbuch der Gewebelehre des Menschen. Engelmann, Leipzig.

Kubota Y, Shigematsu N, Karube F, Sekigawa A, Kato S, Yamaguchi N, Hirai Y, Morishima M, Kawaguchi Y (2011) Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. Cereb Cortex 21:1803-1817.

Larson SD, Martone ME (2009) Ontologies for neuroscience: What are they and what are they good for? Front Neurosci 3:60-67.

López-Cruz PL, Bielza C, Larrañaga P, Benavides-Piccione R, DeFelipe J (2011) Models and simulation of 3D neuronal dendritic trees using Bayesian networks. Neuroinformatics 9: 347-369.

Malach R (1994) Cortical columns as devices for maximizing neuronal diversity. Trends Neurosci 17:101-104.

Marín-Padilla M (1998) Cajal-Retzius cells and the development of the neocortex. Trends Neurosci 21:64-71.

Melzer S, Michael M, Caputi A, Eliava M, Fuchs EC, Whittington MA, Monyer H (2012). Long-range-projecting GABAergic neurons modulate inhibition in hippocampus and entorhinal cortex. Science 335:1506-1510.

Meyer HS, Schwarz D, Wimmer VC, Schmitt AC, Kerr JN, Sakmann B, Helmstaedter M (2011) Inhibitory interneurons in a cortical column form hot zones of inhibition in layers 2 and 5A. Proc Natl Acad Sci U S A 108:16807-16812.

Meyer G, Goffinet AM, Fairén A (1999) What is a Cajal-Retzius cell? A reassessment of a classical cell type based on recent observations in the developing neocortex. Cereb Cortex 9:765-75.

Meynert T (1871) Vom Gehirne der Säugethiere. In Stricker S (Ed.): Handbuch der Lehre von den Geweben des Menschen und der Thiere Vol. 1. Leipzig: Verlag von Wilhelm Engelmann, pp. 694-808.

Mountcastle VB (1998) Perceptual Neuroscience: The Cerebral Cortex. HUP: Cambridge.

Pearl J (1988) *Probabilistic Reasoning in Intelligent Systems*. Morgan Kaufmann.

Peters A, Jones EG, eds (1984) Cerebral Cortex. Cellular Components of the Cerebral Cortex. Vol. 1. New York: Plenum Press.

PING (Petilla Interneuron Nomenclature Group): Ascoli GA, Alonso-Nanclares L, Anderson SA, Barrionuevo G, Benavides-Piccione R, Burkhalter A, Buzsaki G, Cauli B, DeFelipe J, Fairén A, Feldmeyer D, Fishell G, Fregnac Y, Freund T.F., Karube F, Gardner D., Gardner EP, Goldberg JH, Helmstaedter M., Hestrin S, Kisvarday Z, Lambolez B, Lewis D., Marin O., Markram H, Muñoz A, Packer A, Petersen C, Rockland K, Rossier J, Rudy B., Somogyi P., Staiger J.F., Tamas G., Thomson A.M., Toledo-Rodriguez M, Wang Y, West DC, and Yuste R (2008) Petilla Terminology: Nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci 9:557-568.

Porter JT, Cauli B, Staiger JF, Lambolez B, Rossier J, Audinat E (1998) Properties of bipolar VIPergic interneurons and their excitation by pyramidal neurons in the rat neocortex. Eur J Neurosci 10:3617-3628.

Raykar VC, Yu S, Zhao LH, Hermosillo-Valadez G, Florin C, Bogoni L, Moy L (2010) Learning from crowds. J Mach Learn Res 11: 1297-1322.

Rakic P (1976). Local circuit neurons. The MIT Press

Rakic P (2008) Confusing cortical columns. Proc Natl Acad Sci U S A. 105:12099-12100.

Rockland KS (2010) Five points on columns. Front. Neuroanat. 4:22.

Sherwood CC, Stimpson CD, Butti C, Bonar CJ, Newton AL, Allman JM, Hof PR (2009) Neocortical neuron types in Xenarthra and Afrotheria: implications for brain evolution in mammals. Brain Struct Funct 213:301-328.

Staiger JF, Flagmeyer I, Schubert D, Zilles K, Kötter R, Luhmann HJ (2004) Functional diversity of layer IV spiny neurons in rat somatosensory cortex: quantitative morphology of electrophysiologically characterized and biocytin labeled cells. Cereb Cortex 14:690-701.

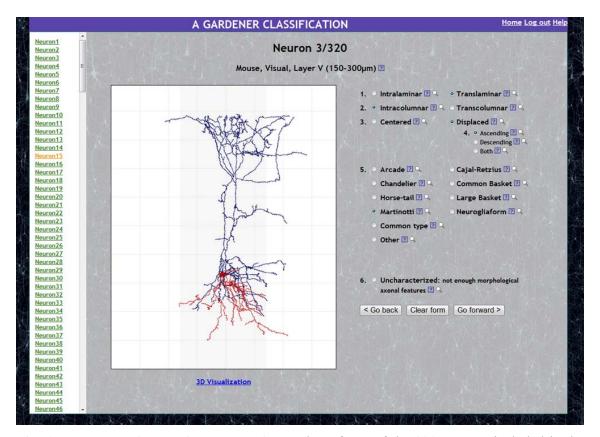
Tomioka R, Rockland KS (2007) Long-distance corticocortical GABAergic neurons in the adult monkey white and gray matter. J Comp Neurol 505:526-538.

Tricoire L, Pelkey KA, Erkkila BE, Jeffries BW, Yuan X, McBain CJ (2011) A blueprint for the spatiotemporal origins of mouse hippocampal interneuron diversity. J Neurosci 31:10948-10970.

Valverde F (1978) The organization of area 18 in the monkey. A Golgi study. Anat Embryol (Berl) 154:305-334.

Welagen J, Anderson S (2011). Origins of neocortical interneurons in mice. Dev Neurobiol 71:10-17.

# Figure legends



**Fig. 1. Web-based interactive system.** Screenshot of one of the 320 neurons included in the web-based interactive system, along with the categories (each possible value for each feature) of the six axonal features displayed for the experts to select as most appropriate to describe the illustrated morphology.

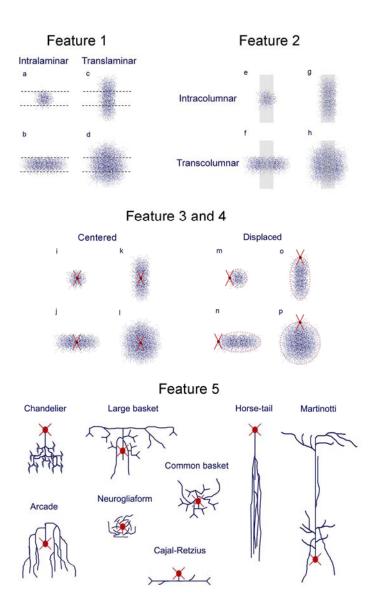


Fig. 2. Schematics of the morphological features. Feature 1, Intralaminar (a,b) vs Translaminar (c,d). Feature 2, Intracolumnar (e,f) vs Transcolumnar (g,h). Feature 3, Centered (i-l) vs Displaced (m-p). Feature 4, Ascending vs Descending vs Both (only when neurons are translaminar and displaced; o, p). Feature 5, interneuron types: Arcade vs Common basket vs Large basket vs Cajal-Retzius vs Chandelier vs Horse-tail vs Martinotti vs Neurogliaform vs Common type (not shown) vs Other (not shown). When an insufficient number of morphological axonal features are visualized for a given interneuron the cell is considered anatomically uncharacterized (Feature 6; not shown). Dashed horizontal lines indicate the extent of the layer. Vertical grey shadows indicate the extent of the column. Axonal arborization is represented by blue dots. Soma and dendritic arborization is represented in red. Possible variations on the relative position of the somata with respect to the axonal arborization of displaced neurons are represented by red dotted ovals.

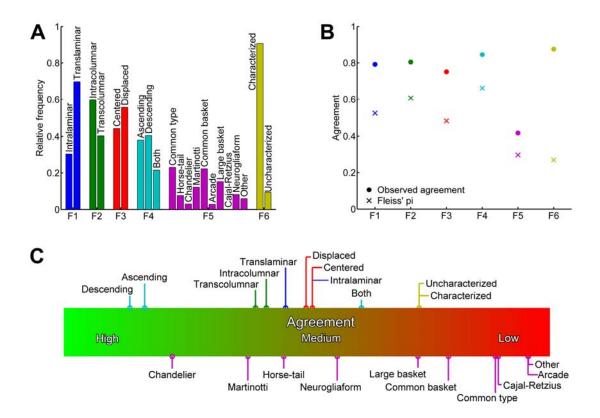


Fig. 3. Agreement analysis. (A) Relative frequency of each category for each feature (F1 to F6), i.e., the number of times a category was selected divided by the total amount of ratings for the feature. (B) Overall observed agreement (circles) and chance-corrected Fleiss' pi index (crosses; Supplementary Online Information S1) for each feature, indicating the degree of concordance between the experts. (C) Chance-corrected (Fleiss' pi index) agreement achieved in each category of each feature. Categories of the same feature are shown using lines with the same color, e.g., categories Intracolumnar and Transcolumnar corresponding to the second axonal feature are shown with green lines. Interneuron types easily distinguished by the experts yielded high agreement (e.g., Chandelier or Martinotti categories), whereas confusing categories such as Common type, Common basket or Large basket yielded low chance-corrected agreement values.

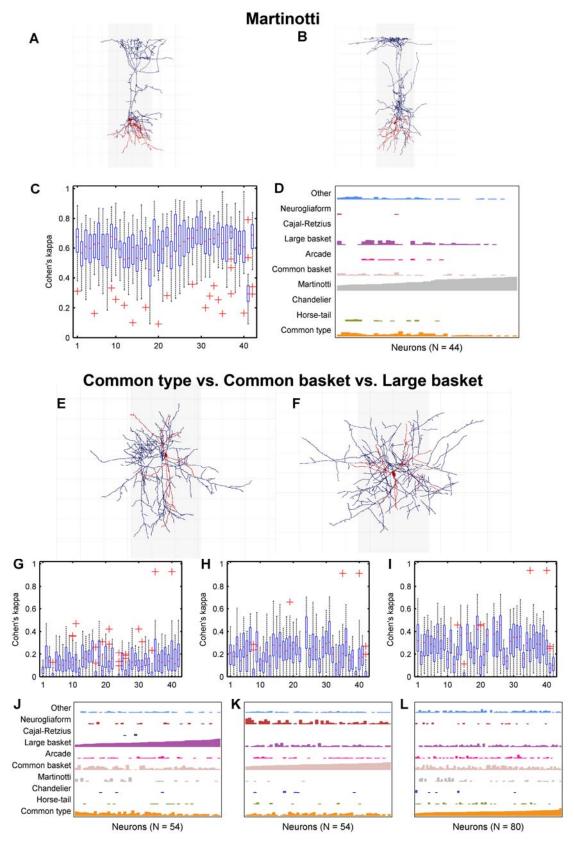


Fig. 4. Examples of agreement (Martinotti category) and disagreement (Common type, Common basket, and Large basket categories). (A,B) Examples of neurons (neurons 3 and 272) categorized by 41 out of 42 experts as Martinotti. (C) Agreement between pairs of experts,

quantified by Cohen's kappa index, when comparing cells categorized as Martinotti against all the other interneuron types. For example, the first blue box shows the agreement values between the first expert and the other 41 experts when classifying interneurons as Martinotti cells. High values of the kappa index yield high agreement. Apart from expert 41, all the other experts yielded fairly high agreement when categorizing interneurons as Martinotti cells. (D) Representative cluster of neurons (44 neurons) indicating the way each neuron was assigned to one of ten categories (each in different color) in Feature 5 by every expert. A vertical bar is shown for each category and each neuron (x axis), representing the number of experts (y axis) who selected the category. High bars (e.g., for category Martinotti) show high agreement when classifying the neurons in this neuronal type. Contrarily, short bars (e.g., for categories Common basket or Other) represent low agreement. Neurons are sorted in ascending order according to the category with the majority of votes (Martinotti). (E) Example of a neuron (neuron 31) that was categorized by 12 experts as Common type, 12 as Common basket, 15 as Large basket, and 2 as Arcade. (F) Example of a neuron (neuron 274) that was categorized by 11 experts as Common type, 12 as Common basket, 14 as Large basket, 1 as Chandelier, 1 as Arcade, and 1 as Other. (G-I) Low agreements between pairs of experts, quantified by Cohen's kappa index, when categorizing interneurons as Common type (G), Common basket (H), and Large basket (I) against all the other interneuron types. (J-L) Examples of clusters of neurons (54, 54 and 80 neurons, respectively) that show no unique category with high bars (cf. panel **4D**). The neurons included in each cluster were categorized as Common type, Common basket, and Large basket by different experts. Note that a high number of experts also categorized neurons as Neurogliaform or Common basket (high bars in panels K and L, respectively). Also, note that the category with the majority of votes (Large basket, Common basket and Common type in panels J, K and L, respectively) show shorter bars than the ones shown in panel D for category Martinotti.

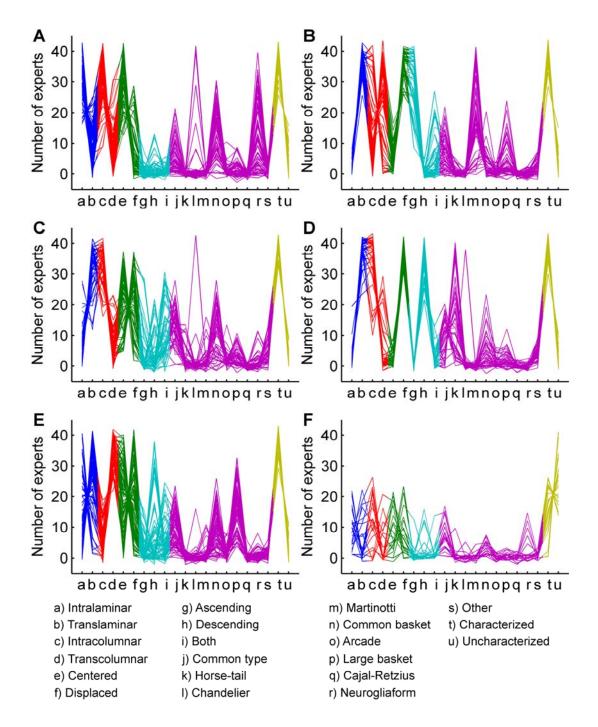


Fig. 5. Clustering of neurons considering all features. (A-F) Parallel coordinates diagrams of clusters of neurons obtained with the k-means algorithm (k = 6) considering all the features at the same time. Each line represents one neuron, showing the number of experts who selected each category of every feature when classifying that neuron. For example, panel B shows a cluster where the majority of neurons were categorized by many experts as Translaminar (blue), Transcolumnar (red), Displaced (green), Ascending (light blue), Martinotti (purple) and Characterized (ochre).

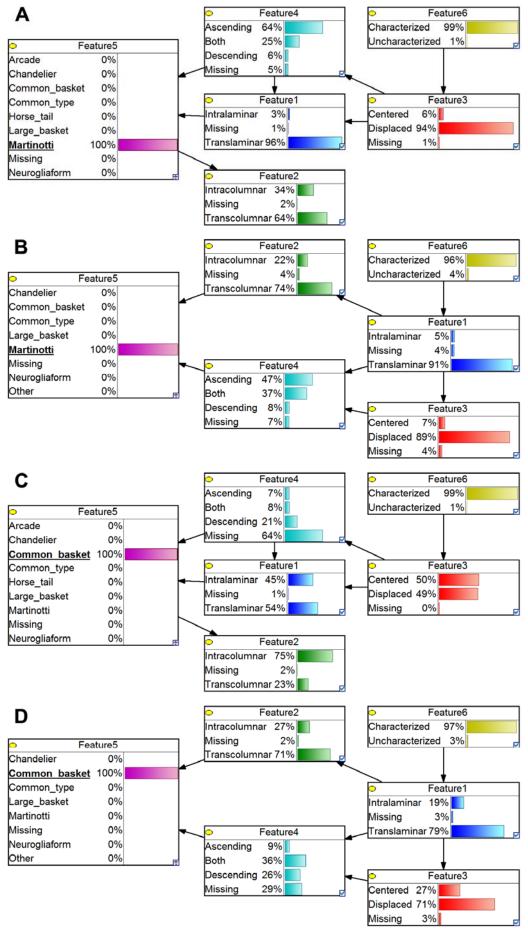


Fig. 6. Examples of Bayesian networks. Bayesian network models when selecting Martinotti (A, B) or Common basket (C, D) categories in Feature 5 for expert 16 (A, C) and expert 27 (B, D). In a Bayesian network structure, each feature is represented with a node (box) in the graph, and an arrow from one node X to another node Y in the graph represents the probabilistic dependence of Y on X (see Supplementary Online Information S1 for further details). Note that the direction of an arrow between two nodes does not necessarily reveal causality or hierarchy. Instead, an arrow connecting two nodes (independent of its direction) shows a probabilistic relationship between the two corresponding features. When a category is selected (e.g., Martinotti as neuron type in panels A and B), probabilistic rules are used to propagate this information and to compute the conditional probability of any other node (e.g., ascending as Feature 4), shown by bar charts in this figure. Thus, the blue bar in Feature 4 of panel A means that if expert 16 called a neuron 'Martinotti', there would be a 64% probability s/he would consider it ascending. Similarities and differences between experts can be identified by comparing their Bayesian networks. For instance, arrows connecting Feature 4 to Feature 5 appear in both Bayesian networks, showing a common relationship for experts 16 and 27. Also, the propagated conditional probabilities can be used to compare experts' opinions. When selecting Martinotti, the propagated probabilities (shown by percentages and colored bars) are similar in the two Bayesian networks, e.g., Translaminar in Feature 1 has 96% probability in panel A and 91% probability in panel B. In contrast, the propagated probabilities when selecting Common basket differ greatly, e.g., Intracolumnar in Feature 2 has 75% probability in panel C and 27% probability in panel D.