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RESEARCH TOPICS

THE NEOCORTICAL COLUMN

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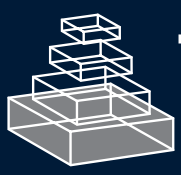
Javier DeFelipe, Henry Markram and
Kathleen S. Rockland



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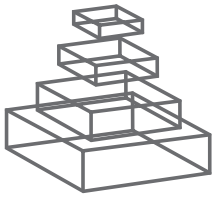

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THE NEOCORTICAL COLUMN

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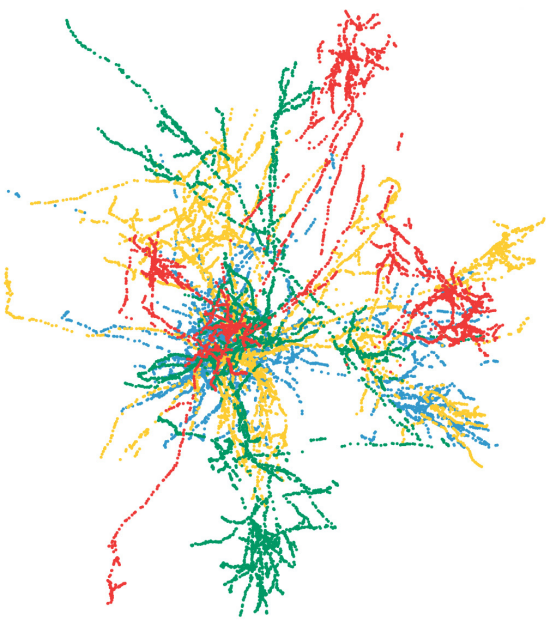

The columnar organization is currently the most widely held hypothesis to explain the cortical processing of information, making its study of potential interest to any researcher interested in the cerebral cortex, both in a healthy and pathological state. Enough data are now available so that the Blue Brain Project can realistically tackle a model of the sensory column in rat. Few will deny however, that a comprehensive framework of the function and structure of columns has remained elusive. One set of persistent problems, as frequently remarked, is nomenclature. "Column" is used freely and promiscuously to refer to multiple, distinguishable entities; for example, cellular or dendritic minicolumns (<50um), and afferent macrocolumns (200-500um). Another set of problems is the degree to which the classical criteria (shared response properties, shared input and common output) may need to be modified and, if so, how. A third, related set of problems is to define area-specific and species-specific variations. Finally, more of an ultimate goal than a problem, is to achieve fundamental understanding of what columns are and how they are used in cortical processes. Therefore, one of the major objectives is to translate recent technical advances and new findings in the neurosciences into practical applications for the neuroscientist, the clinician, and for those interested in comparative anatomy and brain evolution.

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EDITORIAL
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The neocortical column

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In the middle of the twentieth century, Rafael Lorente de Nó (1902–1990) introduced the fundamental concept of the "elementary cortical unit of operation," proposing that the cerebral cortex is formed of small cylinders containing vertical chains of neurons (Lorente de Nó, 1933, 1938). On the basis of this idea, the hypothesis was later developed of the columnar organization of the cerebral cortex, primarily following the physiological and anatomical studies of Vernon Mountcastle, David Hubel, Torsten Wiesel, János Szentágothai, Ted Jones, and Pasko Rakic (for a review of these early studies, see Mountcastle, 1998). The columnar organization hypothesis is currently the most widely adopted to explain the cortical processing of information, making its study of potential interest to any researcher interested in this tissue, both in a healthy and pathological state. However, it is frequently remarked that the nomenclature surrounding this hypothesis often generates problems, as the term "Column" is used freely and promiscuously to refer to multiple, distinguishable entities, such as cellular or dendritic minicolumns or afferent macrocolumns, with respective diameters of <50 and 200–500 μm. Another problem is the degree to which classical criteria may need to be modified (shared response properties, shared input, and common output) and if so, how. Moreover, similar problems arise when we consider the need to define area-specific and species-specific variations. Finally, and what is more an ultimate goal than a problem, it is still necessary to achieve a better fundamental understanding of what columns are and how they are used in cortical processes. Accordingly, it is now very important to translate recent technical advances and new findings in the neurosciences into practical applications for neuroscientists, clinicians, and for those interested in comparative anatomy and brain evolution.

This volume contains nine articles that are intended to provide a summary of our current thoughts on the neocortical column. Three of them (those written by Rockland, da Costa and Martin, and by Rinkus) deal with the nomenclature and more theoretical issues, while the remaining articles include studies on comparative (Raghanti et al.) and developmental (Costa and Hedin-Pereira) aspects, as well as on the normal and altered cortical organization of these columns (Innocenti and Vercelli, Smit-Rigter et al., and Thomson). Finally, we have included a commentary on the analytical and quantitative tools that are currently available to define the diverse morphological patterns and functional parameters that characterize neurons (Losa et al.). The content of each of these articles is briefly summarized in more detail below.

The first article, by *Rockland*, mainly deals with the deceptively simple question "what is a column?" identifying five points for further discussion and re-evaluation: that anatomical columns are not solid structures; that they are part of locally interdigitated systems; that any delimited column also participates in a widely distributed network; that columns are not an obligatory cortical feature; and that columns (as "modules") occur widely in the brain even in non-cortical structures.

In the second article, *da Costa and Martin* describe the historical origins of the concept of the cortical column and the struggle that the pioneers faced to define its architecture. They suggest that within the concept of a "canonical circuit," we may find the means to reconcile the structure of the neocortex with its functional architecture. They propose that the concept of canonical microcircuit respects our understanding of the connectivity in the neocortex, and that the cortical column, as proposed, is sufficiently flexible to transiently adapt the architecture of its network in order to perform the required computations.

The third article by *Rinkus* is a hypothetical approach to examine the function of columns. The author proposes that the minicolumn has a generic functionality that only becomes clear when seen in the context of the higher-level functional unit: the macrocolumn. He proposes that a macrocolumn's function is to store sparsely distributed representations of its inputs and to recognize those inputs. Moreover, he claims that the generic function of the minicolumn is to enforce macrocolumnar code sparseness.

The fourth article is by *Raghanti et al.* and it is a review of the differences among species in minicolumns and GABAergic interneurons, discussing the possible implications for signaling between and within minicolumns. Furthermore, the authors discuss how abnormalities of minicolumn disposition and those of inhibitory interneurons might be associated with neuropathological processes, such as Alzheimer's disease, autism, and schizophrenia. Specifically explored is the possibility that the phylogenetic variability in the calcium-binding proteins expressed by distinct interneuron subtypes is directly related to differences in minicolumn morphology among species, as well as the possibility that this phenomenon might contribute to neuropathological susceptibility in humans.

In the fifth article, *Costa and Hedin-Pereira* address the relationships between cell lineage in the developing cerebral cortex and columnar organization. The authors describe cell lineage experiments that use replication-incompetent retroviral vectors to show that the progeny of a single neuroepithelial/radial glial cell in the dorsal telencephalon become organized into discrete radial clus-

ters of excitatory neurons. These siblings have a higher propensity to develop chemical synapses with one another rather than with neighboring unrelated neurons. The authors discuss the possibility that the lineage of single neuroepithelial/radial glia cells could contribute to the columnar organization of the neocortex by generating radial columns of interconnected sibling neurons.

The sixth article by *Innocenti and Vercelli* reviews the proposal that the bundles of apical dendrites from pyramidal neurons belong to neurons projecting their axons to specific targets. The authors suggest that another structural and computational unit of the cerebral cortex is the cortical output unit. This output unit is an assembly of bundles of apical dendrites and their parent cell bodies, including each of the outputs to distant cortical or subcortical structures of a given cortical locus (area or part of an area).

The seventh article by *Smit-Rigter et al.* deals with the alterations in apical dendrite bundling in the somatosensory cortex of 5-HT3A receptor knockout mice. Using microtubule associated protein-2 immunostaining to visualize the apical dendrites of pyramidal neurons, the authors compare the apical dendritic bundles of wild-type and 5-HT3A receptor knockout mice. In the 5-HT3A receptor knockout mice, the surface of the dendritic bundle was larger than in the wild-type mice, while the number and distribution of reelin-secreting Cajal–Retzius cells was similar in both phenotypes. Along with the previously observed differences in the dendritic complexity of cortical layer 2/3 pyramidal neurons and in the cortical reelin levels, the authors propose that the 5-HT3 receptor fulfills an important role in determining the spatial organization of cortical connectivity in the mouse somatosensory cortex.

In the eighth article, *Thomson* reviews aspects of layer VI and after briefly summarizing the development of this layer, describes and compares the major pyramidal cell classes found in layer VI. The connections made and received by these different classes of neurons are then discussed, as are the possible functional consequences of these connections, with particular reference to the shaping of physiological responses in the visual cortex and thalamus. Inhibition in layer VI is discussed where appropriate. Many types of interneurons can be found in each cortical layer and layer VI is no exception, although the functions of each type of interneuron remain to be elucidated.

The ninth article is by *Losa et al.* and it addresses the issues raised in an interesting article by Romand et al. (2011) on the morphological development of thick-tufted layer V pyramidal cells in the rat somatosensory cortex. These pyramidal neurons are key elements of the columnar organization and Romand et al. used 3-D model neurons, reconstructed from biocytin-labeled cells, to study the principles that govern the dendritic and axonal arborization of these neurons. The methods used by Romand et al. are those employed commonly in many laboratories to analyze the neuronal components of the column and therefore, this article is of general interest to any researcher interested in column organization. However, the conclusions reached in their article are criticized by Losa et al. on the basis of the analytical procedure applied. According to Losa et al. the morphometric approaches used by Romand et al. (and as a consequence those used in most studies on cortical neurons) are usually termed conventional, as they are based on single scale measuring. Such an approach may be well suited to evaluate biological objects assumed to be, or arbitrarily approximated to, regular Euclidean structures, yet it is inappropriate to quantitatively describe the morphology of thick-tufted layer V pyramidal cells, which are characterized by complex functional properties and irregular morphological features. Thus, Losa et al. propose that an objective estimation can only be reached by applying the principles and rules of Fractal geometry. Nevertheless, Losa et al. conclude "that fractal and conventional morphometric approaches, built up on distinct epistemological principles, may set the understanding of the biologic reality at a different level. The former describes the morphological complexity within an experimental interval of observation scales that obviously encompasses the Euclidean dimension, while the latter proceeds at a primary level, i.e., by reducing cellular shapes and tissue structures to monotone elements which could be described by means of deterministic rules. Nevertheless, fractal and conventional morphometry may represent complementary analytical/quantitative tools to elucidate the diversity of morphological patterns and functional parameters which characterize neural cells and brain structures."

In summary, due to the general interest in the cortical columns, thousands of articles have been dedicated to this structure. Indeed, the article of Vernon Mountcastle describing the columnar organization of the cortex (Mountcastle, 1957) has been cited over 1462 times! Thus, while it is obvious that there are many issues and different points of view that have not been dealt with in the present e-book, we believe it fulfills our main intention of providing the reader with some interesting articles addressing different aspects and concepts associated with the organization of the neocortical column.

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Five points on columns

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"Column," like "gene," has both conceptual and linguistic shortcomings. The simple question "what is a column" is not easy to answer and the word itself is not easy to replace. In the present article, I have selected five points, in no way comprehensive or canonical, but which may nevertheless serve as a prompt and aid for further discussions and re-evaluation. These are: that anatomical columns are not solid structures, that they are part of locally interdigitating systems, that any delimited column also participates in a widely distributed network, that columns are not an obligatory cortical feature, and that columns (as "modules") occur widely in the brain in non-cortical structures. I focus on the larger scale macrocolumns, mainly from an anatomical perspective. My position is that cortical organization is inherently dynamic and likely to incorporate multiple processing styles. One can speculate that the distributed mappings within areas like piriform cortex may resemble at least one mode of neocortical processing strategy.

Keywords: collateralization, cytochrome oxidase, layer 2 honeycomb, modularity, terminal arbors, thalamocortical modules, zinc-positive connections

INTRODUCTION

The column as basic unit and defining cortical attribute has been a compelling, not to say seductive idea. Thus, "column" has persisted both conceptually and linguistically, despite significant problems in both domains. (1) "Column" is ambiguous. 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 (Jones 2000; Rockland and Ichinohe, 2004; DeFelipe, 2005; Horton and Adams, 2005). It can refer to a functional or to an anatomical grouping. (2) "Column" invites over-simplification. Few if any structures extend from pia to white matter, or even from layer 2 to layer 6; and few if any have definable, "solid" borders.

The simple question, what is a column?, however, is not easy to answer and the word itself is not easy to replace. The column hypothesis is often summarized as stating that "the fundamental unit of cortical organization is a group of interconnected neurons that share a certain set of properties and extend vertically through the cortical layers to form a column" (e.g. Krieger et al., 2007). Key defining features have come to be (1) interconnected neurons (2) with common input, (3) common output, and (4) common response properties; but these criteria have remained difficult to prove. On the one hand, there is overwhelming experimental support in favor of vertical organization ("the cortical column"). On the other, the confirmation of strictly "common" input, output, and response properties has proved elusive (Krieger et al., 2007).

In the present article, I will focus on the larger scale macrocolumns, mainly from an anatomical perspective. I will not address the broader issue of whether or how anatomy predicts function, since this seems best postponed until results from the newer techniques, such as optogenetics, can be assimilated. Issues concerning minicolumns have been discussed previously (Jones, 2000; Rockland and Ichinohe, 2004), and will be considered in other articles in this issue. My case will be that "column," as word and concept, has become too rigid, and has lost the ability to convey the complex and dynamic aspects of cortical organization. Something of this view has been proposed as "emergent properties" of columnar organization (Markram, 2008), and is included in the idea of "cortical cloud" of local synaptic connectivity (Horton and Adams, 2005).

In this brief review, I have selected five points toward a critical re-evaluation of columnar organization. In no way comprehensive or canonical, these are simply intended as a prompt and discussion aid: anatomical columns are not solid structures, they form locally interdigitating systems, any delimited column participates in a widely distributed network, columns are not an obligatory cortical feature, and columns (as "modules") occur widely in the brain, in non-cortical structures. I am not distinguishing among columns, patches, or modules, and will mainly continue to use the term "column", having no obvious alternative to offer.

These same five points can be applied to minicolumns. Minicolumns, by any of the various definitions, also are not "solid." Whether there are different types of minicolumns is unknown, although the literature has tended to favor the view that they are fundamentally repetitive and uniform, as opposed to consisting of distinctly interdigitated systems. The literature has also tended to view minicolumns as local structures, not particularly inter-cooperative or influenced by a spatially distributed network. There is often an assumption that minicolumns are an obligatory cortical feature, although variability across areas has been documented (Peters et al., 1997). That minicolumns are a properly cortical feature is commonly viewed as true, although minicolumn-like dendritic bundles can be found in non-cortical structures (e.g., Roney et al., 1979).

A first section gives background, with a bias toward structural aspects. This is necessarily brief, since a full review is beyond the scope of the present article.

BACKGROUND

"Macrocolumns" include metabolic zones (e.g., zones of heightened cytochrome oxidase levels), connectional columns or patches, and functional or activity columns. Many, but not all systems of anatomical connections show some degree of columnarity. Thalamic

and cortical terminations, as visualized by various anterograde tracers injected *in vivo*, are often dramatically columnar in cross section, especially in layer 4 and adjacent layers. By contrast, cortical or thalamic terminations in layer 1 are in fact transcolumnar, typically diverging over several millimeters. Retrogradely labeled cortical projection neurons in layers 3 and 5 are often patchy or columnar; but patchiness has not been reported for corticothalamic or other projection neurons in layers 5 or 6, even in highly topographic areas. Thus, at the scale of macrocolumns, multiple mappings can be distinguished, only some of which are columnar (**Figure 1**).

Serial reconstructions or tangential sections, parallel to the pia and layer 1, reveal that what appear to be columns in cross section have a variety of shapes and sizes. Ocular dominance columns, one of the textbook examples of columnar organization, are actually slab-like domains; and column width is variable as a function of the visual field; that is, larger in the foveal representation. In the peripheral visual field representation, the slab-like configuration breaks up into patches (Adams et al., 2007). Size and shape variability has been documented across species, and also across individuals within one species (Horton and Hocking, 1996; Adams et al., 2007). Similarly, the barrels in rodent somatosensory cortex are not stereotyped. Hollow barrels, with cell sparse cores, are typical of mice, young rats, and the anterolateral subfield of mature rats, but solid columns, with cell dense cores, are typical of the main posteromedial field in rats (Rice, 1995). Variability is not reported for other columnar systems of connections, but this is likely because many of the systems are harder to visualize globally or require specialized tissue processing.

In primary sensory cortex, stimulation of an eye (in primates) or whisker (in rodents) results in distinct functional ("activity") columns, conspicuously vertical through the cortical layers. These are associated with thalamocortical terminations, but it is important to recognize that the relationship is not direct. First, thalamocortical terminations are layer specific and targeted mainly to layer 4. Other layers receive thalamic terminations, but none of these extend throughout the cortical depth. Consequently, activity columns result from a mix of direct thalamocortical terminations and subsequent intrinsic, intra- and inter-laminar processing (Sato et al., 2007, among others). Secondly, terminal arbors of individual thalamocortical axons are often smaller than the cross-sectional width of activity columns (in monkey: Blasdel and Lund, 1983; Freund et al., 1989). Thus, activity columns result from several factors, both molecular and activity-related (Inan and Crair, 2007), which bring about the convergence of smaller arbors in a 300–500 μm wide space.

In association cortices, activity columns are more difficult to visualize and their identification tends to be based on electrophysiological criteria (in primate inferotemporal cortex: Tanaka, 2003). There have been no reports so far of patchy thalamocortical connections to higher order association areas; and the anatomical substrate of functional columns is usually attributed to extrinsic and/or intrinsic cortical connections. These often do have a patchy distribution. In cross section, anterogradely labeled cortical terminations can have a dramatically through-layer columnar appearance. (e.g., autoradiography images in Rockland and Pandya, 1979; Selemon and Goldman-Rakic, 1988). These images, however, can be deceptive. The deeper layer component, as shown by higher resolution Golgi-like markers, may be predominantly axons rather than terminations; and single axon analysis has shown that a terminal patch is composed of individual arbors that are not stereotyped, but rather have different size, shape, and number of terminal boutons (Rockland, 2002).

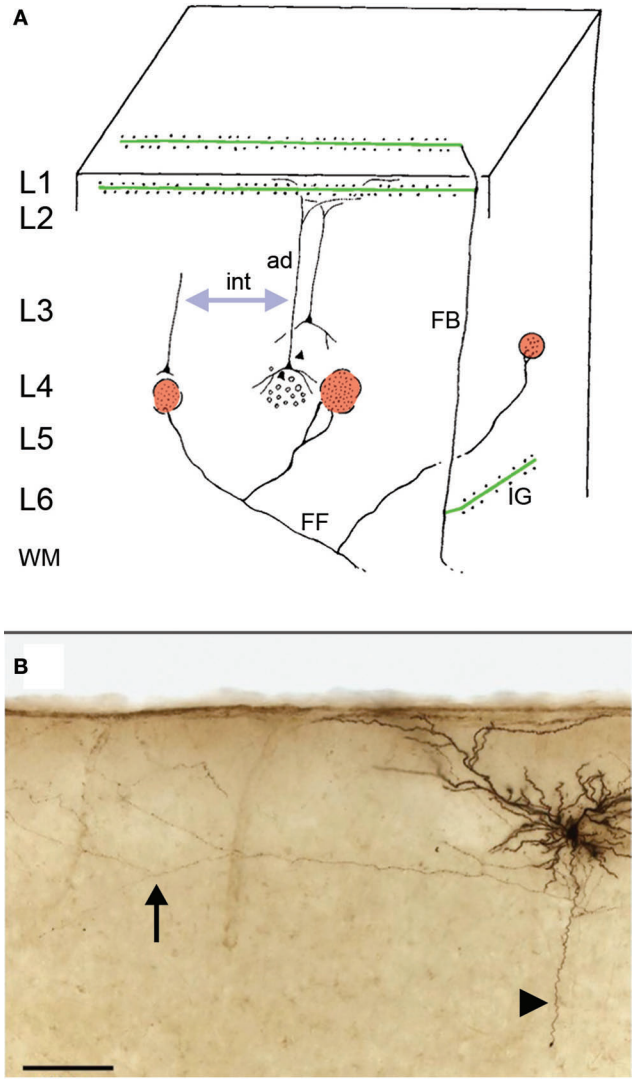

FIGURE 1 | Connectional building blocks: feedforward (FF), feedback (FB), and intrinsic (int) connections. (A) Feedforward (red) and intrinsic (blue arrow) connections are both modular ("columnar"). Pyramidal neurons postsynaptic to feedforward connections presumably are themselves interconnected, but how these extrinsic and intrinsic connections interact is poorly understood. Feedback connections (green) are typically divergent, presumably crossing over a territory corresponding to multiple columns. Modified from Rockland and Drash (1996). Ad=apical dendrite; ig=infragranular. **(B)** Pyramidal neuron in layer 2 of rat visual cortex (intracellularly filled with biocytin *in vitro*). Arrowhead indicates descending axon (truncated in the slice preparation), and vertical arrow points to distal part of an intrinsic axon collateral in layer 2, 600 μm from the cell body (courtesy of Dr. Tohru Kurotani). Scale bar = 100 μm.

Cross-sectional cortical columns line up as a series of discontinuous stripes (prefrontal cortex: Pucak et al., 1996). In auditory cortex, both callosal and ipsilateral cortical connections have a com-

plex configuration in relation to tonotopic organization (Imig and Reale, 1981). Overall, however, compared with thalamocortical terminations, less information is available concerning the size, shape, substructure, and variability of cortical columns; and detailed data concerning their interconnectivity are still lacking.

FIVE POINTS ABOUT STRUCTURAL MACROCOLUMNS

POINT 1

Columns are not solid structures (**Figure 2**). First, dendrites cross-apparent borders in both directions. Pyramidal neurons within a defined column have basal dendrites, oblique dendrites, and apical tufts that extend beyond the home column, and conversely, there will be invading dendrites from neurons in adjoining columns (also: DeFelipe, 2005). In rodent barrel cortex, dendrites of neurons in layer 4 conform to barrel limits (Harris and Woolsey, 1979), but this seems to be an exceptional case. Neurons in layer 4 of primate visual cortex do not comparably conform to ocular dominance boundaries (Katz et al., 1989), and pyramidal neurons in layer 3 extend their dendrites independently of the patches defined by cytochrome oxidase (Hubener and Boltz, 1992; Malach, 1994). This means that some proportion of synapses will contact dendrites whose soma is internal or external to the column, but the actual proportion is not known. Still to be determined as well, is whether contacts are specifically targeted or "randomly" distributed among postsynaptic populations. An interesting question then becomes, how do functionally distinct columns result from an underlying "messy" anatomy.

Second, columns have substructure. In rodent sensory cortex, 2–3 sub-barrel domains have been described on the basis of cytochrome oxidase inhomogeneities and local enrichment of thalamocortical terminations (Land and Erickson, 2005). Similarly, for ocular dominance columns of primate visual cortex, classical anatomical and physiological studies identified core and edge regions, functionally distinguished by different degrees of monocular bias (LeVay et al., 1975). More recently, different conditions of visual deprivation have revealed functional sub-compartments within ocular dominance columns, visualized either by changes in cytochrome oxidase activity (Horton and Hocking, 1998) or by differential expression of immediate-early genes (Takahata et al., 2009).

At the cellular level, there is growing evidence that cortical columns contain multiple, highly specific, fine-scale subcircuits (Yoshimura et al., 2005; Otsuka and Kawaguchi, 2008). This result, which correlates with reports of locally heterogeneous response properties (Sato et al., 2007) weakens arguments in favor of strong intra-columnar homogeneity.

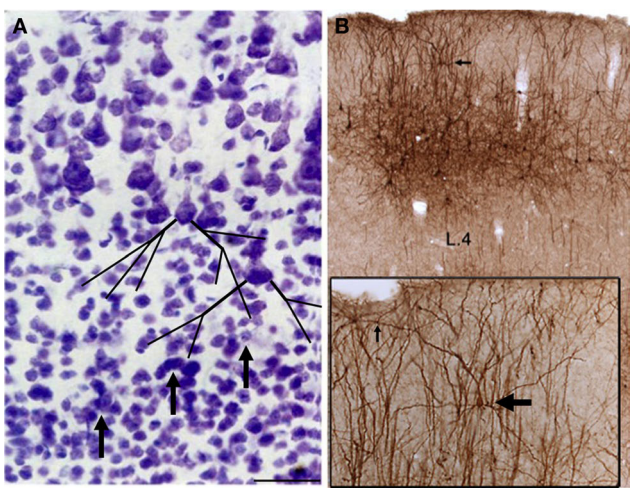

FIGURE 2 | Columns are not solid structures. (A) Cell stain of layer 4 and adjacent layer 3 (macaque temporal association cortex; coronal section). Three distinct cellular rows are apparent in layer 4 (arrows). However, these will be interpenetrated by dendritic and axonal neutrophil; for example, basal dendrites of layer 3 pyramidal neurons, as drawn schematically. **(B)** A large patch of neurons in anterior temporal cortex, retrogradely labeled by an injection of EGFP-adenovirus (immunoreacted for DAB) in posterior temporal cortex. A layer 2 neuron (horizontal arrow) has a laterally divergent apical dendrite (vertical arrow) extending over 250 μm from the soma. Scale bar = 40 μm in **(A)**, 500 μm for **(B)**, and 100 μm for inset.

POINT 2

Columns are partly defined by their neighbors (**Figure 3**). That is, macrocolumns result from several kinds of interdigitation. This is often considered a mode of parallel processing. It is also related to differential processing by segregated dendritic populations.

Interdigitation of different thalamic nuclei

The best documented example of two thalamic systems in the same layer is from rodent barrel cortex (Alloway, 2008). In layer 4, thalamocortical projections from the ventral posterior medial or posterior nuclei respectively target the barrels (lemniscal pathway) and their intervening septa (paralemniscal pathway). A similar segregation occurs more generally, but with segregation in different layers. In the primate somatosensory system, calbindin-positive thalamocortical projections terminate in layer 1, and parvalbumin-positive projections terminate in layer 4 (Rausell and Jones, 1991). In this instance, the projections to layer 4 are topographic and patchy, while those to layer 1 are divergent, crossing across multiple columns. In primate visual cortex, divergent thalamic terminations from the lateral geniculate and inferior pulvinar both terminate divergently in layer 1 (Rockland et al., 1999).

Interdigitation of thalamic and cortical systems

In primary visual cortex of macaques, thalamocortical terminations, visualized as zones of heightened cytochrome oxidase activity, interdigitate with a subset of corticocortical terminations, that contain synaptic zinc, a neuromodulator. This complementarity occurs in layer 4A and in layer 3 (Dyck et al., 2003).

In rat, a similar complementarity has been demonstrated, but in a different laminar location, at the border of layers 1 and 2. VGLUT-2, a global marker for thalamocortical terminations, forms patches in coronal sections that interdigitate with histochemically reacted zinc, a marker for zinc-positive cortical terminations (Ichinohe et al., 2003). In tangential sections, the patches have a honeycomb or reticular configuration. This is pronounced in rat visual cortex. It is detectable in other areas, at the same superficial level, in both rat and macaque monkey (Ichinohe et al., 2003; Ichinohe and Rockland, 2004).

The functional significance of the thalamocortical–corticocortical honeycomb is unknown, but could be related to differential processing by distinct postsynaptic populations. Apical dendrites of layer 2

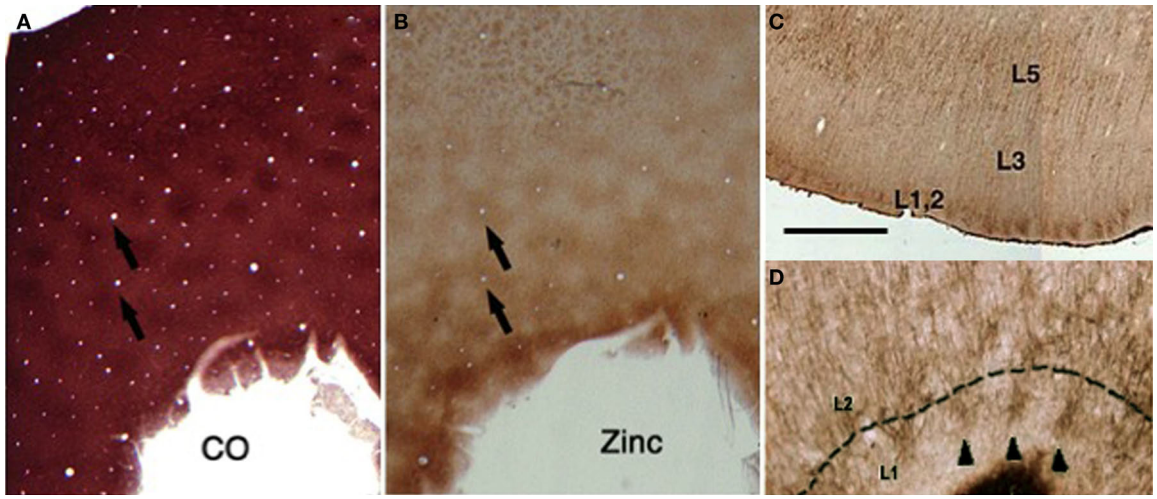

FIGURE 3 | Interdigitating systems. (A) Tangential section through monkey primary visual cortex, reacted for cytochrome oxidase (CO). Obvious patches correspond to thalamocortical terminations. **(B)** Adjacent section reacted for synaptic zinc, where patches correspond to a subset of corticocortical terminations. The top of the photo is cut tangential through layer 4A. The zinc-positive patches are complementary to the CO-patches in layer 3 (see arrows), and layer 4A. **(C)** Coronal section through the posterior orbitofrontal cortex of macaque, where MAP2 immunohistochemistry reveals distinct clusters of apical dendrites at the border of layers 1 and 2. These are likely to co-localize with zinc-positive terminations. **(D)** Higher magnification of C, where three dendritic clusters are indicated by arrowheads. **(C)** and **(D)** are modified from Figure 3 of Ichinohe and Rockland (2004). Scale bar in **(C)** = 1.0 mm, 160 μm for **(D)**, and 600μm for **(A)** and **(B)**.

pyramids have been found to co-localize with zinc-positive cortical projections whereas those of layer 5 pyramids co-localize with the VGLUT-2 labeled thalamocortical projections (Ichinohe et al., 2003). Presumably, the two "streams" are integrated in a next step during local, intra- and inter-laminar processing. Why they need to be initially segregated is not clear.

The location of a honeycomb modularity at the border of layers 1 and 2 points to an independent role for this uppermost cortical stratum, distinguishable from that of layer 4, and thus potentially a different system, embedded within a thalamic-based columnarity in layer 4. Several recent reports have emphasized layer specificity on the basis of differential plasticity properties. A zone of distinctive structural plasticity, coinciding with layer 2 of mouse visual cortex, has been identified by pronounced remodeling of interneuron dendritic tips (Lee et al., 2008). Pharmacological blockade of cannabinoid receptors, also in mouse visual cortex, prevents the ocular dominance shift induced by monocular deprivation in the uppermost layers, but not in layer 4 (Liu et al., 2008).

Interdigitation of cortical connections

Both intrinsic and extrinsic cortical connections are often columnar in cross section. Despite decades of work, the organization of these connections, singly and in relation to each other, is only poorly understood. Global markers do not distinguish between intrinsic and extrinsic cortical projections, and double anterograde tracer injections may not be successful unless the appropriate placement within the target areas is achieved. Intracellular or juxtacellular injections potentially can display the total intrinsic and extrinsic arborizations for a single neuron or small number of neurons, but these techniques do not yield large numbers. Intracellular fills are often carried out in *in vitro* preparations, which are not suitable for long-distance extrinsic connections.

For extrinsic connections, *in vivo* injections of different anterograde tracers have identified two distinct patterns. In certain common cortical target areas, frontal and parietal projections were found to terminate in an array of interdigitating columns (in cross section); in others, the terminations had a laminar, not columnar complementarity (Selemon and Goldman-Rakic, 1988). A columnar termination pattern implies an initial segregation by laterally displaced populations of postsynaptic dendrites whereas laminar segregation implies, as in the hippocampus, segregation by depth along spatially intermingled dendritic populations.

POINT 3

Individual columns are embedded within distributed networks (**Figures 4 and 5**). Although column formation is often treated as a local event, columns might be best viewed as part of an interconnected network ("diaschisis"). This higher order connectivity is not easily accessible to experimentation, although it may become more so with the development of reliable transneuronal techniques. Intra-areal coordination of columnar architectures has been investigated during development by comparing layouts of orientation activity columns in areas V1 and V2 in 2-deoxyglucose experiments (Kaschube et al., 2009). One review of corticothalamic projections has proposed a "rule of parity." The authors propose to modify the idea of "reciprocity" to "parity," where the distribution of corticothalamic projections is seen as determined by convergence with branching patterns of prethalamic afferents (Deschenes et al., 1998).

Distributed organization occurs at several levels. First, cortical projection neurons have intrinsic collaterals. Intrinsic collaterals, as demonstrated most clearly by intracellular injections, extend 2–3 mm from the soma, and for neurons in layers 3 and 5, form multiple patches of terminations (d~250–500 μm) in monkey and cats (Gilbert and Wiesel, 1983; McGuire et al., 1991; Ojima and Jones, 1991; Yabuta

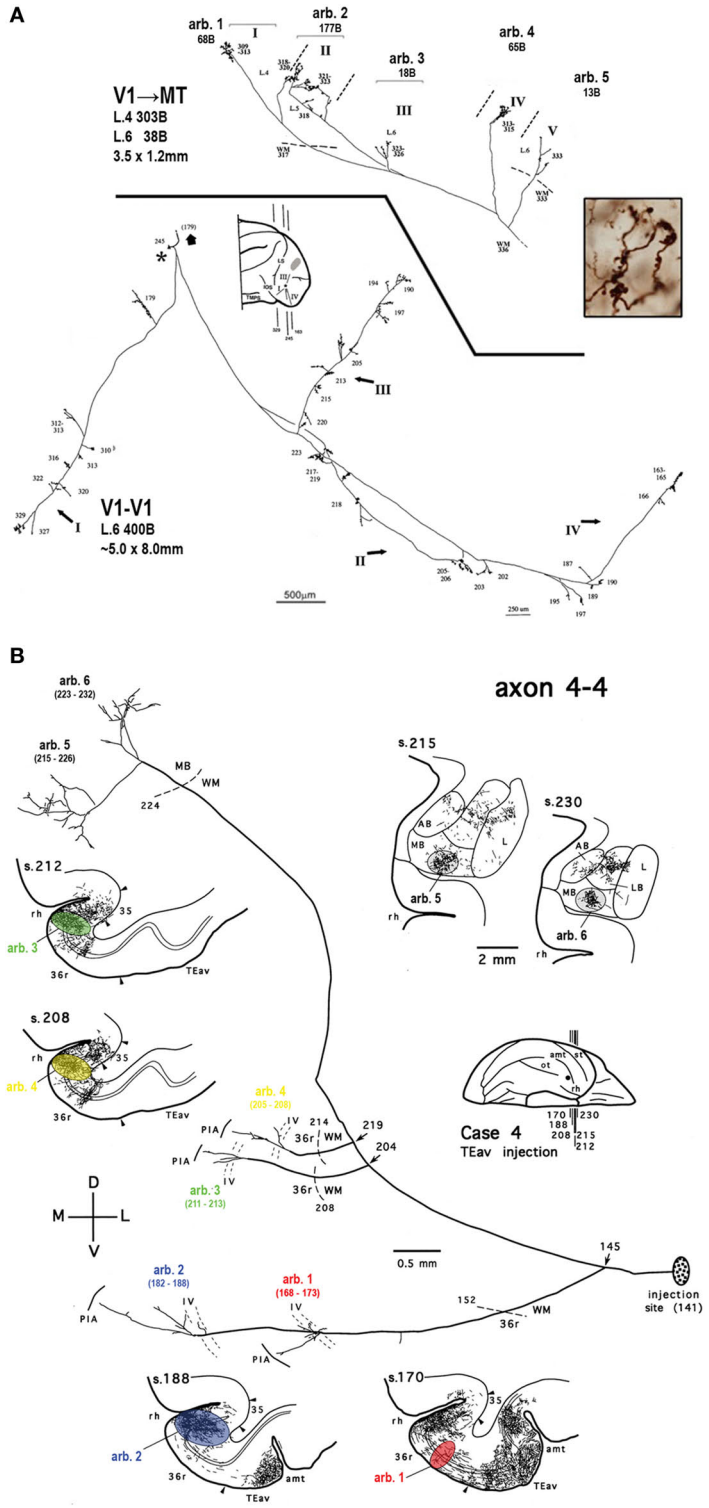

FIGURE 4 | Columns are part of distributed networks. (A) Top: Meynert cells in area V1 project to extrastriate area MT where they form multiple arbors in layers 4 and 6. One axon with five arbors (arb.) is illustrated. Large numbers = number of terminal specializations (boutons, b); and smaller numbers indicate individual tissue sections, where larger numbers are more anterior. Bottom: The same neurons form extensive intrinsic connections within area V1. For this neuron, four extended collaterals (I–IV) were identified. The anterior–posterior position of three coronal sections is indicated by lines on the schematic of the posterior half of the cerebral hemisphere, and the approximate position of the collaterals is indicated by I–IV. A BDA injection site is indicated by the shaded oval. Terminal specializations in MT are illustrated in the inset. Modified from Figure 1 of Rockland (2002). **(B)** A neuron anterogradely labeled by an injection of PHA-L in macaque area TEav has four arbors in adjoining parts of area 36, and two additional arbors in the amygdala. Arbors were followed to denser projection patches (color-coded in selected coronal sections) in different sections. Sections correspond to the numbered lines on the schematic of the monkey hemisphere. Modified from Figure 12 of Cheng et al. (1997).

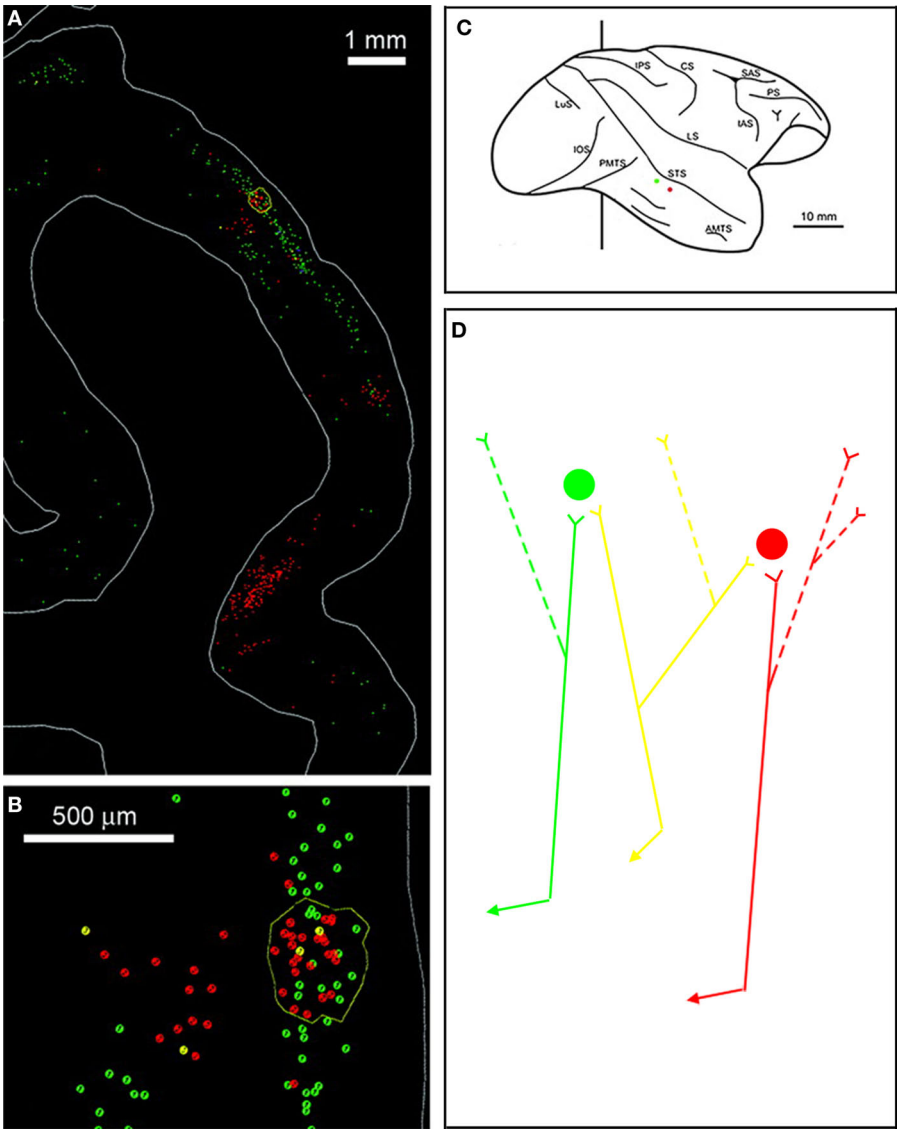

FIGURE 5 | Distributed terminations shown by retrograde tracers. (A) Small injections of two retrograde tracers in monkey temporal cortex result in large patches of red or green projection neurons, which converge to the respective injection sites. **(B)** Schematic of the monkey right hemisphere with the two injections (cholera toxin subunit B conjugated with alexa 488 (green fluorescence) or alexa 555 (red fluorescence). The line indicates the level of the coronal section illustrated in **(A)**. **(C)** Small clusters occur where single-colored neurons are intermixed and where there are also double-labeled neurons. **(D)** The interpretation, consistent with analysis of anterogradely labeled single axons, is that neurons have branched arbors. Three neurons are represented schematically by colored triangles, and their branched axons by corresponding colored lines. Only some of the arbors (solid lines) will be labeled by a given injection, while others will fall outside the injected area (dashed lines). Modified from Figure 5, Borra et al. (2010).

and Callaway, 1998). Terminations are preferentially concentrated in certain layers, and are thus more strictly "patchy" than "columnar. The number and spacing of terminal patches is documented for only a small number of pyramidal neurons, so that the degree of variability is unclear. Neurons in layer 6 have local collaterals, but these do not typically form patches (Anderson et al., 1993).

Second, extrinsically projecting axons typically have 2–4 arbors, each about 200–400 µm in diameter, which distribute over a 2×3 mm hollow space within the target area. Current thinking on columnar organization would predict that the multiple arbors innervate columns with similar response properties. However, the multiple arbors are not stereotyped, but are heterogeneous, possibly having different postsynaptic targets and synaptic effects. One arbor is often identifiable as "principal," on the basis of size and number of boutons (Zhong and Rockland, 2003), and could potentially have different, and even opposite effects from the secondary arbors. For example, axons projecting from V1 to extrastriate area MT in macaque typically have two spatially separated arbors to layer 4 (>1.0 mm apart), and another spatially offset arbor in layer 6 (**Figure 4**). If, as has been proposed, area MT has a compartmental organization according to directionality preference, the two layer 4 arbors might target two functionally similar columnar domains,

but with functionally different consequences, while the intervening arbor in layer 6 could be within a functionally dissimilar domain (Rockland, 1989).

Third, a single axon can have branched collaterals to multiple cortical areas (Cheng et al., 1997; Mitchell and Macklis, 2005). How are the multiple projection foci, widely separated in space, determined? This is unknown, but one can think of piriform cortex, where odorants appear to be represented by unique and distributed ensembles of neurons, modeled as random convergent excitatory inputs from the olfactory bulb (Stettler and Axel, 2009). A not dissimilar organization may exist in sensory and association cortices, with respect to distributed ensembles of neurons, even though it has become more customary to assume a significantly contrasting organization for neocortex (**Figures 4 and 5**).

POINT 4

Columns are not obligatory to cortex (**Figure 6**). In piriform (olfactory) cortex and other limbic areas, layer 1 is a major input layer, and layer 4 is either absent or poorly developed (Neville and Haberly, 2004). In these areas, it is layer 1 that has a complex organization; but this is in the tangential dimension, where layers 1a, 1b, and 1c are connectionally distinguishable strata. An interesting possibility is that features of "older," non-layer 4 cortex may be incorporated or embedded within neocortex. These features would include a lack of modularity, at least in layer 1, and a heightened importance for apical dendritic tufts of underlying pyramidal cells.

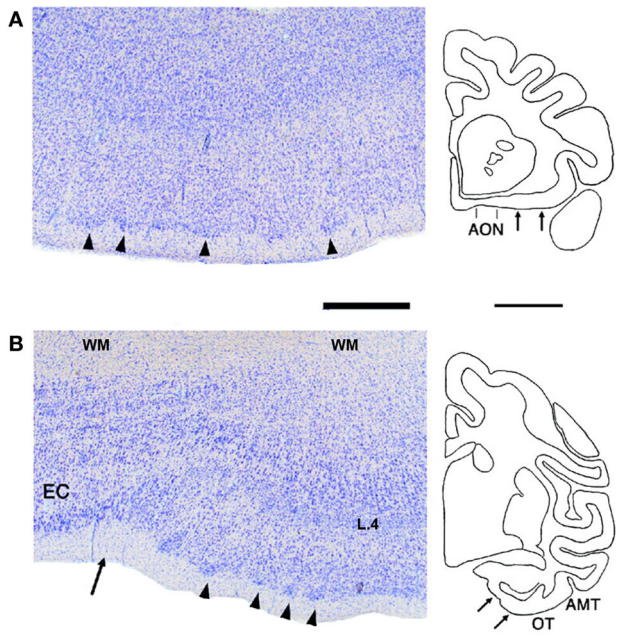

FIGURE 6 | Columns are not obligatory to all cortical areas. Nissl stains of three areas with low overall columnarity and accentuated layer 2. **(A)** Posterior orbitofrontal cortex in monkey. **(B)** Perirhinal cortex, adjacent to posterior entorhinal cortex. Entorhinal cortex (EC) is remarkable for conspicuous lamination, as well as cell islands in layer 2. Coronal section outlines at the right show the areas (arrows) from which the photos were taken. Modified from Figure 4, Ichinohe and Rockland (2004). AMT, anterior middle temporal sulcus; AON, anterior olfactory nucleus; OT, occipitotemporal; WM, white matter. Scale bars = 1 mm for photomicrographs, 5 mm for section outlines.

The entorhinal cortex has prominent cell islands ("modules"), but these are confined to layer 2. The presubiculum has pronounced cell islands, again confined to the upper layers. These co-localize or interdigitate with patches visualized by several neurochemical markers (Ding and Rockland, 2001). In the presubiculum, the development of the patchy organization has been attributed to reelin secreted by Cajal–Retzius cells, seemingly cooperating with the influences of early serotonergic projections (Nishikawa et al., 2002; Janusonis et al., 2004).

Comparative anatomy provides many examples of cortex apparently without anatomical columns or with dramatically modified columns. Whales and dolphins have a cortex with a small layer 4 and enlarged layer 1 (Hof and Van der Gucht, 2007). Connectional data are not available, but the prediction would be of a divergent, layer-1 dominant architecture, resembling that of piriform cortex in rodents. In the same species, the insular cortex has distinct cellular modules, but these are concentrated in layer 2, similar to the general pattern in primate and rodent entorhinal cortex (layer 2) or rodent barrel cortex (layer 4) (Manger et al., 1998). The occipital cortex in giraffe has distinct cellular modules, but these are again concentrated in islands in layer 2 (DeFelipe, 2005).

Finally, cortical architecture can be significantly disrupted and yet apparently remain functionally intact. The disrupted barrel cortex in the reeler and in other mutant or transgenic mice is not associated with marked somatosensory deficits. The degree to which cortex is modifiable, and by what mechanisms, has been extensively investigated under various environmental manipulations. With the development of finer techniques, one can anticipate a new generation of genetic and molecular manipulations. Overexpression of NT3 is reported to result in an enhanced expression of dendritic bundles ("minicolumns") in rat barrel cortex (Miyashita et al., 2010).

POINT 5

Columns outside cerebral cortex (**Figure 7**). While vertical "columns" of terminations or projection neurons have been considered hallmarks of cortical structure, modularity per se is a common organizational principle in the brain. The periaqueductal gray contains longitudinal columns of afferent inputs, output neurons and intrinsic interneurons thought to co-ordinate different strategies for coping with different types of aversive stimuli (Bandler and Shipley, 1994; Keay and Bandler, 2001). The lateral septal nucleus is reported to have a complex system of chemically and connectionally distinct zones of transverse sheets (Risold and Swanson, 1998). Some thalamic nuclei have distinct domains, which are neurochemically and connectionally distinguishable (Rausell and Jones, 1991). The basal ganglia are organized into neurochemically and connectionally distinct striosomes and matrix (Graybiel and Ragsdale, 1978).

The superior colliculus (SC), a laminated subcortical structure, has an intricate tiered and mosaic modularity in the intermediate layers (Illing and Graybiel, 1986; Harting et al., 1992). These have been conveniently referenced to 200–600 μm wide patches

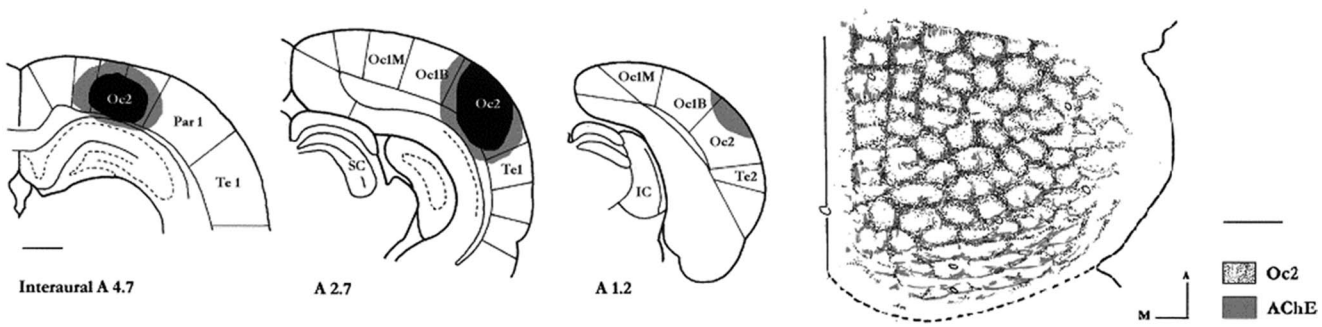

FIGURE 7 | Columns, as "modules", exist outside cerebral cortex. Three coronal sections (rat) to illustrate injection of WGA-HRP in visual cortex. This results (at right, tangential view of the superior colliculus) in a honeycomb pattern of cortical terminations (Oc2), which overlaps with a similar pattern shown by histochemistry for acetylcholinesterase (AChE). A, anterior; m, medial. Modified from Figure 1 from Mana and Chevalier, 2001. Scale bars = 1 mm for section outlines; 500 μm for tangential colliculus.

of acetylcholinesterase reactivity, corresponding to cholinergic inputs from the pedunculopontine nucleus (Harting et al., 1992; Mana and Chevalier, 2000). Both cortical and subcortical inputs contribute to the organization. AChE rich zones, predominantly in the dorsal SC, co-localize with nigrotectal and frontotectal inputs (i.e., motor-related), whereas somatosensory cortical and trigeminotectal projections (i.e., sensory-related), themselves overlapping, target the AChE reduced zones.

More recent investigations of collicular geometry have demonstrated that the neurochemical and input patches form a 3-D honeycomb lattice. This has been modeled as a high resolution matrix for the generation of directed orienting movements, where the multimodal nature of objects in the environment necessitate a complex array of multiple channels (Mana and Chevalier, 2001). A relatively constant number of 80–100 AChE compartments has been identified in a cross-species comparative study; and this has been taken to indicate a common genetic program.

In the cerebellar cortex, an elaborate array of modular subdivisions is revealed by histochemical markers, the topography of afferent projections and some efferent projections, and by gene expression in subpopulations of Purkinje cells (Voogd and Glickstein, 1998; Sillitoe and Joyner, 2007). *Zebrin II* expression reveals a parasagittal pattern of Purkinje cell stripes (PC), each consisting of a few hundreds to thousands of PCs, that is highly reproducible, activity independent, and conserved across species. Other molecular and connectivity markers have an orderly relation to zebrin + or zebrin-stripes (Larouche and Hawkes, 2006). The functional importance of this striking organization remains to be elucidated, but, similar to the mosaicism of the superior colliculus, has been suggested to subserve a massively parallel architecture with a high number of processing channels (Larouche and Hawkes, 2006). By contrast, the number of markers revealing a stripe-like organization in the cerebral cortex, at least at present, is limited.

CONCLUSION

As a term, column is imperfect. The word inevitably has connotations of something solid, repetitive, and static; columns "of something." The common alternatives – "module" or "patch" or "domain" to varying degrees suffer from the same problem. None succeed in conveying the fluid and dynamic properties which may more properly be quintessential cortical attributes.

With the acquisition of more detailed data combined with an openness to new interpretations and models, "column" may follow something of the evolution of "gene." At one point equated with an indivisible, discrete unit of genetic transmission, "gene" now implies an interleaved continuum of coding and regulatory information, where gene expression is controlled by combinatoric actions of transcription factors and other regulatory proteins (Gerstein et al., 2007; Mattick et al., 2009). An equivalent semantic expansion for "column" might come about when we have more information about types of columns and the range of operations. Are there "regulatory" columns? "silent" columns?

Progress can be expected to come from better characterization of cell types (Brown and Hestrin, 2009). This could take us beyond macrocolumns, which are now often visualized by global patterns and averaged connectivity. Re-examination would also be helped by full consideration that small areas of cortex may use different rules for connectivity and the associated columnarity. In a recent report, small injections of two retrograde tracers in monkey inferotemporal cortex appeared to result, in downstream visual areas, in small clusters with an elevated percentage of double-labeled neurons. This would correspond to clusters of more highly collateralized neurons or neurons with preferential connectivity to the two injection sites, as opposed to one of these (Borra et al., 2010).

Unfortunately, there is no easy alternative to "column," and no more specific terminology. But, perhaps under the influence of new data, the word and concept can change, so that the connotation is not crystalline, static, and repetitive, but more dynamic and variegated. For now, best may be to continue using the term, but more and more with something in mind that is closer to Sherrington's "enchanted loom," except that both structure as well as function should be imagined as in a dynamically fluid state.

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Whose cortical column would that be?

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The cortical column has been an invaluable concept to explain the functional organization of the neocortex. While this idea was born out of experiments that cleverly combined electrophysiological recordings with anatomy, no one has 'seen' the anatomy of a column. All we know is that when we record through the cortex of primates, ungulates, and carnivores in a trajectory perpendicular to its surface there is a remarkable constancy in the receptive field properties of the neurons regarding one set of stimulus features. There is no obvious morphological analog for this functional architecture, in fact much of the anatomical data seems to challenge it. Here we describe historically the origins of the concept of the cortical column and the struggles of the pioneers to define the columnar architecture. We suggest that in the concept of a 'canonical circuit' we may find the means to reconcile the structure of neocortex with its functional architecture. The canonical microcircuit respects the known connectivity of the neocortex, and it is flexible enough to change transiently the architecture of its network in order to perform the required computations.

Keywords: cortical column, Daisy, bouton cluster, neuroanatomy, canonical microcircuit

ORIGINS

Columns are fatally attractive. To Western eyes reared on classical and neoclassical forms, they seem an existential necessity of the built world. For the youthful reader of any neuroscience textbook, they are one of the few memorable facts about the architecture of the neocortex. So convincing are they, and so central to our present day concepts, that vast resources in human and machine time and are being devoted to defining every element and every connection in the cortical column so that a facsimile can be recreated '*in silico*' (Markram, 2006; Helmstaedter et al., 2007). Peering down a microscope, squinting at a computer monitor, or listening to the activity at the tip of a microelectrode, one no longer needs the eye or ear of faith to see columns almost everywhere. But it was not always thus: Mountcastle (2003), reminiscing about his work in the 1950s, wrote, 'When in 1955–1959 I described the columnar organization of the somatic sensory cortex on the basis of observations made in single neuron recording experiments in cats and monkeys (Mountcastle et al., 1955; Mountcastle, 1957; Powell and Mountcastle, 1959a), the report was met with disbelief by many neuroanatomists.' The reason was simple. The horizontally layered iso-cortex of Oskar Vogt and its cytoarchitectonic divisions into 'cortical organs' made vertical subdivisions a *non sequitur*.

DISCOVERY OF CORTICAL 'COLUMNS'

Mountcastle claimed that he was not the first to discover columns in the cortex (Mountcastle, 1997). He generously gave Lorente de Nó (1949) credit for having imaginatively conjured vertical chains of neurons from his Golgi studies of what Lorente de Nó then thought was the mouse's 'acoustic' cortex [misidentified by Rose (1912), actually the somatosensory cortex]. However, Lorente de Nó's data were far from convincing and hardly pointed to the receptive field properties that were mapped by Mountcastle's electrophysiology. The crucial observation of Mountcastle and colleagues was that although successive cells in a penetration originated from the same receptive field location, the two modalities of light touch and light skin pressure were represented independently within 'narrow vertical columns or cylinders extending from layer II through layer VI' (Mountcastle, 1957).

Mountcastle (1957) thought that his cortical 'minicolumns' had dimensions 30–50 μ in diameter and extended throughout the full thickness of the cortex. The dimensions of the functional columns in the cat were guessed at between one cell and 0.5 mm in diameter, because Mountcastle and his colleagues had great difficulty in finding their electrode tracks in histological sections. When he extended his studies in the monkey with the help of the Oxford anatomist Tom Powell (Mountcastle and Powell, 1959a,b; Powell and Mountcastle, 1959a,b), their Methods section revealed an extraordinary concern about the accuracy and detail of identifying the electrode tracks. From these analyses, however, they made the far-reaching observation that neurons recorded from penetrations made perpendicular to the surface of the cortex are 'modality pure', while penetrations made at an angle showed higher modality change.

Perhaps the most important feature of Mountcastle's concept of functional columns was its ease of generalization. Thus not only did he demonstrate columns in both cat and monkey, he also initiated a paradigm for probing the functional architecture of any area of the neocortex. A key element was the stability of recordings from single units, which allowed the receptive fields of a sequence of neurons to be mapped in detail. His new neighbors, David Hubel and Torsten Wiesel, who had been hired by Steven Kuffler in 1958, rapidly adopted his paradigm and began to map receptive fields in the cat's visual cortex in their basement laboratory in the Wilmer Institute of Ophthalmology. Because of

Mountcastle's proximity, columns were in their thinking, but even after their early breakthrough in discovering that the receptive fields of cortical neurons were orientation selective and binocular, they struggled to make sense of how different orientations were represented in the visual cortex (Hubel and Wiesel, 1962, 1963). Following Mountcastle's experience, their one certainty was that the cells of like orientation selectivity were found a single radial penetration from surface to white matter. By the simple expedient of making multiple electrolytic lesions along an electrode track, they avoided the struggles that Mountcastle and colleagues had had in finding the electrode tracks in histological sections. This ability to have accurate histology of the electrode tracks was an essential component of their entire oeuvre. Their most valuable data was gained from experiments in which they combined anatomy and physiology (Hubel and Wiesel, 2005, pp. 244–245). The contribution of a long list of anatomists to their work was absolutely key, for these data could not have been obtained had they been using chronic recording techniques and it is unlikely that the ice-cube model would have come into existence at all.

OCULAR DOMINANCE AND ORIENTATION SEQUENCES

The notion of ocular dominance columns remained a glint in the eyes of Hubel and Wiesel until, by accident, they discovered firmer evidence for them after inducing an artificial divergent squint in young kittens (Hubel and Wiesel, 1965). When they recorded from area 17, they found that virtually all cells were monocular, with left or right eye dominated cells being found in equal proportions. In normal controls cats 85% of the cells were binocular. Many years later they recollected that they almost did not begin this recording experiment, because when they tested the kittens' visual behavior it seemed so normal (Hubel and Wiesel, 2005). And as if this were not enough for a single experiment, they made another key discovery: 'The grouping of the cells into separate eye domains was almost as surprising as the fact they were monocular, for until then we had only been vaguely aware of the division of the cortex into left-eye and right-eye domains – the ocular dominance columns' (Hubel and Wiesel, 1998). With new eyes they returned to the normal adult cat and found sequences of cells strongly dominated by one eye, although at this early stage they described these as a 'system of parcellation by ocular dominance', rather than ocular dominance columns (Hubel and Wiesel, 1965).

A further crucial observation followed: that the ocular dominance of a neuron was not correlated with its orientation preference. In this respect the columnar systems they described in the visual cortex were quite unlike the somatosensory cortex, in that every neuron in the visual cortex was a member of both columnar systems, whereas neurons in the somatosensory cortex responded to light or deep touch, but not both. Their struggles to understand the representation of orientation were not unexpected, given that their attempts to understand the map of retinotopy were also proving difficult. In their epic 1962 paper on the cat they noted that even within a column defined by common orientation preference, the retinotopic positions of the of successive units showed 'apparently random staggering of receptive field positions', and also could change eye dominance. This last observation was puzzling if one imagined the column to be a radial string of cells.

Given the difference in the estimated dimensions of an ocular dominance column (0.5 mm) and an orientation column in the cat (0.1 mm) (Hubel and Wiesel, 1963), the ocular dominance should be more stable in a radial penetration than iso-orientation. In both cat and monkey they observed large variations in the size of the receptive field even in radial penetrations (Hubel and Wiesel, 1962, 1968, 1974a). Except in the special case of the whisker representation, they did not regard the topographic representation by itself as a columnar system (Hubel and Wiesel, 1968, 1974a), because it is continuous. They interpreted Mountcastle's concept of the column as a 'discrete aggregation of cells, each aggregation being separated from its neighbors by vertical walls that intersect the surface (or a given layer) in a mosaic' (Hubel and Wiesel, 1968). On this interpretation, the representation of the whiskers in the somatosensory cortex of the rodent, would qualify as a columnar system, because each whisker is discretely represented. However, in most other respects the columns of the topographic representation of the whiskers are different from the functional columns seen in cat and monkey sensory cortex, which are not created by the topographic map, but emerge from it.

COLUMNS IN THE ROLLER

Even in the monkey's area 17, which Hubel and Wiesel described as a Rolls Royce compared to the Model T Ford of the cat's (Hubel and Wiesel, 2005), the issue of the organization of the orientation columns was puzzling. Their legendary 5-hour-long penetration in area 17 of a squirrel monkey named 'George', where they found an exquisitely ordered sequence of clockwise and counter-clockwise changes in orientation through a continuous penetration of 53 recording sites, was also not without mystery, not least because the sequence of orientation was uninterrupted by the non-oriented cells that they had shown in the same paper to be a feature of layer 4 of rhesus monkey cortex (Hubel and Wiesel, 1968). In their discussion of these results they expressed their bafflement that the striate cortex seemed to contain regions where orientation columns were orderly, and regions where they were not. Their bafflement was compounded by their observation that there was no hint of such differences structurally. When they looked at their Nissl-stained sections they saw radial fascicles everywhere. Did columns look like cylindrical pillars, or slabs? Did they alternate like a checkerboard, or were the pillars embedded in a matrix of parallel, swirling slabs? These were questions that preoccupied them to such a degree that they employed every old and new technique they could to satisfy their curiosity. The result was the most comprehensive description of the structural and functional architecture of any area of neocortex (Hubel and Wiesel, 1977).

What Hubel and Wiesel could show in the visual cortex, but Mountcastle for the somatosensory cortex could not, was that their description of 'column' was a misnomer. What the anatomical and physiological methods showed was that the columns were not Greek pillars, but swirling slabs. But by the time their revisionist discovery hit the presses, the term 'column' was indelible and the belief in the existence of such a mythical beast clearly remains. The revisionist view of the two systems of ocular dominance and orientation was captured in the 'ice-cube' model of the visual cortex, which was first unveiled by Hubel and Wiesel in their Journal of Comparative Neurology paper of 1972 (Hubel and Wiesel, 1972). In that paper they had made electrolytic lesions in single laminae of the dor-

sal lateral geniculate nucleus and induced terminal degeneration in layer 4. This study was one of the rare examples of work that they first reported in a letter to Nature (Hubel and Wiesel, 1969). Although their summary diagram had an accelerated entry to the textbooks and remains a perennial favorite, their path to the first ice-cube model was far from fast or easy, as we have seen.

OBITUARY: COLUMNS?

The simplest conclusion from this brief history is that there is no cortical column, or at least, if there is, it is a structure without a function, as Horton and Adams (2005) poignantly concluded. But although such reports of the death of the column have proved premature, it is clear that there is no single anatomical entity about which there is general agreement. Here we continue to use the term, but only in its historical or metaphorical sense.

A more nuanced view, however, is that in addition to its layered structure, the cortex also organizes its functionality in the vertical dimension, but, as with the layers, the size and shape of these vertical organizations varies greatly. At the most basic level, a cortical area is often defined as the region containing a single topographic representation of a sensory surface, like the retina, skin, and cochlear. These topographic maps are represented vertically in all layers, but not with the same degree of fidelity in each layer. In the unusual case of the discrete sensory representation of the whisker array in rodents, the patch representing a single whisker in layer 4 is elongated – this anisotropy in 'magnification factor' presumably reflects the receptor density at the periphery. The closest equivalent to the whisker representation in the visual system is the segregation of the left and right eye inputs to layer 4 – the ocular dominance system of cat and monkey. However, the ocular dominance stripes are highly variable structures and not present in all species (LeVay et al., 1980, 1985; see critique by Horton and Adams, 2005). In the rhesus striate cortex (LeVay et al., 1985), and in enucleate humans (Adams et al., 2007), they are heterogenous in their spacing and vary over a factor of two in their dimensions even in a single hemisphere, whereas the Nissl-stained densities of the cortical cells appear uniform throughout. However there is a larger problem to worry about.

THE HARSH REALITY OF BIOLOGY

'There is one puzzling discrepancy between these physiological results and the morphology. The orientation column thickness is at most the order of 25–30 μm, yet from sections of Golgi material most cells are known to have dendritic and axonal arborizations that extend, apparently in all directions, for distances of up to several millimetres' (Hubel and Wiesel, 1974a).

How do cortical neurons organize themselves into the networks that express not only individual properties like orientation selectivity or ocular dominance, but arrange these circuits to express a precise 3-D map of these properties? This central question has never been better posed than in the passage above from Hubel and Wiesel. The second of the two papers that Hubel and Wiesel published in the journal of Comparative Neurology in 1974, is arguably their masterpiece (Hubel and Wiesel, 1974b). In its palpably deep thought, it synthesized 15 years of intensive description of what they called the 'machinery' of striate cortex. In the monkey they had seen the left and right eye ocular dominance columns as having some degree of exchange, so that the monocular layer 4 neurons became progressively more binocular in superficial and deep layers. 'This is in sharp contrast to the orientation columns, since for these there is no evidence to suggest any cross-talk between one column and its immediately adjoining neighbors' (Hubel and Wiesel, 1968). One of their major interpretations for the existence of columns rested on the concept of economy of connections (Hubel and Wiesel, 1963). Their model of serial processing required interconnections between neurons with the same orientation and receptive field position. Hence locating them all in the same column would provide the most economical means of connecting neighbors that needed the same set of thalamic inputs.

FINDING FORM

Here we highlight some of the problems in achieving this specificity, using some of our own data from the cat. In *in vivo* experiments we recorded from single cells in cat area 17, classified them physiologically, filled them with horseradish peroxidase, and reconstructed them in 3-D (Martin and Whitteridge, 1984a). In separate experiments we used optical imaging of the intrinsic signal to obtain 2-D orientation maps (for methodology see Bonhoeffer and Grinvald, 1996). **Figure 1** shows the boutons of four different neurons from four different cortical layers of area 17. In all these neurons the bouton distribution is not homogenous through space, but instead the axons form clusters of boutons. Binzegger et al. (2007) developed a method to identify these clusters objectively. The results of their algorithm applied to the neurons of **Figures 1A,B** are shown in **Figure 1C**. The cluster of boutons surrounding the cell body is of particular interest since it forms synapses in the neuron's 'own' minicolumn (we call this cluster 'proximal'). The proximal clusters not only extend beyond several minicolumns, but are not spatially restricted to the diameter of the dendritic arbor of the minicolumn. This implies that not even specificity of connections could restrict the connections to neurons within a minicolumn. Moreover, if the proximal cluster was the anatomical correlate of columnar organization, the proximal clusters of different neurons would be of similar sizes. Instead, we find that the size of the 'proximal' clusters vary greatly between different neurons (**Figures 1D and 2**).

We pursued this comparison between the anatomy of visual cortex and its functional vertical organization by comparing the bouton cluster size with the width of the active patches seen with the optical imaging when a single orientation is displayed. Neurons in the visual cortex are not selective to just a single orientation as implied by the ice-cube model, but have tuning curves that extend over thirty or more degrees of visual angle. Consequentially the region of the cortex that generates a response to any given orientation is necessarily larger than a single minicolumn. The change in preferred orientation over cortical space in the cat and monkey is about 10° every 50 μm, with a complete orientation cycle taking 500–1200 μm (Hubel and Wiesel, 1974a; Albus, 1975). As described by Binzegger et al. (2007), the proximal cluster of layer 2/3 pyramidal neurons have a lateral extent of about 600 μm, which is sufficient to cover a complete 'hypercolumn' – the set of a dozen or more columns representing a full 180° cycle of orientation (Hubel and Wiesel, 1974b). Here we superimpose the proximal clusters of the excitatory neurons in the database of Binzegger et al. (2007) on a map of a single

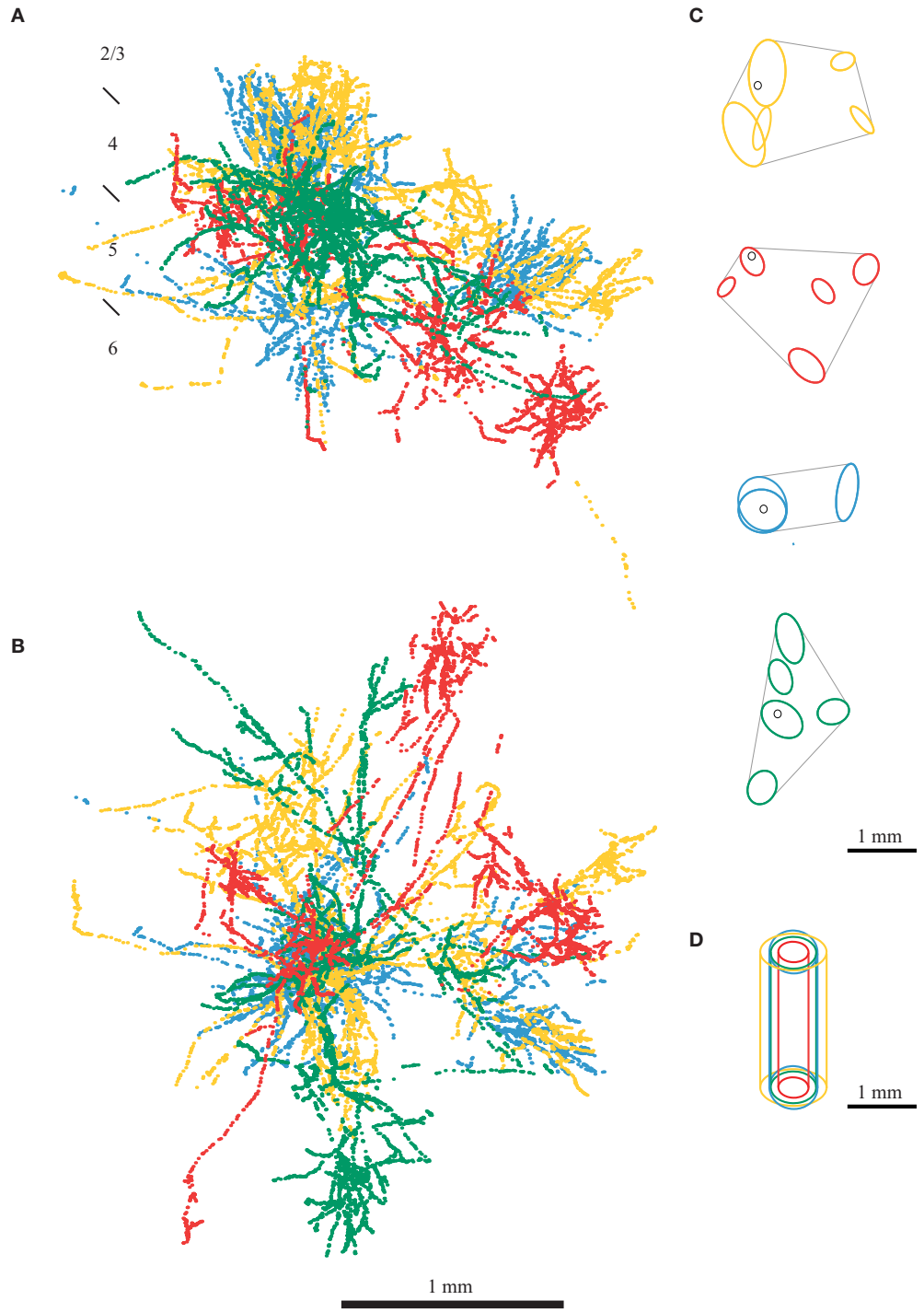

FIGURE 1 | Bouton distribution of four neurons from the primary visual cortex of cat. Axons of neurons from all layers spread over a distance covering the dimensions of many minicolumns. The boutons from a layer 2/3 pyramidal neurons are shown in yellow, from a layer 4 spiny stellate in red, from a layer 5 pyramidal neurons in blue and from a layer 6 pyramidal neuron in green. **(A)** Coronal view. **(B)** Top view. **(C)** Bouton clusters of the axons shown in **(A)** (adapted from Binzegger et al., 2007). **(D)** Comparison of the size of a cortical column cover by the proximal cluster of boutons of each neuron (Binzegger et al., 2007). A cluster is considered proximal if it intersects with the vertical axis running through the soma.

orientation in area 17 obtained using optical imaging of intrinsic signal (**Figure 2**). The proximal clusters of layer 4 neurons, which project within layer 4 and to the superficial layers, are similar in size to the functional orientation domains (**Figures 2B–D**). This correspondence between the size of a single orientation patch and the proximal cluster seen for layer 4 neurons is not apparent for

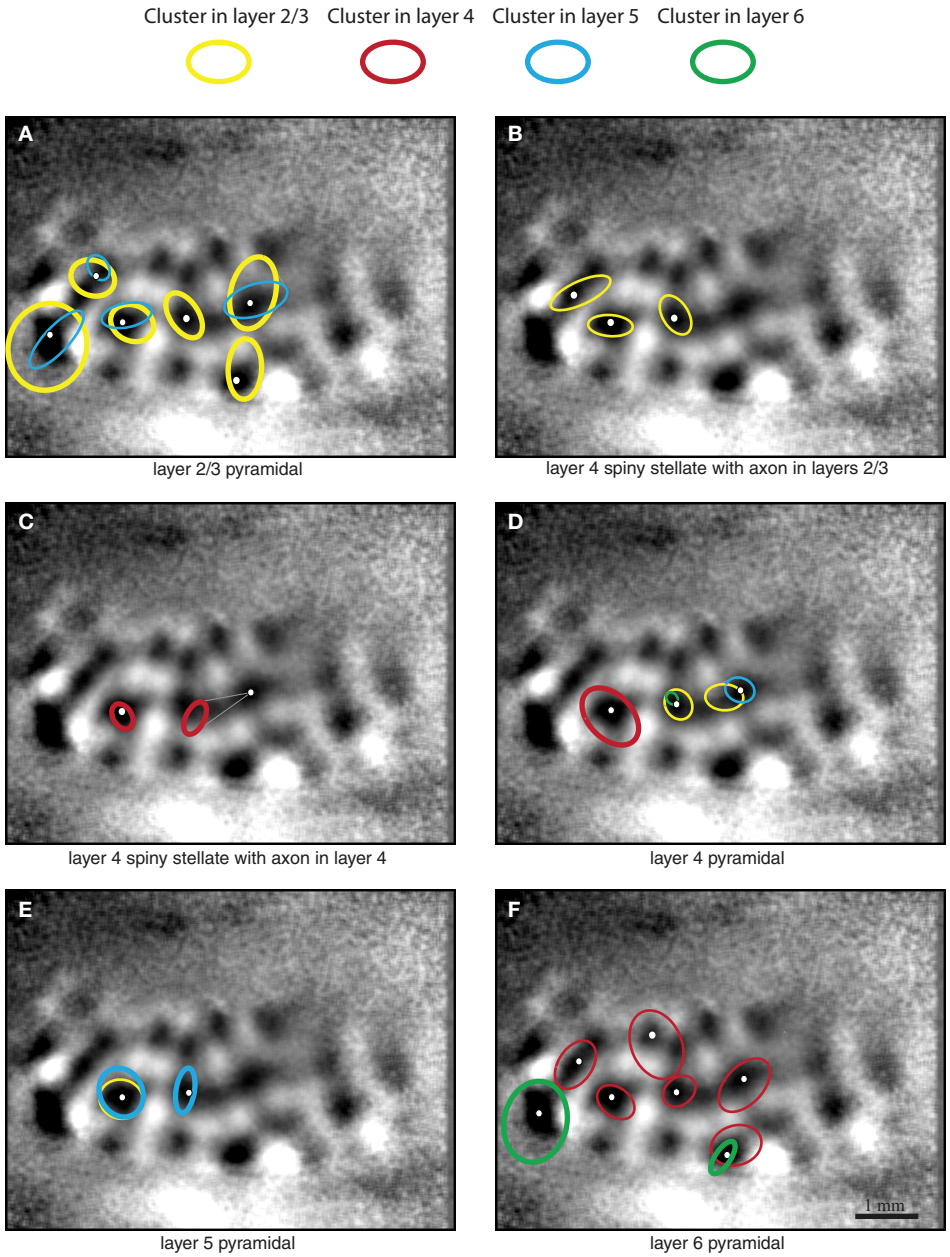

FIGURE 2 | Comparison of the size of the proximal cluster of boutons and functional domains for a single orientation recorded with optical imaging. Proximal clusters formed by neurons of layer 2, 3 and 6 are often larger than the orientation domains. Also apparent is the fact that the size of the proximal clusters varies between different neuronal types. **(A–F)** Show proximal clusters of different neurons (the cell bodies are shown as white dots) from a single cell type. The clusters are color-coded according to the layer in which they are located. In **(C)** one of the spiny stellates does not have any proximal cluster, and we show the closest cluster to the cell body. The optical imaging map was obtained by dividing the response to the preferred orientation by the sum response of all orientations (cocktail blank). The neurons had receptive fields that lay within 14° of the fovea. Clusters taken from Binzegger et al. (2007).

neurons of other layers, especially in the pyramidal neurons of layer 2/3 and 6 (**Figures 2A,F**), whose proximal clusters spread beyond the region of active cortex.

In **Figure 3** we show a schematic representation of a typical dendritic spread (white circles on the left) together with the smallest and largest diameters of the proximal cluster of layer 2/3 pyramidal neurons (black ellipse). We overlap the schematic of the arbors with a functional map of orientation. The overlap of structure and function indicates that the proximal clusters of layer 2/3 pyramidal neurons (and also some layer 6 pyramidal neurons) form synapses with neurons that lie in domains of the orientation map that have orthogonal orientation preferences to the domain that contains the cell body. Thus, from a simple consideration of the dimensions of the axonal clusters and the

functional orientation patches, the situation outlined by Hubel and Wiesel is at least as bad as they imagined. The situation worsens when we consider singularities in the orientation map where the hypercolumn is effectively rotating around a point and regions with different orientation preferences are in very close proximity. Because of their appearance in false color images, these are called 'pinwheels' (Bonhoeffer and Grinvald, 1991; Maldonado et al., 1997; Ohki et al., 2006).

DAISY FIELDS FOREVER

'There is of course no reason why an orientation column should not have rich connections with another column of identical field orientation even though the two might be separated by as many as 15–18 different columns. Indeed, if eye preference columns are interconnected, and if one eye preference column does contain many orientation columns, then the interconnections must be highly specific, one orientation column being connected to another some distance away' (Hubel and Wiesel, 1968). This prediction was vindicated by the experiments of Rockland and Lund, who made bulk injections of tracers into the shrew and primate cortex (Rockland and Lund, 1982, 1983; Rockland et al., 1982). They discovered patchy labeled around the periphery of the injection. This patchy connectivity was subsequently found in many cortical regions (Rockland et al., 1982; Luhmann et al., 1986; Burkhalter and Bernardo, 1989; Kisvarday and Eysel, 1992; Yoshioka et al., 1992; Lund et al., 1993; Levitt et al., 1994; Fujita and Fujita, 1996; Pucak et al., 1996; Kisvarday et al., 1997; Tanigawa et al., 2005). Intracellular studies confirmed that lateral axonal projections of cortical neurons and cortical afferents are patchy (Gilbert and Wiesel, 1979, 1983; Martin and Whitteridge, 1984a). This clustering is especially prominent for the thalamic afferents and pyramidal cells, but is also true of the smooth neurons.

In their quantitative analysis of the distribution of clusters, Binzegger et al. (2007) discovered that the number of boutons in a cluster is exponentially related to the number of clusters the individual neurons forms. The largest cluster in terms of number of boutons is almost always the proximal cluster. From simply knowing the total number of boutons and the number of clusters, one can predict how many boutons are in the proximal cluster and successive clusters. Regardless of the number of clusters, however, between 30% and 90% of the boutons formed by a superficial layer pyramidal are in the proximal cluster. In a related, but more procrustean analysis, Stepanyants et al. (2009) estimated that 92% of the boutons that lie within a minicolumn originate from cells located more than 100 μm away. Thus, as was evident even from the early intracellular labeling studies (Gilbert and Wiesel, 1979, 1983; Martin and Whitteridge, 1984a), Hubel and Wiesel (1968) had been mistaken to suppose that neurons within a 30-μm column are much more strongly connected than the connections between these cortical columns.

Outside the proximal cluster, the remaining boutons formed by a superficial layer pyramidal cell are found in layer 5 and in the distal clusters in the superficial layers, where their collaterals form a structure known as the cortical 'Daisy' (Douglas and Martin, 2004, 2007). The cortical Daisy is not found in rodents, but appears to be ubiquitous in all cortical areas in other species. It has one interesting property relevant to the discussion, which is that it scales in an interesting, species independent way across cortex. The diameter of the distal clusters (the 'petals' of the Daisy), which are formed by the convergence of the axons of many pyramidal cells, is proportional to the distance between the clusters (Douglas and Martin, 2004; Binzegger et al., 2007) (**Figure 4**). In the Macaque monkey, where the Daisy system has been most intensively studied, the dimensions of the Daisy increase from the occipital cortex to the prefrontal cortex. In the visual cortex, Hubel and Wiesel's intuition that lateral projections connect like-to-like seems to be borne out in the Daisy (Livingstone and Hubel, 1984; Malach et al., 1993; Bosking et al., 1997; Kisvarday et al., 1997), but direct correlations of functional maps with the Daisy structure have not been done for any other areas, because the relevant functional properties are unknown.

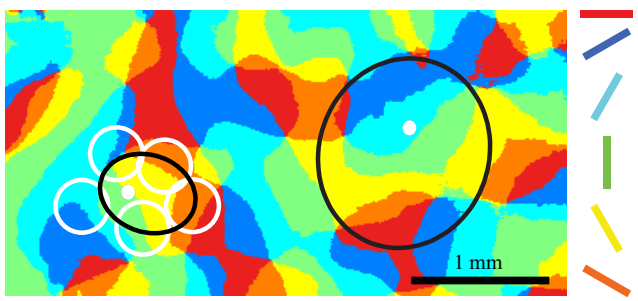

FIGURE 3 | Spread of proximal boutons over multiple orientation domains. The proximal clusters of neurons in layer 2, 3 and 6 can overlap with dendrites of functional domains representing orthogonal orientations. Proximal cluster of two layer 2/3 pyramidal neurons (black ellipse, the cell body is shown as a white dot) superimposed on an orientation map of area 17. Each region of area 17 is color-coded for its preferred orientation. The white circles surrounding the left cluster represent the coverage of a typical dendritic arbor.

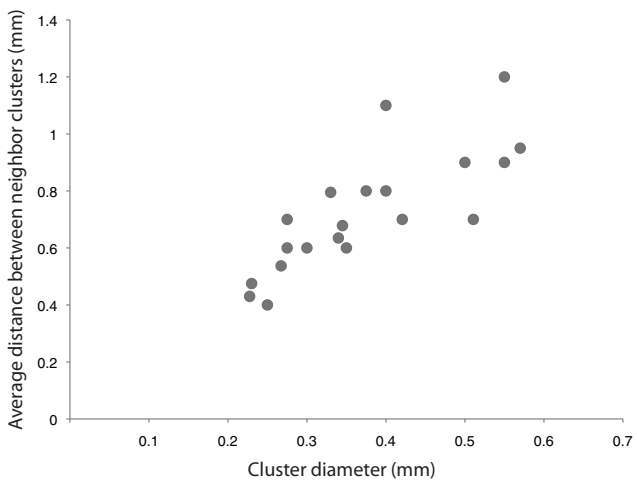

FIGURE 4 | The diameter of the distal bouton clusters, scales with the distance between the clusters (adapted from Binzegger et al., 2007). Average measurement taken from various cortical areas and species (Rockland et al., 1982; Luhmann et al., 1986; Burkhalter and Bernardo, 1989; Kisvarday and Eysel, 1992; Yoshioka et al., 1992; Lund et al., 1993; Levitt et al., 1994; Fujita and Fujita, 1996; Kisvarday et al., 1997).

FUNCTION STRUCTURE = ?

The data presented above indicates that Hubel and Wiesel's puzzle, with which we began Part II, is real and remains unsolved. Given this mismatch between the size of individual neurons and the regularity of the orientation map, how is it that we find well-tuned oriented cells in the superficial layers? In layer 4 we can always assume as many have (Hubel and Wiesel, 1962; Reid and Alonso, 1995; Ferster et al., 1996; Chung and Ferster, 1998) that the orientation selectivity is determined by the thalamic input. This is not so for the superficial and deep layers. We know that pyramidal cells are excitatory and that the major connections made by pyramidal cells are with each other. This is particularly relevant to the pyramidal cells of the superficial layers, where we estimate that most of the excitatory synapses a superficial pyramidal cells forms are with other superficial layer pyramidal cells (Binzegger et al., 2004). Thus, the envelope of excitatory input that any one superficial layer pyramidal cells receives must strongly reflect the axonal spread of the superficial layer neurons.

PENELOPE'S TAPESTRY

THICKETS OF 'MINICOLUMNS'

In the cat (as well as in primate and rodent) the apical dendrites of pyramidal cells form bundles that extend radially through the cortex. These have been called 'minicolumns' as they are clear anatomical evidence for columnar organization. The question is whether they bear any relation to the radial columns seen functionally (Peters and Yilmaz, 1993; see review by Rockland and Ichinohe, 2004). Although Mountcastle (Mountcastle, 1957, 2003; Powell and Mountcastle, 1959a) was convinced that 'minicolumns' were the basis of his functional columns Rockland and Ichinohe (2004) have discussed in some depth why these dendritic bundles do not reflect the functional columns. Moreover, while it is true that apical dendrites are radially aligned, the basal dendrites and axons of cortical pyramidal cells spread laterally over a distance of many minicolumns. This structural organization of the cortical wiring predicts abundant recurrence between different dendritic bundles. An *in vitro* study in the mice somatosensory cortex indicated that neurons within one bundle are as likely to be connected as neurons between adjacent bundles (Krieger et al., 2007).

The concept of the minicolumn highlights again the fundamental discrepancy between structure and function. For Hubel and Wiesel, the column was the structural means whereby the cortex could 'digest' the information arising from each small region of the visual field. This phagous process required that the relevant connections were made vertically between the thousands of neurons who shared receptive field locations and other aspects of receptive field specificity, and which could be connected serially to create the simple and complex receptive fields. It is worth noting that the 'jitter' in the visual receptive field positions along any radial column does not seem to be accompanied by a comparable jitter in the orientation preference (Hubel and Wiesel, 1962, 1974b). This is a paradox if one reflects that the standard feedforward model of orientation selectivity, and indeed the ON and OFF subfield organization of simple cells, requires a very high degree of retinotopic precision and that this precision needs to be propagated in the whole orientation column.

It is ironical that Mountcastle identified Lorente de Nó work as the origin of the concept of the cortical column, when recent evidence indicates that nothing like our textbook view of cortical columns is found in the mouse. In the rodent visual cortex the lack of columns, or indeed any apparent regularity in the map of orientation, is striking when compared to precision in the maps of orientation in carnivores, ungulates and primates (Hubel and Wiesel, 1962, 1963, 1968; Clarke and Whitteridge, 1976; Clarke et al., 1976; Girman et al., 1999; Ohki et al., 2005). The closest approximation to the cortical column is the somatosensory cortex of the mouse and other rodents, where the somatotopic representation of the whiskers is mapped in discrete patches, at least in layer 4. These were the patches that Lorente de Nó described in his study of the mouse cortex (Lorente de Nó, 1922). But these whisker representations are the equivalent to the map of visual space in the visual cortex and not at all equivalent to the segregated receptor specific 'columns' seen by Mountcastle in the somatosensory cortex of cat and monkey. Nor are they similar to the emergent properties of orientation or binocularity, arranged in swirling slabs, as seen in the cat and monkey visual cortex by Hubel and Wiesel. Indeed, for Hubel and Wiesel, 'Whether they (the layer 4 whisker patches) should be considered columns seems a matter of taste and semantics' (Hubel and Wiesel, 1974a).

NEURAL ECONOMIES

In the visual cortex of whisking rodents, single unit recording provided no indication of columns, orientation or otherwise (Girman et al., 1999), although dendritic bundles are present (Peters and Kara, 1987). The imaging with calcium indicators confirmed the single unit results in showing an apparently random, column-less distribution of orientation preferences (Ohki et al., 2005), so that in the false color representations it looked like a spilled box of Smarties ("M 'n M's" in the USA). In appearance this is quite unlike the equivalent representation of candy stripes and colored pinwheels of the orientation maps in tree shrew, cat, ferret and monkey. Koulakov and Chklovskii (2001) suggested that different patterns of orientation columns reflect the operation of a wire minimization constraint in the lateral connections. Interestingly, Hubel and Wiesel (1962, 1974a) had previously introduced this constraint of 'economy of wiring', as an organizing principle for a regular map of orientation. However, the rodent arrangement of spilled Smarties provides efficient wiring only under the constraint that every location has a random mix of neurons of all orientation preferences and that each neuron is required to connect equally to neurons of all orientation preferences. If this latter constraint is relaxed and neurons are allowed to connect more often to other neurons of like preference, then the pattern formed is more like the candy stripes of the ice cube model. The pinwheel/candy stripe patterns arise when both constraints exist and compete – connect to all versus connect only to like.

However, it may be that the problem of explaining the apparent disorder of the rodent orientation system is little different from that of explaining the emergence of a highly ordered orientation maps in the cat, sheep, tree shrew, and monkey. Both systems seem to require the notion of physiological discreteness, whether it be of individual cells, 'minicolumns', ice cube slabs, or pinwheels. For example, Hubel and Wiesel (1962, 1963, 1968, 1974a) were impressed by the abrupt discontinuities they occasionally discovered in tangential penetrations, which they felt was one strong argument for discreteness. Yet, from mouse to monkey visual cortex, the orientation selectivity of individual neurons cannot be accounted for by any

evident structural patterning of the dendritic arbor (Martin and Whitteridge, 1984b; Anderson et al., 1999), neither has such discrete patterning has been described for the proximal regions of the axon. Nor is it helpful to appeal to some hidden selectivity of connections that ensures that only like connects to like, since this is excluded by the spill over of the proximal axon cluster into unlike territory (as indicated above and in previous studies, Kisvarday et al., 1997; Yousef et al., 2001). The intracellular studies also show that like can be synaptically connected to unlike, yet still be well tuned for orientation (Schummers et al., 2002; Monier et al., 2003).

CONJECTURES AND REPRESENTATIONS

One route to understanding this complexity of circuitry is to remind ourselves that each cortical neuron represents not just a receptive field position and an orientation, but is multifunctional. Each neuron represents an array of different functional attributes. 'Compared with cells in the retina of lateral geniculate body, cortical cells show a marked increase in the number of stimulus parameters that must be specified in order to influence their firing' (Hubel and Wiesel, 1962). This combinatorial property, which was so apparent in the early single unit recordings, is also clearly evident in population recordings. This combinatorial power of the receptive field is revealed in the studies of DeAngelis (1999) and Yen (2007) who confirmed and extended Hubel and Wiesel's observations that neighboring neurons may share some receptive field properties, but have other properties that very different. Thus they may share orientation and ocular dominance, but differ in the substructure of their receptive fields, or direction preference, or strength of binocular disparity tuning.

Another example is that of Basole et al. (2003) who used electrophysiological and optical recordings of ferret area 17 to show that the same neuronal population could respond to multiple combinations of orientation, length, motion axis and speed. The tuning to each of the stimulus properties was dependent on the others, and the lateral clusters formed by the axons of superficial layer pyramidal cells is one means by which stimulus features from different orientations, directions, etc., are combined within the same region of the visual field. Detailed modeling would be very helpful here to clarify the constraints on the wiring. This view on the responses of cortical neurons might solve the riddle of the elusive and illusive anatomical column, since the location of the columnar response to a particular stimulus feature is not fixed in the cortical sheet, one should not expect either to find anatomical boundaries of the column.

For 50 years, the neocortical column has been our model for the computational unit of the cortex. One very important implication of the columnar model is that the small computational unit is repeated throughout the visual cortex. In moving away from this rather static image of the functional architecture to the idea of repeated canonical circuits, it is not a great leap of the imagination to suppose that all of cortex carries a similar computation on its inputs, whether it be for perception, or more complex cognitive judgements (Barlow, 1980).

With this in mind we have developed the concept of a 'canonical circuit' for cortex, which embodies the idea of a repeated local circuit that performs some fundamental computations that are common to all areas of neocortex (Douglas et al., 1989; Douglas and Martin, 1991). The canonical circuit (**Figure 5**) is firmly based on an analysis of the statistics of the connections between the different types of cortical cells and their physiology. The vast majority of these connections are intracortical, either interlaminar or within the same lamina. However, the canonical microcircuit is not a 'module', nor does it specify a particular dimension, or number of neurons. Instead it captures some of essential attributes of the rules that govern the connections between different cell types that permit the multiple functions of cortical circuits such as recurrent excitation and inhibition, the amplification of weak inputs from thalamus or other cortical areas, and the balance of excitation and inhibition. How these attributes are employed and deployed, depends of course on the demands of a specific cortical area. An example of the implementation of the idea of the canonical circuit to other cortical regions, is the work of Heinzle et al. (2007) who used the canonical circuit derived from cat visual cortex to successfully model the function of the primate frontal eye field.

'AUTOPOIETIC' CIRCUITS

The dynamical properties of such recurrent networks generate interesting behaviors, when we consider that the cortical circuit is not a static entity, but is a transient entity formed by the subset of currently active neurons (e.g. Binzegger et al., 2009; Haeusler et al., 2009). Neurons that are below spike threshold are transiently disconnected from the circuit, so through activity the circuit changes its network architecture dynamically. In this sense the circuits are autopoietic: creating themselves by their own interactions and by the transformations of the representations embedded in their connections. An example is the emergence of orientation selectivity from the non-oriented precursors in the thalamus.

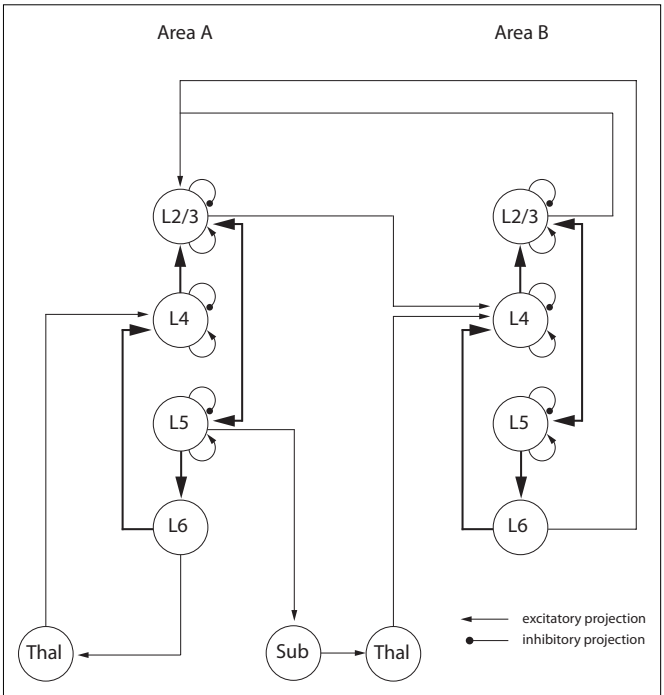

FIGURE 5 | Representation of the major connections in the canonical microcircuit (adapted from Douglas et al., 1989; Douglas and Martin, 1991, 2004). Excitatory connections are represented by arrows and inhibitory ones as lines with round ends. Neurons from different cortical layers or brain structures are represented as circles. 'L*x*' designates the cortical layer where the cell body is located, 'Thal' designates the thalamus and 'Sub' designates other subcortical structures.

Within the canonical cortical circuit, the inhibitory threshold depends on the overall network activity. In the example of orientation selectivity, this inhibitory threshold ensures that only features of the input that match patterns embedded in the weights of the cortical excitatory connections are amplified by the recurrent circuits. Weakly-active neurons are suppressed due to action of the inhibitory network. Thus, the cortical network actively imposes an interpretation on an incomplete or noisy input signal. Different patterns of inputs drive the network towards different fundamental distributions of activity that reflects different aspects of the map of its excitatory connections. It is this dynamic aspect of cortical function that is inherent in the canonical circuit and offers a core circuit that can be replicated throughout neocortex.

Coda

How fortunate is it for us that Mountcastle and Hubel and Wiesel did not begin their seminal single unit studies in the rodent cortex! Any counterfactual history will indicate the significance of the loss that would have been incurred by cortical studies if they had not created a conceptual framework centered on the concept of the cortical column. Through their own studies on cat and monkey cortex they revealed a rich world of cortical structure and function – the 'functional architecture' of the cortex. Within this framework, studies of the development and plasticity of cortical columns flourished. Studies of cortical plasticity due to altered rearing provided crucial evidence that there were critical periods during development. The ocular dominance system, which is strongly plastic, and the orientation system, which is not, have both played major roles in understanding the role of visual experience in the maturation of the sensory cortex. It is difficult to see how the enormous expansion of cortical neuroscience would have occurred without their lead and example. Even now a new generation of muscular youth are applying their approach to probe the cortex of *Mus musculus*, trying to answer the same questions, exchanging optical and genetic methods for the gold-standards of tract-tracing and electrophysiology. Without this paradigm for studying the cortex, and without the central concept of the cortical column, much of the most influential work on neocortex in many different species over the past 50 years simply could not have happened. The column hypothesis has greatly enriched our understanding of the neocortex by providing a coherent description of the functional architecture of the cortex. However, the evident complexity of the structure and function of the component neurons, extracellular matrix, and glia that form the cortical circuits requires a comparable complexity of concepts. This is our Grand Challenge for the 21st century.

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A cortical sparse distributed coding model linking mini- and macrocolumn-scale functionality

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No *generic* function for the minicolumn – i.e., one that would apply equally well to all cortical areas and species – has yet been proposed. I propose that the minicolumn does have a generic functionality, which only becomes clear when seen in the context of the function of the higher-level, subsuming unit, the macrocolumn. I propose that: (a) a macrocolumn's function is to store sparse distributed representations of its inputs and to be a recognizer of those inputs; and (b) the generic function of the minicolumn is to enforce macrocolumnar code sparseness. The minicolumn, defined here as a physically localized pool of ~20 L2/3 pyramidals, does this by acting as a winner-take-all (WTA) *competitive module*, implying that macrocolumnar codes consist of ~70 active L2/3 cells, assuming ~70 minicolumns per macrocolumn. I describe an algorithm for activating these codes during both learning and retrievals, which causes more similar inputs to map to more highly intersecting codes, a property which yields ultra-fast (immediate, first-shot) storage and retrieval. The algorithm achieves this by adding an amount of randomness (noise) into the code selection process, which is inversely proportional to an input's familiarity. I propose a possible mapping of the algorithm onto cortical circuitry, and adduce evidence for a neuromodulatory implementation of this familiarity-contingent noise mechanism. The model is distinguished from other recent columnar cortical circuit models in proposing a generic minicolumnar function in which a group of cells *within* the minicolumn, the L2/3 pyramidals, compete (WTA) to be part of the sparse distributed macrocolumnar code.

Keywords: sparse distributed representations, minicolumn, macrocolumn, novelty detection, population coding, learning, memory, winner-take-all

INTRODUCTION

The columnar organization of neocortex at the minicolumnar (20–50 μm) and macrocolumnar (300–600 μm) scales has long been known (see Mountcastle, 1997; Horton and Adams, 2005 for reviews). Minicolumn-scale organization has been demonstrated on several anatomical bases (Lorente de No, 1938; DeFelipe et al., 1990; Peters and Sethares, 1996). There has been substantial debate as to whether this highly regular minicolumn-scale structure has some accompanying generic dynamics or functionality. See Horton and Adams (2005) for a review of the debate. However, thus far no such *generic* function for the minicolumn – i.e., one that would apply equally well to all cortical areas and species – has been determined.

One basis upon which a functionality for the minicolumn has been suggested is possession of highly similar receptive field characteristics, or tuning, by the cells comprising the minicolumn, e.g., V1 orientation columns (Hubel and Wiesel, 1962, 1968) and minicolumn-sized regions innervating cutaneous zones (Favorov and Diamond, 1990). The reasoning here appears to be that because a group of cells all have very similar tuning to a particular feature, α, e.g., an edge at a particular orientation, they form a unit whose function is to recognize α. However, in searching for a possible generic minicolumn function, we need not limit ourselves to considering only recognition functions. Furthermore, possession of highly similar tuning cannot be a basis for a *generic* minicolumn functionality since in many cortical areas, the cells encountered along vertical penetrations can have quite variable tuning (cf. Sato et al., 2007, 2008). On closer analysis, this is true in orientation column data as well (Hetherington and Swindale, 1999; Ringach et al., 2002). If there is a generic minicolumn functionality, then it must be compatible with varying degrees of tuning correlation amongst the minicolumn's cells.

I propose that the minicolumn does have a generic functionality (given shortly), but one that only becomes clear when seen in the context of the function of the higher-level, subsuming unit, namely, the macrocolumn, which has been demonstrated anatomically (Goldman and Nauta, 1977; Lübke et al., 2003; Egger et al., 2008) and functionally (Mountcastle, 1957; Woolsey and Van der Loos, 1970; Hubel and Wiesel, 1974; Albright et al., 1984). I propose that the function of a macrocolumn (e.g., hypercolumn, segregate, barrel) is to store *sparse distributed representations* of its *overall* input patterns, and to act as a recognizer of those patterns. By "overall input" pattern, I mean the macrocolumn's overall input at a given moment, including not only its bottom-up (BU) inputs from thalamus or lower cortical areas, but also its top-down (TD) inputs from higher cortical areas and its horizontal (H) inputs, which I propose carry temporal (sequential) context information (recurrently) from the representations previously active in the same and nearby macrocolumns. Thus, an "overall input pattern" can equally well be termed, a "context-dependent input". Thus, it is in fact the macrocolumn whose generic functionality is appropriately characterized as recognition; i.e., recognition of a class determined by the history of context-dependent inputs to which it has been exposed.

A distributed representation of an item of information is one in which multiple units collectively represent that item, and crucially, such that each of those units generally participates in the representations of other items as well. Distributed representations are also referred to as cell assemblies, population codes, or ensembles. In this paper, "representation" and "code" will be used interchangeably. A *sparse* distributed code (SDC) is one in which only a small fraction of the pool of available representing units is part of any particular code (Palm, 1982; Lynch et al., 1986; Kanerva, 1988).

If the macrocolumn stores SDCs, then there must be some mechanism that enforces sparseness and this, I propose, is the generic function of the minicolumn. Specifically, I propose that small, physically localized pools of L2/3 pyramidals, e.g., ~20 such cells, act as winner-take-all (WTA) *competitive modules* (CMs). This implies that macrocolumnar codes should consist of about 60–80 active L2/3 cells, one per minicolumn: for simplicity, assume 70 minicolumns per macrocolumn hereafter. Defined in this way, the minicolumn has a more flexible relation to the ontogenetic column, the apical dendrite bundle, the DBC horsetail, etc. For example, a given minicolumn might include L2/3 pyramidals from more than one apical dendrite bundle, consistent with the findings of Yoshimura and Callaway (2005) of fine-scale networks of preferentially interconnected L2/3 pyramidals.

There is increasing evidence for the use of SDC in the cortex and other brain structures; e.g., auditory cortex (Hromdka et al., 2008), visual areas (Young and Yamane, 1992; Vinje and Gallant, 2000; Waydo et al., 2006; Quian Quiroga et al., 2008), zebra finch neopallium (Hahnloser et al., 2002), olfactory structures (Jortner et al., 2007; Linster and Cleland, 2009; Poo and Isaacson, 2009), and hippocampus (Leutgeb et al., 2007). Particularly supportive of the proposed hypothesis is the Reid Lab's calcium imaging data of rat V1 during stimulation by drifting square-wave gratings (Ohki et al., 2005). Their movie (http://reid.med.harvard.edu/movies.html) shows sparse collections of L2/3 cells extending over an approximately macrocolumn-sized region synchronously turning on and off in response to particular grating orientations. **Figures 1A,B** (two frames from the movie) show distinct sets of cells, i.e., codes, responding to bars moving left and right, respectively, and emphasize that individual units may participate in multiple codes (red-circled cells). In terms of the proposed model, the active neurons would be the winners in their respective minicolumns, as suggested in **Figures 1C,D**. **Figure S1** in Supplementary Material provides another view of how the proposed model maps onto cortex.

If the macrocolumn does indeed function as a SDC field in the way suggested here, then we must answer two key questions regarding its dynamics.

1. How is any particular set of winners, one in each of the 70 minicolumns, initially chosen in response to an input pattern and bound into a holistic code? That is, how are macrocolumnar codes *learned*?
2. How is a previously learned code reactivated in response to future presentations of the input pattern that it was initially chosen to represent? That is, how are stored macrocolumnar codes *retrieved* (reactivated)?

In the next section, I describe an algorithm, referred to as the *code selection algorithm* (CSA), which answers both questions. A key property of the CSA is that it causes similar inputs to be assigned to similar, i.e., more highly intersecting, codes. This property, which will be referred to as SISC (similar inputs map to similar codes), is very important in terms of computational efficiency (see Discussion) and is possessed by most distributed coding schemes. However, the CSA achieves it in a novel, probabilistic fashion, which can be summarized as follows:

1. Determine the *familiarity* of a macrocolumn's input. To a first approximation, an input's familiarity is its maximum similarity to any input previously stored in the macrocolumn.
2. Set the amount of randomness (noise) in the process of selecting winners in the WTA modules in inverse proportion to the input's familiarity.
3. Select the winners.

The algorithm's rationale is described in detail in the next section, but broadly, the idea is as follows. When an input, α_1, is familiar, we want to reactivate the code, β_1, to which α_1 was

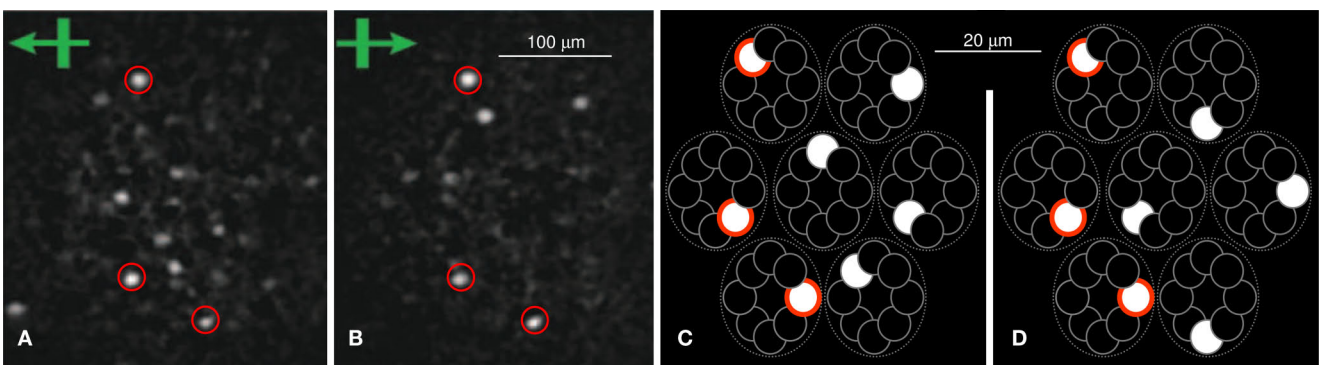

FIGURE 1 | Calcium (tangential) images of L2/3 of rat visual cortex showing sparse sets of cells activating in unison (see movie link in text) in response to leftward (A) and rightward (B) drifting gratings. From Ohki et al. (2005). Red circles highlight some cells common to both codes. **(C,D)** Sketch of proposed sparse distributed coding concept, which could plausibly give rise to data like **(A)** and **(B)**. Note different scales. Red borders emphasize intersections between codes. N.b.: To make the sketches look more like the calcium images, black is used for inactive units and white for active: this is reversed in subsequent figures.

previously assigned. The cells comprising β_1 will have had their synapses (from the cells comprising α_1) increased during learning. Thus, if α_1 is presented again, the cells of β_1 will have the highest synaptic input summations in their respective WTA modules. In this case, winners should be chosen on the *deterministic* basis of these summations: no noise should be present in the winner selection process. On the other hand, increasingly novel inputs should be assigned to increasingly distinct codes, i.e., codes having progressively smaller intersection with existing codes. This can be achieved by increasing the noisiness of the winner selection process in each WTA module, which can be achieved by suppressing the influence of the deterministic synaptic inputs (which reflect prior learning) relative to baseline random (spontaneous) activity. By adjusting parameters that control the global (i.e., across the whole macrocolumn) noise level, we can modulate the expected intersection between the set of cells which have the maximal input summations in their respective WTA modules and the set of winners that are actually chosen, thus implementing SISC.

Many experimental and theoretical studies implicate neuromodulators, notably norepinephrine (NE) and acetylcholine (ACh), in functionality similar to the above, which can be described generally as modulating signal-to-noise ratio (SNR). Doya (2002) proposed that NE levels control the amount of noise in a process of choosing output actions. However, Doya's model assumes a *localist* representation of the choices, which precludes possession of the SISC property (see Discussion). In addition, increased ACh has been shown to selectively increase the strength of afferent relative to intrinsic inputs in piriform cortex (Hasselmo and Bower, 1992) and other brain structures (see Hasselmo, 2006 for review). These ACh findings have been summarized as showing that increased ACh adjusts network dynamics to favor encoding new memories compared to retrieving old memories, which fits well with the proposed CSA functionality. Following the model description, I offer a speculative mapping of my proposed model onto neural circuitry and discuss evidence for novelty-contingent noise modulation by both NE and ACh. However, the specifics of this mechanism are a subject of ongoing research and likely will involve interactions between neuromodulatory systems (cf. Briand et al., 2007).

Any discussion of columnar function of course centrally concerns cortical circuitry, and more specifically, the putative canonical cortical microcircuit (Rockland and Pandya, 1979; Douglas et al., 1989; Douglas and Martin, 1991). I therefore want to finish the Introduction with the following point. We have made huge progress in understanding many of the components of cortical microcircuitry – a tiny sample of which includes (DeFelipe et al., 1990; Larkum et al., 2001; Beierlein et al., 2003; Schubert et al., 2003; Zhu et al., 2004; Feldmeyer et al., 2006; Fukuda et al., 2006; Krieger et al., 2007; Egger et al., 2008; Hirata and Castro-Alamancos, 2008; Berger et al., 2009; Murayama et al., 2009; Symes and Wennekers, 2009; Briggs, 2010; and see Thomson et al., 2002; Bannister, 2005; Silberberg et al., 2005 for reviews). Nevertheless, we remain far from any sort of comprehensive and consensual understanding of how cortical columnar circuitry manipulates, i.e., stores and retrieves, *specific* items of information. In the main, only very broad conclusions regarding information processing are asserted in the experimental literature, e.g., horizontal connections between fast-spiking L4 interneurons and pyramidals are involved in formation of L4 assemblies and sharpening of tuning (Sun et al., 2006); that both the populations of L4 pyramidals and of L5A pyramidals have transcolumnar connectivity patterns allowing them to act as integrators of information coming in from multiple vibrissae in parallel, or in close sequence (Schubert et al., 2007); that the receptive fields of barrel-related L2/3 pyramids are dynamic and thus may reflect learning to recognize spatiotemporal patterns of vibrissae deflections (Brecht et al., 2003); that WTA competition occurs in the supra- and infragranular layers (Douglas and Martin, 2004); and that local (~100 μm) L2/3-to-L2/3 connections might serve to synchronize firing of L2/3 cell assemblies (Lübke and Feldmeyer, 2007); etc. I believe the hypothetical model described herein to be a significant contribution because it goes beyond broad conclusions and offers a mechanistic explanation of how *specific* informational items are learned and retrieved and in so doing, proposes a generic function for the minicolumn, i.e., that it functions as a WTA module in support of manipulating SDCs at the next higher, i.e., macrocolumnar, scale.

RESULTS: MODEL DESCRIPTION

Figure 2 shows the functional architecture of a *simplified* version of the model. In particular, it was stated in the Introduction that sparse macrocolumnar codes are chosen in response to a macrocolumn's *overall* input, which includes its BU, H, and TD inputs. However, illustrations of the model in operation in that general case become rather complex, particularly since the H (and TD) weights carry temporal information, which necessitates showing the model at multiple successive time steps while processing spatiotemporal patterns. More importantly, the core elements of the hypothesis – which are: (a) that the macrocolumn stores SDCs consisting of one winning L2/3 cell per minicolumn; and (b) that the SISC property is achieved by modulating the amount of randomness (noise) present in the winner selection process in inverse proportion to input familiarity – can be clearly and more simply described for the BU-only case (i.e., where inputs are purely spatial patterns). Therefore, the model description in this paper will be limited to the BU-only case. However, the generalized model (with BU, H, and TD inputs) and the accompanying generalized version of the CSA are given in **Figures S2 and Table S1** in Supplementary Material.

In **Figure 2**, the input field, F1, is comprised of 12 binary units and can be considered analogous to a specific thalamic nucleus, though topographical organization is not assumed. The coding field, F2, consists of $Q = 4$ WTA CMs, each containing $K = 3$ binary units. Complete (all-to-all) BU connectivity from F1 to F2 is assumed for simplicity. These BU weights (synapses) are binary, initially 0, and are permanently set to a weight of 1 the first time the pre- and postsynaptic units are co-active (i.e., Hebbian learning).

MODEL DYNAMICS: THE CODE SELECTION ALGORITHM

The model's core algorithm, the CSA, determines which cells are chosen to represent an input, during both learning and retrieval. A single iteration of the algorithm involves two rounds of competition in the CMs of F2. The first round is a *hard WTA* competition (represented by boxes labeled "Max" in **Figure 2**). The purpose of the first round is to compute a *global familiarity* measure, G, of the input pattern. G then drives a global modulation of the F2 unit

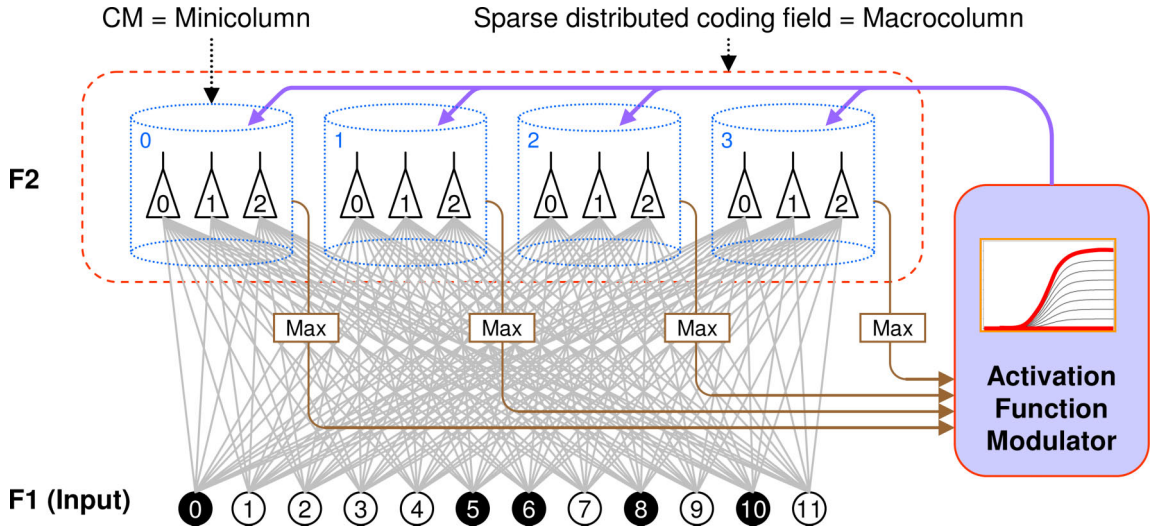

FIGURE 2 | Functional architecture. The input field (F1) consists of binary feature detectors: a particular input consisting of five active features is shown. The coding field, F2, is proposed as a macrocolumn analog. It consists of winner-take-all competitive modules (CMs) proposed as analogous to minicolumns. Each CM has $K = 3$ binary units. Bottom-up connectivity is all-to-all: gray lines signify initially 0 weights. The familiarity (G) of the input is proposed to be computed via a subcortical, neuromodulator-based mechanism, which then modulates the F2 unit activation function parameters (e.g., sigmoid height) contingent on G (purple arrows). See text.

activation function (**Figure 2**: purple arrows) in preparation for the second competitive round, which is a *soft WTA* competition, the intent of which is that:

1. as G goes to 1 (indicating a completely familiar input), the probability that the unit with the highest input summation in a CM wins approaches 1, and
2. as G goes to 0 (indicating a completely novel input), all units in a CM become equally likely to win (regardless of their input summations).

This policy ensures, statistically, the SISC property. The steps of the CSA are as follows.

1. Each F2 unit i computes its raw input summation, $u(i)$.

$$u(i) = \sum_{j \in \alpha_n} w(j,i) \quad (1)$$

where α_n is the current input (F1) pattern. Because unit activations are binary, we can simply sum the weights, $w(j,i)$, which are also binary.

2. Normalize $u(i)$ to [0..1], yielding $V(i)$.

$$V(i) = u(i)/S \quad (2)$$

S is the number of active units in the input pattern. $V(i)$ is a local measure of support, or likelihood, that F2 unit i should be activated. It reflects how well unit i's receptive field (RF), specified by its afferent weight vector, matches the current input vector.

3. (Round 1 competition) The maximum $V(i)$, $\hat{V}_x$, is found in each of the Q CMs.

$$\hat{V}_x = \max_{i \in C_x} \{V(i)\} \quad (3)$$

where x indexes the CMs and i indexes the units in a CM, C_x.

4. Average the Q $\hat{V}_x$ values, yielding G, a *global* measure of the *familiarity* of the current input.

$$G \equiv \sum_{x=1}^{Q} \hat{V}_x / Q \quad (4)$$

5. The *expansivity*, η, of the probabilistic activation function (which is implemented via steps 6–8) is set as an increasing nonlinear function of G (Eq. 5, expressed as a table).

G	0.0	0.2	0.4	0.6	0.8	1.0
η	0	0	0.2	5	12	100

(5)

η corresponds to sigmoid height (in Eq. 6). The idea is to increase the range of *relative win likelihoods*, $\psi(i)$ (defined in step 6) over any given CM's units as G goes to 1. This in turn, serves to nonlinearly exaggerate the difference in the *final win probabilities* (Eq. 7) between F2 units with low and high V values. The specific parameters of any instance of the G-to-η mapping will determine the specifics of the relation between input similarity and code similarity, i.e., the expected code intersection as a function of input similarity. The specific η values in Eq. 5 were chosen to yield the ρ-distributions in the examples of **Figures 3 and 4**.

6. The V values of all units in all CMs are then passed through the sigmoidal activation function (Eq. 6) whose shape/scale reflects G. Again, particular parameter values affect the relation of input similarity to code similarity (and therefore, storage capacity): values of $\lambda = 28$ and $\phi = -5$ produce the V-to-ψ mappings in **Figure 4**. As noted above, within each CM, the output variable, $\psi(i)$, can be viewed as a relative likelihood that unit i should be chosen winner. The ψ-distributions in each CM are normalized to final probabilities in step 7.

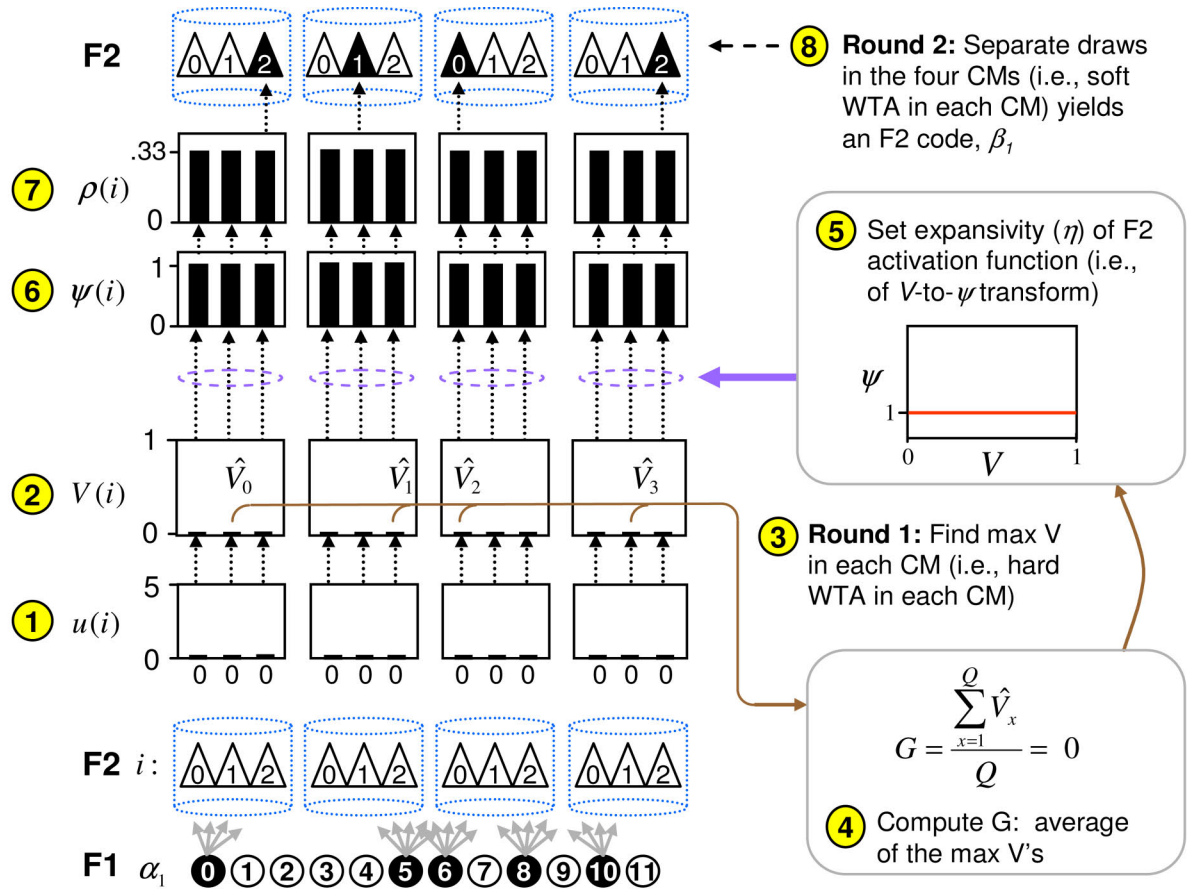

FIGURE 3 | Graphic illustration of the CSA. Circled numbers refer to algorithm steps in text. Here, I show the case of the first input, α_1, presented to the model. The algorithm detects that α_1 is completely unfamiliar, i.e., G is computed to be 0, and sets the F2 activation function to a constant function (red line), which makes the choice of winner in each CM completely random, and thus, the overall F2 code, β_1, also completely random.

$$\psi(i) = \frac{\eta}{1 + e^{-(\lambda V(i) + \phi)}} + 1 \quad (6)$$

When $G = 1$ (perfectly familiar), η is maximized (in Eq. 5), which maximizes relative and total (once normalized, via Eq. 7) probabilities of winning for units with the maximum V value in their respective CMs. In contrast, when $G = 0$ (completely novel), $\eta = 0$, which collapses the sigmoid to the constant function, $\psi = 1$, thus making all units in a CM equally likely to win. This causes the expected intersection of the code being chosen in the current instance with any previously assigned code to be at chance level. In general, this modulation of the sigmoid activation function tends toward *code completion* in proportion to the familiarity of the input and *code separation* in proportion to its novelty.

7. Transform relative likelihood distribution (ψ) in each CM to true probability distribution (ρ).

$$\rho(i) = \frac{\psi(i)}{\sum_{k \in \text{CM}} \psi(k)} \quad (7)$$

8. (Round 2 competition) Choose an F2 code by drawing a winner from the ρ-distribution (soft max) in each CM. Thus, choosing an F2 code is actually performed as Q separate draws. When $G = 0$, these draws are statistically independent, as in **Figures 3 and 4D**. As we consider increasingly familiar inputs, i.e., for G approaching 1 (and, assuming the model is still operating in a regime where crosstalk is sufficiently low), the draws become increasingly correlated (dependent), as can be seen in going from **Figure 4C** to **4B** to **4A**.

Figure 3 graphically illustrates the operation of the CSA in the case of the model being presented with its first input, α_1. The gray arrows indicate that the BU signals propagating from the active F1 units will be traversing connections with zero synaptic strength. This leads to unnormalized (u) and normalized (V) input summations of 0 for all 12 F2 units (steps 1,2). In step 3, the max V, $\hat{V}$, in each CM is found (ties broken at random). In step 4, G is computed as the average of the $\hat{V}$ values: in this case all the $\hat{V}$ are 0, so $G = 0$. In step 5, the value, $G = 0$, maps to $\eta = 0$, which causes the activation function of the F2 units to collapse to the constant function, $\psi = 1$. In step 6, each F2 unit applies this activation function to its V value, yielding the uniform relative likelihood distribution in each CM. In step 7, the relative likelihood function in each CM is normalized to a true probability (ρ) distribution, which in this case, is again uniform. Finally, in step 8, a winner is drawn in each CM, resulting in a random F2 code, e.g., β_1.

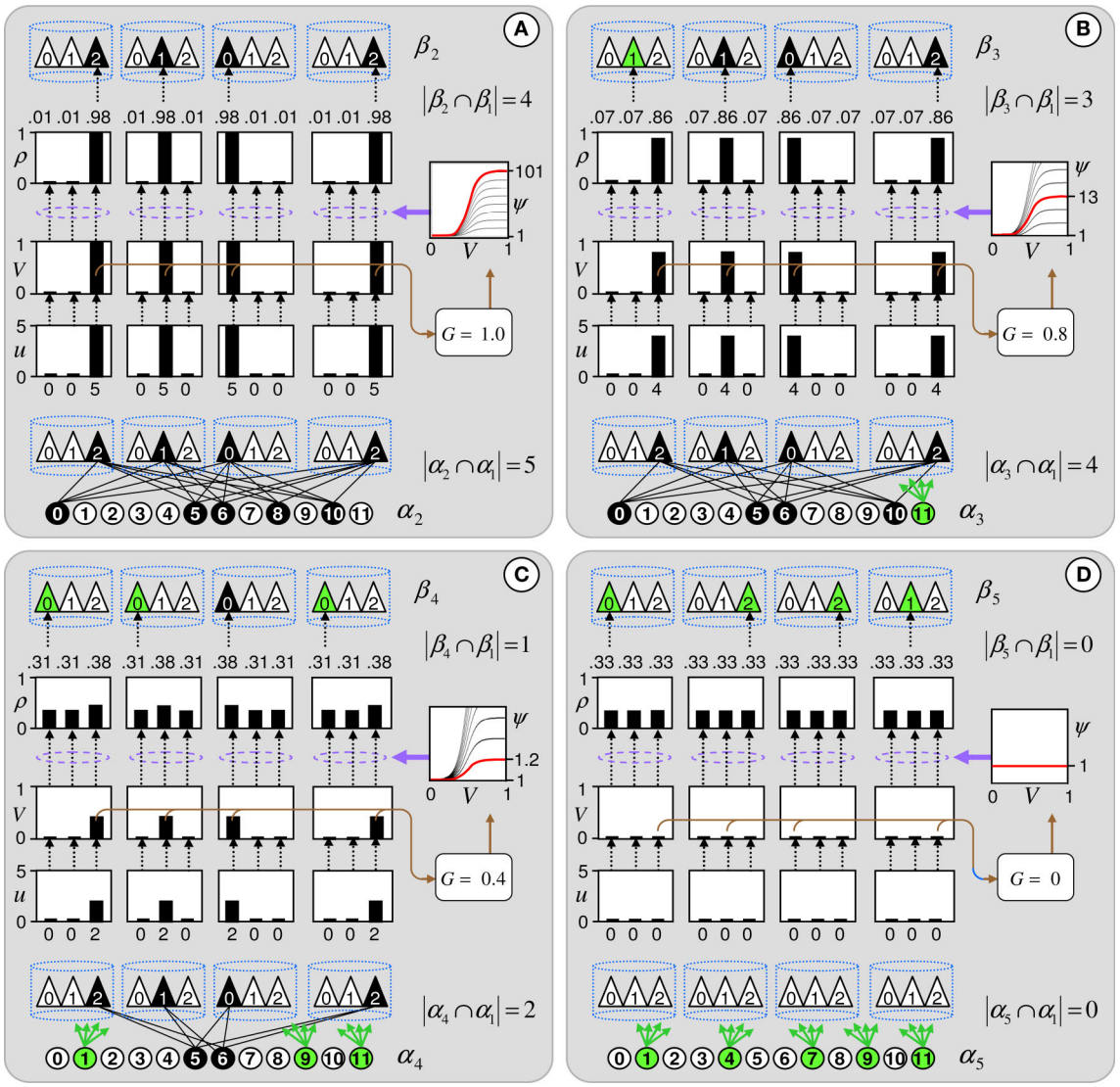

FIGURE 4 |The CSA and the SISC property. Green F1 (F2) units denote units *not* in common with α_1 (β_1). Green arrow sprays represent signals propagating via naïve ($w = 0$) weights. See text.

Figure 4 demonstrates that the CSA realizes the SISC property by considering four possibilities (A–D) for the second input presented to the model of **Figure 3**. These four inputs, α_2–α_5, range from being identical to α_1 (completely familiar) to having zero overlap with α_1 (completely unfamiliar). To save space, the panels of **Figure 4** use an abbreviated version of the format of **Figure 3**. Most noticeably, the intermediate variable, ψ (relative likelihood), is not shown. However, the transform from V through to ρ should still be clear. Black BU connections are ones that were increased to one when α_1 was learned (**Figure 3**). The overall message of **Figure 4** is as follows. Working from **Figure 4A** to **4D**, the inputs have progressively lower similarity (intersection) with α_1: F1 units *not* in common with α_1 are shown in green. As G drops, the sigmoid expansivity drops (note the changing ψ scale). Thus, the ρ-distributions become progressively flatter, which in turn results in F2 codes, β_2–β_5, having progressively smaller intersection with β_1. F2 units *not* in common with β_1 also shown in green.

Figure 4A shows the case of presenting a completely familiar input again, and is thus a recognition test trial, demonstrating retrieval. This leads, via CSA steps 3 and 4, to $G = 1$, which yields, via steps 5 and 6, the expansive nonlinear V-to-ψ mapping shown (red sigmoid). This nonlinearity is applied to every F2 unit, yielding the highly peaked ρ-distributions shown. Finally, one unit is drawn in each CM. The probability of drawing the correct unit in any single CM is approximately 98%. Of course, what's crucial in this case, i.e., when the input is completely familiar ($G = 1$), is that the *entire* correct F2 code is reactivated. In this case, that probability is $(0.98)^4 \approx 92\%$. Thus, the familiarity, G, which depends on the entire F2 layer and is thus *global* information, influences

the *local* activation functions so as to produce the desired overall result, in this case, reactivation of the code (memory trace), β_1, of the familiar input pattern, α_1. The explanations of the remaining panels follow that of **Figures 3 and 4A**. In going from **Figure 4B** to **4D**, one can readily see decreasing intersection with α_1, decreasing *u* and *V* values, decreasing *G*, decreasing sigmoid expansivity, progressively flatter ρ-distributions, and ultimately, decreasing intersection with β_1.

Given a desired probability, *R*, of correctly reactivating an entire code (i.e., of choosing the correct unit in each CM), when $G = 1$, given values for *Q* and *K*, we could compute the needed value of η. However, the macrocolumn model is a content-addressable associative memory and in actual usage, multiple sparse codes will be stored in superposition. Any thorough analysis of the model's expected retrieval error must include the effect of overlap between the stored codes (i.e., cross-talk): this influences the shapes of the ρ-distributions and thus, the expected retrieval accuracy for any given number of stored codes. Such an analysis will be conducted empirically and reported in a separate paper.

Before leaving **Figure 4**, I underscore three important points. First, while the ρ-distributions become flatter as *G* decreases, the units comprising the code of the most similar previously learned input (here, α_1) remain most likely to win in their respective CMs. If we simply *deterministically* chose the unit with maximum $V(i)$ in each CM, we would have chosen the same F2 code, β_1, in response to all four inputs, α_2–α_5. Thus, the computation of a quantity, *G*, which depends on *all* the CMs is essential to achieving the SISC property. It constitutes a channel through which information transfers between *all* F2 units throughout the whole macrocolumn. As noted earlier, the full model also assumes direct "H" connections between F2 units, analogous to the horizontal matrix of L2/3 (see **Figure S2** in Supplementary Material). These also mediate communication, but of the prior code active in the macrocolumn, not of the simultaneous state of all F2 units throughout the macrocolumn.

Second, learning is *single-trial* and involves only one iteration of the CSA. This is largely facilitated by the fact that when a given input-code association, α_j–β_j, is learned, each of β_j's F2 units simultaneously has its afferent weight from *all* of α_j's F1 units increased. The effect of these simultaneous correlated potentiations allows a rapid, even single-trial, formation of an association, even if the individual synaptic potentiations are small, consistent with the recent characterization of thalamocortical learning described in Bruno and Sakmann (2006).

Third, **Figure 4A** shows that recognizing an exact instance of a previous input also requires only one iteration of the CSA. Although this example does not directly show it, this holds for recognition of non-exact matches as well. Evidence for this will be presented in a separate work. That both learning and recognition require only a single CSA iteration is especially significant since, as can readily be seen, none of the CSA steps involves iterations over stored codes: thus, the time it takes for the CSA to either store a new input or retrieve the closest matching stored input remains constant as the number of stored codes increases. This does not imply that an infinite number of codes can be stored: of course, the model has finite storage capacity. This capacity will be characterized in future research, but should be similar to other sparse associative memories (Willshaw et al., 1969; Palm, 1982; Moll and Miikkulainen, 1995; Knoblauch et al., 2010).

PROSPECTIVE MAPPING TO CORTICAL CIRCUITRY

There remain huge gaps in our knowledge of the intrinsic physiological, synaptic, morphological, and connectional properties of all classes of cortical cell and of functional relationships between cortical and sub-cortical structures. Nevertheless, **Figure 5** shows a possible neural realization of the model's WTA CM, i.e., minicolumn, and dynamics (CSA). I have attempted to respect known constraints but the realization is admittedly speculative and ongoing modifications will undoubtedly be required. **Figures 5A–E** show the critical events transpiring in a single minicolumn at five points in time during the CSA's computational cycle. Note that due to space limitations **Figure 5** cannot depict the true extents of the various axonal and dendritic systems of the cells involved. **Figure S3** in Supplementary Material provides a more global context showing these extents.

Figure 5A shows the initial CSA steps 1 and 2 when the L2/3 pyramidals (here only two cells, but in reality, ~20) integrate their inputs. While the CSA explanation in the prior section included only the BU inputs, all three input classes are included here:

- BU inputs (labeled "2") are mediated via L4 (Rockland and Pandya, 1979)
- TD inputs ("1") are mediated via L2/3 apical tufts (Rockland and Drash, 1996)
- H inputs ("3") are mediated via the horizontal matrix of L2/3 (Gilbert and Wiesel, 1989; Feldmeyer et al., 2006)

All three input vectors arrive in parallel and collectively give rise to postsynaptic potentials (PSPs) in the L2/3 pyramidals. The three (normalized) inputs are combined multiplicatively; see the generalized version of the CSA (**Table S1** in Supplementary Material). The chandelier cells (represented by a single red unit labeled "C") are firing at this time, preventing the L2/3 pyramids from firing.

In **Figure 5B**, the chandeliers shut off (grayed out) and the L2/3 pyramid with the highest PSP (cell 2) is assumed to be the first to spike (CSA step 3). This winning cell, and more specifically, its PSP value (*V* in Eq. 2), represents this minicolumn's *local* judgment of how similar the macrocolumn's closest-matching stored input is to the current overall (i.e., BU, H, and TD) input. The output of cell 2 is then communicated to some locus where it is averaged with the round 1 winners from the other ~70 minicolumns of the macrocolumn, yielding *G* (CSA step 4). As noted in the Introduction, the functionality related to *G* seems most consistent with the phenomenology of neuromodulatory systems, especially ACh and NE. Support for this speculation is given in the following sub-section. Note that the communication of cell 2's PSP value is hypothesized to be mediated via L5, one of whose pyramidal cells is seen integrating here (light green); this is based loosely on evidence that L5 cells, specifically L5B pyramidals, almost exclusively target pontine areas (with collaterals to thalamus) (Deschênes et al., 1994; Krieger et al., 2007).

L2/3 pyramidals are the primary source of BU signals to higher cortical areas (Rockland and Pandya, 1979; and see Thomson et al., 2002; Brecht et al., 2003; Schubert et al., 2003; Bannister, 2005;

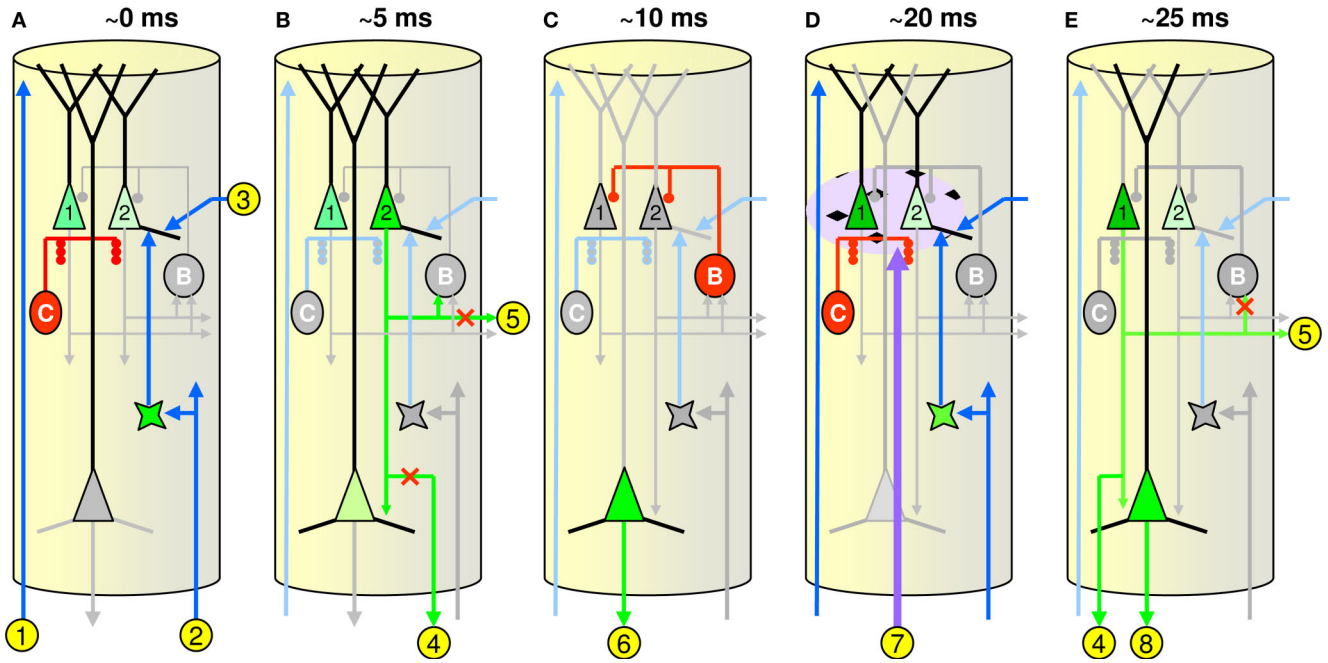

FIGURE 5 | Hypothetical minicolumnar circuit. (A–E) Depict critical events transpiring in a single minicolumn at five points in time during the CSA's computational cycle, which I propose corresponds to one gamma cycle: approximate timings relative to start of CSA cycle are shown across top. The units labeled "C" ("B") represent the local chandelier (basket cell) populations, respectively; See text for detailed explanation.

Helmstaedter et al., 2007; Lübke and Feldmeyer, 2007; Petersen, 2007; Egger et al., 2008; Lefort et al., 2009 for wider background on cortical columnar circuitry relevant to the current proposal). In addition, as stated earlier, the horizontal L2/3-to-L2/3 connections are proposed to communicate this macrocolumn's *final* hypothesis regarding its total input pattern in the current CSA cycle recurrently back to the same (and surrounding) macrocolumns on the next CSA cycle. Hence, it is crucial that since that final hypothesis is not present until the second round of competition completes (**Figure 5E**), the output pathways carrying those signals must be closed (red "x"s on paths "4" and "5"). Though not depicted here, one possible mechanism for selectively preventing horizontal signaling in L2/3 is activation of the double bouquet cells (DeFelipe et al., 1990, 2006; Peters and Sethares, 1997). Their "horsetail" axons, being interdigitated, nearly one-to-one with minicolumns would allow them to make contact with a substantial portion of the horizontally (and obliquely) oriented dendritic and axonal processes, in L2/3, and thus prevent passage of horizontal signals.

In **Figure 5C**, the L5 pyramidal mediating the winning L2/3 cell's PSP value has reached threshold and sends that output to the sub-cortical averaging locus (path "6"). In addition, the winning cell itself has activated the local basket cell network (electrically coupled, cf. Brown and Hestrin, 2009), represented by the unit labeled "B", which rapidly deactivates and re-polarizes (resets) the L2/3 pyramidals (grayed out). This reset need not be back to a completely even baseline: some remnant of the relative PSP distribution prior to basket cell activation might remain, biasing the second round of competition.

In **Figure 5D**, the result of the subcortical computation of *G* is returned to the macrocolumn (path "7") in the form of neuromodulator release (purple cloud surrounding the L2/3 pyramidals). This release modifies the activation functions of the L2/3 pyramidals, as described earlier. Note that this neuromodulatory "cloud" actually extends across a broad, i.e., macrocolumnar (or wider), expanse of L2/3, not just within a single minicolumn as this figure may suggest. The chandeliers are again firing to prevent any L2/3 from firing as the second round of integration occurs. The basket cells are inactive, allowing this integration to take place.

In **Figure 5E**, the chandeliers again deactivate. The L2/3 pyramidal with the highest PSP is the first to spike. In general, the pyramidal cell winning on this second round of competition may differ from the first round winner. In particular, the probability that the second round winner is the same as the first round winner increases with *G*. The set of L2/3 winners, one per minicolumn, across the whole macrocolumn represents that macrocolumn's final decision (hypothesis) as to identity of its current overall (TD, H, and BU) input. Thus, the output of the winning L2/3 cell in each minicolumn is now communicated to:

1. Lower cortical regions via L5 and its backprojections to the lower regions' L1 (labeled "8") (Rockland and Drash, 1996).
2. L2/3 pyramids in the same and neighboring macrocolumns via the local horizontal L2/3 matrix ("5") (Gilbert and Wiesel, 1989; Feldmeyer et al., 2006), thus delivering temporal context information (recurrently) to the local region to be integrated on the next CSA cycle.
3. The L4 layer of higher cortical regions ("4") (Rockland and Pandya, 1979).

Note that the output of the winning L2/3 cell should be prevented from recurring to the local basket network at this time so as to allow the integration period to occur at the beginning of the next computational cycle; hence, the red "x" on the link to basket cell.

I reiterate that the above possible cortical realization of the proposed SDC model is highly speculative. It clearly lacks numerous details, especially regarding processing in the intermediate processing stages, e.g., L4, and output processing involving L5 (and L6). Nevertheless, it is a starting point and can be falsified, especially as experimental methods mature. For example, the many timing relationships in the circuit can be tested. We still have decidedly little in the way of hard constraints on the time courses of activation of the many cell types involved in cortical (and hippocampal) circuits, though progress is being made (Klausberger et al., 2003, 2004; Silberberg et al., 2005; Silberberg and Markram, 2007; Klausberger and Somogyi, 2008; Otsuka and Kawaguchi, 2009; Woodruff et al., 2009).

Moreover, the proposed theory's key assumption that the L2/3 pyramidals are the core repository of information in cortex and that the codes laid down in L2/3 are the context-dependent memories of our experiences, is subject to challenge. Specifically, the anatomy of the L5 thick tufted cells suggests that they too have access to BU (via L4), TD (via their apical tufts in L1), and H (via an extensive intra-L5 horizontal network, Schubert et al., 2007) inputs, and therefore that L5 might store the most detailed and context-dependent codes in cortex, a view supported by findings such as (de Kock et al., 2007). In the end, for the purpose of this "hypothesis and theory" paper, I believe the architecture and algorithm (CSA) to be more important than the specifics of any particular neural realization.

Support for neuromodulator-based implementation of familiarity-contingent noise

In this section, I consider evidence relating to six model predictions:

a} *Signals generated in the macrocolumn [i.e., the $\hat{V}_x$ (Eq. 3)] can influence neuromodulatory systems (brown links in **Figure 2**).* Strictly interpreted, **Figure 2** suggests that the model can only be true of cortical areas that have direct projections to the activation function modulator (AFM), hypothesized to be instantiated in midbrain neuromodulator source areas, e.g., basal forebrain (BF, source of ACh) and locus coeruleus (LC, source of NE). Relatively few cortical areas project directly to BF or LC. Direct cortical afferents to BF arise mainly from prepyriform, anterior insula, orbitofrontal, entorhinal and medial temporal areas (Mesulam and Mufson, 1984). Direct cortical afferents to LC arise from dorsal prefrontal cortex (Arnsten and Goldman-Rakic, 1984), medial prefrontal cortex (Jodo et al., 1998).
While direct projections are limited, a much larger fraction of cortex may be able to influence the hypothesized AFM via multi-synaptic pathways involving thalamus and other subcortical structures, especially via pathways interconnecting BF, LC, and other neuromodulator source areas. For example, BF cholinergic neurons are excited by LC (Jones et al., 2004), which allows dorsal and medial prefrontal areas indirect influence on BF. Similarly, LC receives input from the Raphe nuclei (reviewed in Samuels and Szabadi, 2008) which would allow further extension of the sphere of cortical influence upon the AFM.
This is a subject of ongoing research. However, it is clearly possible that my macrocolumnar model applies only to the smaller set of cortical areas suggested above. There is some merit to this idea. After all, there would be some advantage to deferring the decision as to the overall familiarity/novelty of the current input (moment) to the very highest cortical levels, which are in position to make the most informed decisions. In this case, once G is computed, it is then broadcast pan-cortically, i.e., to all levels of the hierarchy, allowing the local choice of code to proceed accordingly, i.e., with a level of randomness appropriate to G. **Figure S2** in Supplementary Material illustrates this view.

b} *There exists some neuromodulatory signal, η (Eq. 5), which correlates with the detection of familiarity, and/or inversely with the detection of novelty.* Such correlations have been shown for both ACh (Miranda et al., 2000, 2003) and NE (Sara et al., 1994; Vankov et al., 1995).

c} *The signal η can physically reach cortex (purple arrows in **Figure 2**).* LC projects to all of cortex (Foote and Morrison, 1987; Berridge and Waterhouse, 2003; Samuels and Szabadi, 2008). BF projects to almost all cortical areas (reviewed in Briand et al., 2007). The amount of randomness to be added to the winner selection process is a global parameter, which applies *non-specifically* to all the minicolumns. This is consistent with volume transmission believed to be used by neuromodulatory systems (Descarries et al., 1997; see Sarter et al., 2009 for discussion of the complexities of the evidence regarding volume transmission).

d} *The signal η determines (Eqs 6–8) the amount of noise (randomness) in the selection (activation) of cortical (i.e., macrocolumnar) codes.* Controlling the noisiness of a process of choosing a winner from a competing group of neurons can be achieved by some combination of two actions: (i) increasing the spike probability of cells with high input summations relative to those with low summations and (ii) lowering the spike probability of low-input cells relative to high-input cells. Both of these actions can be achieved by uniformly (i.e., to all competing cells in a WTA module) modulating intrinsic cell properties such as excitability. Numerous studies have demonstrated both excitatory and suppressive effects on target cell responses (both principal neurons and interneurons) for both ACh (Kawasaki and Avoli, 1996; Shalinsky et al., 2002; Cobb and Davies, 2005; Tateno et al., 2005; Isakova and Mednikova, 2007; Lawrence, 2008; Eggermann and Feldmeyer, 2009) and NE (Foote et al., 1975; Kawaguchi and Shindou, 1998; Harley and Helen, 2007; Moxon et al., 2007). It is not my intention here to argue for a precise realization of this mechanism. As suggested in many reviews (Berridge and Waterhouse, 2003; Lucas-Meunier et al., 2003; Sara, 2009), the landscape of this research is very complex and we are far from a comprehensive understanding of the how the various neuromodulatory systems affect high-level cognitive processing either alone or in concert (Briand et al., 2007). Nevertheless, the large and varied body of evidence at least admits the possibility that one or more of these neuromodulators could implement the familiarity-contingent AFM mechanism (CSA steps 5–8; see Doya, 2002, p. 502, for similar speculation).

e} *Decreased* η, *i.e., increased noise, leads to greater code separation (decreased intersection).* Recently, Goard and Dan (2009) showed that increased BF stimulation decreased the correlation amongst a population of rat V1 cells. This decreased correlation essentially shows increased separation between population codes, which in the model proposed here, would manifest as decreased intersection between sparse codes.

f} *Disabling of the brain's ability to produce high noise, i.e., causing* η *to be permanently high, should reduce the ability to learn new inputs, while sparing or having much less effect on recognition of known items.* Looking at **Figure 4**, if the AFM was "stuck" in the highly expansive sigmoid condition (low noise), all four inputs, α_2–α_5, would have high probability of mapping to the same code, β_1. This would prevent the model from being able to distinguish them in future presentations. However, in general, inputs that were mapped to unique codes prior to such a disabling event will reliably activate those codes on future presentations. In accord with this, Browning et al. (2010) found that severely diminishing cholinergic inputs to inferotemporal cortex severely reduced macaques' performance on a visual episodic memory task, while having little effect on a DNMS task. McGaughy et al. (2005) found a similar effect: cholinergic deafferentation of entorhinal cortex reduced performance on DNMS tasks involving novel odors but not familiar odors.

DISCUSSION

I have described a theoretical model of cortical function that explains the functional relationship between the minicolumn and macrocolumn. Specifically:

a} The macrocolumn (in any of its forms) is proposed to store information in the form of SDCs, and

b} The minicolumn (specifically, its L2/3 pool of pyramidals) is proposed to operate as a WTA CM, the purpose of which is to enforce the sparseness of the macrocolumnar code.

Two key motivations for this model are the computational advantages of SDC and the increasingly strong evidence for SDC in the brain, cited in the Section "Introduction". One important advantage of SDC over a localist code is that the number of unique items that can be stored can be far larger than the number of representing units. For example, the 12 F2 units of the model in **Figure 2** allow $3^4 = 81$ unique codes, though in realistic systems, e.g., with less than complete connectivity leading to and from a coding field like F2, the number of those codes that can safely (i.e., while maintaining retrieval error rates below some acceptable criterion) be assigned will be substantially lower than 81. Nevertheless, if the number of input items that will need to be distinguished is not known *a priori*, SDC is more flexible.

A second computational advantage of SDC is that, if used in conjunction with an appropriate storage/retrieval algorithm it possesses the SISC property. I demonstrated, with the small but statistically reasonable example of **Figures 3 and 4**, that the CSA yields the SISC property. The SISC property strongly differentiates SDC from localist models: it is not even defined for a localist model since every code is formally disjoint with every other code. Hence, there is no structural way to represent degrees of similarity in a localist code. If there is no way to represent a measure, e.g., similarity, structurally, then whenever that measure is required – e.g., when the closest matching stored item in a database (i.e., macrocolumn) to an input must be returned – it must be computed, which takes time and energy. In contrast, when items' codes are stored in physically overlapped fashion such that the degree of code overlap represents item similarity, as is the case for the proposed model, the most closely matching stored item will be returned *immediately*, i.e., without requiring any serial search through the stored items. **Figure S4** in Supplementary Material shows test retrievals of the four unique codes stored in the model of **Figures 3 and 4**, demonstrating possession of this immediate access property for this small example. Empirical proof of this property based on larger scale simulations is currently being developed.

I consider the representation and the CSA to be the most important contributions of this paper because of the computational advantages just described. However, I believe the suggestion that the minicolumn's *generic* function is to act as a WTA CM is also important. Saying only that a group of L2/3 units forms a WTA CM places no *a priori* constraints on what their tuning functions or receptive fields should look like. This is what gives that functionality a chance of being truly generic, i.e., of applying across all areas and species, regardless of the observed tuning profiles of closely neighboring units. Indeed, a recent calcium imaging study of mouse auditory cortex by Rothschild et al. (2010) shows highly heterogeneous small-scale (even immediately adjacent cells) tuning even though the large-scale tuning is tonotopic. Experimental methods are only now just reaching the point where this hypothesis might be directly testable, e.g., modifying calcium imaging methods to have millisecond temporal granularity; see Ohki and Reid (2007).

In a sense, the main point of this paper is that a *generic* minicolumnar function becomes apparent as soon as we postulate that what the cortex, i.e., a macrocolumn, generally does is store and retrieve (access) SDCs of *specific* context-dependent inputs. As noted in the Section "Introduction", the experimental literature contains little in the way of proposals linking the formation and retrieval of *specific* SDCs (i.e., of specific input items, especially of temporal context-dependent items) to the cortical microcircuitry. My proposed model goes beyond broad conclusions and offers a mechanistic explanation of how *specific* informational items are learned and retrieved and in so doing, proposes a generic function for the minicolumn, i.e., that it functions as a WTA module in support of manipulating SDCs at the next higher, i.e., macrocolumnar, scale.

There have been several recent models linking formation/retrieval of specific items to cortical circuitry and which describe specific roles for the minicolumn (Kupper et al., 2007; George and Hawkins, 2009; Litvak and Ullman, 2009; Schrader et al., 2009). However, all of these models use localist representations and therefore would not possess the advantages of SDC described above. The Cortext model (Kupper et al., 2007; Schrader et al., 2009) assumes that each minicolumn in a hypercolumn represents one particular input feature. On each computational cycle, a WTA process runs within each *hypercolumn*, such that exactly one minicolumn wins, which would be strongly at odds with the calcium image data (Ohki et al., 2005). A second problem is that the assumption that whole minicolumns compete with each other implies that any given hypercolumn (at any level of the cortical hierarchy) can represent only ~70 unique features (equivalence classes), which seems severely restrictive, especially for hypercolumns at higher cortical levels, e.g., IT. The Litvak and Ullman (2009) model

also postulates that the L2/3 pool of neurons in a minicolumn implements a max function. However, their model proposes that each single minicolumn (specifically, its L2/3 pool) is partitioned into several *disjoint* groups ("cliques") of cells, each representing a different item. Since any particular cell can participate in only one clique, this constitutes a localist code. George and Hawkins (2009) also assume that minicolumns represent informational items in a localist fashion. Note however that both George and Hawkins (2009) and Litvak and Ullman (2009) explicitly mention moving to a more general combinatorial code, a.k.a. SDC, as a future research direction.

A core principle of the proposed model is this notion of controlling the amount of noise in the process of choosing (activating) a macrocolumnar code as an inverse function of input similarity. Doya (2002) uses the same principle, referred to as "Boltzmann selection", to modulate the amount of noise in the process of choosing amongst output action actions. Doya specifically hypothesizes that NE controls the noise whereas I can assert only that it is some neuromodulator-based mechanism. In Doya's model, as NE levels increase, the action with the greatest expected reward is chosen with probability approaching 1. This is suggested as corresponding to the "exploitation" end of the exploitation–exploration continuum. As NE levels drop to 0, all actions become equally probable, i.e., "exploration", which is appropriate if no single action has a particular high expected reward, which generally correlates with the condition of novelty, i.e., of being in a novel environment wherein it is harder to anticipate the outcome of known actions. The analogy to high expected reward in my model is a highly familiar input ($G \approx 1$) in which case we want the stored code for that familiar input to be reactivated with probability approaching 1; the condition where no action has a high expected reward is analogous to low familiarity, i.e., where no stored input is very similar to the current input, in which case we want to lay down a new memory trace for the novel input. Despite the similarities, Doya's model also assumes a *localist* representation of the choices and, like the other models just mentioned, cannot realize the advantages of SDC.

I have identified several avenues of active and future research at various points in the text and as noted in the previous section, the prospective neural realization is highly speculative and very incomplete. Several additional questions/issues for future research are:

1. Is the current proposal that the L2/3 cells engage in two rounds of competition in each computational (putatively, gamma) cycle plausible?
2. For simplicity, I have described the model in the simplest case of having only one internal coding field (F2) and processing only purely spatial input patterns. However the core model was originally developed for the spatiotemporal pattern (sequence) case (Rinkus, 1996) and was generalized some time ago to have an arbitrarily deep hierarchy of coding fields (Rinkus and Lisman, 2005). See **Figure S2** in Supplementary Material. How do these generalized versions of the model map to neural structures?
3. Is there evidence that chandeliers become active twice as frequently as baskets, as the proposed realization predicts? Is there evidence for the converse?
4. Although not elaborated herein, the proposed mini-/macrocolumn model is easily generalized to allow substantial overlap between minicolumns (see **Figure S5** in Supplementary Material) and multiple winners in a minicolumn on each computational cycle. These degrees of freedom need to be explored.
5. We know of the fast, phasic, time scales of operation for NE (Rajkowski et al., 2004) and DA (Schultz, 1998) and of slightly slower but still phasic mode for ACh (Gulledge and Kawaguchi, 2007), but these have been proposed as signaling other measures besides pure novelty (Redgrave et al., 1999; Bouret and Sara, 2005; Dayan and Yu, 2006). How might all these signals conspire to implement a pure novelty signal?

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/neuroscience/neuroanatomy/paper/10.3389/fnana.2010.00017/

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A comparative perspective on minicolumns and inhibitory GABAergic interneurons in the neocortex

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Neocortical columns are functional and morphological units whose architecture may have been under selective evolutionary pressure in different mammalian lineages in response to encephalization and specializations of cognitive abilities. Inhibitory interneurons make a substantial contribution to the morphology and distribution of minicolumns within the cortex. In this context, we review differences in minicolumns and GABAergic interneurons among species and discuss possible implications for signaling among and within minicolumns. Furthermore, we discuss how abnormalities of both minicolumn disposition and inhibitory interneurons might be associated with neuropathological processes, such as Alzheimer's disease, autism, and schizophrenia. Specifically, we explore the possibility that phylogenetic variability in calcium-binding protein-expressing interneuron subtypes is directly related to differences in minicolumn morphology among species and might contribute to neuropathological susceptibility in humans.

Keywords: calcium-binding proteins, calbindin, calretinin, parvalbumin, neuropathology, evolution

INTRODUCTION

Vernon Mountcastle was the first to describe the cortical minicolumn (Mountcastle et al., 1955), providing what appeared to be a simple means by which to understand the cerebral cortex. According to this model of cortical organization, neurons, glia, and their connections form part of an all-encompassing vertical system which unites the cells of each minicolumn into a coordinated functional unit (Mountcastle, 1997). In this context, the smallest unit of cortical anatomy is the minicolumn, which is defined by a narrow radial array of single neurons. Minicolumns are arranged within larger macrocolumns (e.g., barrel somatosensory cortex of the rodent).

While the minicolumn has arguably brought a coherent sense of structure to our thinking, the pursuit of simple explanations has been fueled by an ancillary argument for regularity in cortical minicolumn morphometry. Influential in this regard has been the highly cited paper by Rockel et al. (1980) that reinforced the claim that there is a fundamentally uniform architecture to the cortical minicolumn, reporting that the number of neurons within a minicolumn (defined as the number of neurons within a strip of tissue 30-μm wide and 25-μm thick from pial surface to white matter) is nearly invariant at 110 neurons across cytoarchitectonic areas and species, suggesting that functional differences were principally a result of wiring (Rockel et al., 1980). The claim that cortical minicolumns are uniform reified the philosophical idea that human and animal differences can be reduced to purely quantitative measures (Rakic, 2008) and added validity to the widespread use of macaque and mouse models in neurobiology. In addition, it seemed, at least in principle, to be congruent with the existence of the ontogenetic columns described in Pasko Rakic's "radial unit hypothesis", which provided a basis for understanding how evolutionary changes in the neocortex occur through modifications of cell cycle dynamics of founder neurons in the ventricular zone during development.

Subsequent work demonstrated that the estimates of neuron numbers for the cortical minicolumn as described by Rockel et al. (1980) were flawed for various reasons, including problems with extrapolating from counts in different cytoarchitectonic areas without corrections for cell size and arbitrary designations for the expected dimensions of a cortical minicolumn (DeFelipe et al., 2002; Rakic, 2008). Further empirical evidence has also shown that there is much greater phylogenetic variation in the number of neurons underneath 1 mm^2 of cortex than was previously thought (e.g., Herculano-Houzel et al., 2008). Although several investigators have directly refuted the validity of the Rockel et al. (1980) claim (e.g., Preuss and Goldman-Rakic, 1991; Beaulieu, 1993; Skoglund et al., 1996; DeFelipe et al., 2002; Casanova et al., 2009), the concept of uniformity in cortical minicolumn structure still remains popular and is widely used in computational models of cortical operation.

Recent research on the histological organization of the neocortex across mammals, however, has demonstrated a rich diversity of structural variants, ranging from the morphology of astrocytes to the packing density of neurons. In this review, we will examine species diversity in two key elements of neocortical structure – interneurons and minicolumns. Variability in minicolumn structure will be linked to known phylogenetic differences in calbindin- (CB), calretinin- (CR), and parvalbumin (PV)-immunoreactive (ir) interneuron populations with the aim of assessing the possible implications of variation in minicolumn subcomponents on the evolution of inter/intracolumnar communication. Furthermore, we will discuss how

an appreciation for minicolumn and interneuron variability may shed light on neuropathologies associated with cognitive deficits. We propose that the appearance of a specific GABAergic interneuron subtype within the Primate Order, the double bouquet cell, has made a significant contribution to unique minicolumn functions and human susceptibility to specific neuropathologies.

COMPARATIVE ASSESSMENT OF MINICOLUMNS: DIVERSITY AMONG MAMMALS

A major obstacle in dispelling the assertion that there is uniformity in minicolumn size is the lack of systematic, standardized data on minicolumn dimensions across a wide range of species (Buxhoeveden and Casanova, 2002a). The data that are currently available represent different methodological and measurement techniques from tissues that were prepared in a manner that might be susceptible to differential shrinkage of histological components. Further, cortical minicolumns have not been examined in a very wide range of brain sizes, so we currently do not know if there are upper and lower bounds for cortical minicolumn dimensions or if there are regular patterns related to the extent of gyrification (Buxhoeveden and Casanova, 2002a). Although current comparative data on minicolumn width are not ideal, they do permit a tentative examination of variability within a limited range of species. As reported from the literature, minicolumn width appears to vary substantially among species and across cortical areas (**Figure 1**).

If we limit ourselves to considering only minicolumn widths reported for the visual cortex using the apical dendrite bundle as the defining feature, it is apparent that the primary visual cortex of the rhesus macaque (*Macaca mulatta*) has, on average, a smaller minicolumn width (23–30 μm) compared to that reported for

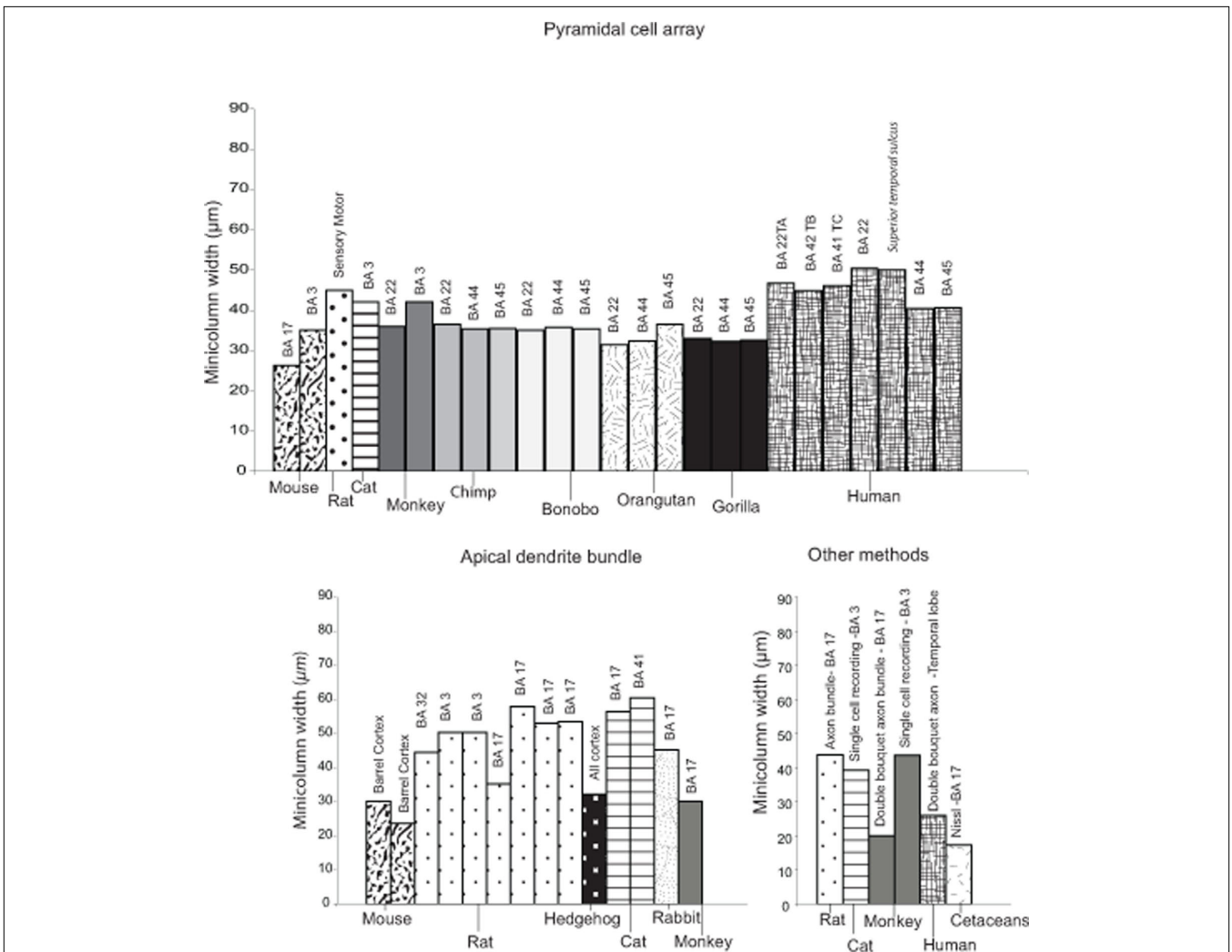

FIGURE 1 | Minicolumn widths collated from the literature for the Class Mammalia. The following sources were used: Escobar et al. (1986), White and Peters (1993), Peters and Yilmaz (1993), Feldman and Peters (1974), Favorov and Diamond (1990), Tommerdahl et al. (1993), Gabbott and Bacon (1996), Peters and Walsh (1972), Peters and Kara (1987), Kohn et al. (1997), Fleischauer et al. (1972), DeFelipe et al. (1990), Peters and Sethares (1991), Peters and Sethares (1996), Peters and Sethares (1997), Buxhoeveden et al. (2001a), Von Bonin and Mehler (1971), Kaas et al. (1981), Buxhoeveden et al. (2002a), Schlaug et al. (1995), Seldon (1981), del Rio and DeFelipe (1997a), Buldyrev et al. (2000); and Casanova and Tilquist (2008). Note that in certain cases an average minicolumn width had been computed by calculating the mean based on the minimum and maximum values reported in each study. Numbers refer to Brodmann areas.

rats (30–40 μm, average 35 μm), cats (55–60 μm, average 57.5 μm) and rabbits (40–50 μm, average 45 μm). Interestingly, even though the brain of the rhesus macaque is approximately 4-fold larger in size than that of a cat (macaque: 106.4 cc; cat: 25.3 cc), there is not a concomitant increase in minicolumn width (Peters and Yilmaz, 1993). Peters and Yilmaz (1993) speculated that this may be the case because macaques might possess more complex cell columns, which render a more detailed specification of information, thereby permitting a reduction in overall minicolumn width. A more recent analysis of the morphometric variability of minicolumns among macaque monkeys, humans, and chimpanzees (Casanova et al., 2009) again confirmed that there are differences in minicolumn width across species, but concluded that the core column space (i.e., the space that contains the majority of neurons and fibers and is distinguishable from the adjacent, cell-poor neuropil space) remains relatively invariant, suggesting that this subcomponent of the cortical minicolumn may be evolutionary conserved (Casanova et al., 2009). It is possible that conservation of the minicolumn core dimension in these three species may be confined to this particular clade, and only further comparisons to other species will elucidate whether this apparent uniformity is indicative of an upper or lower bound for all primates or mammals as a whole. The above examples, although tentative, highlight the need for casting the comparative next even wider to include other as yet unexamined species and emphasize how an analysis sensitive to diversity in minicolumn attributes may provide an interpretation which is more functionally and ecologically linked to the environments within which species evolved.

For example, it is notable that a similar "primate-like" pattern of smaller minicolumn sizes (19.9 μm) were reported for the visual cortex of large brained Cetacea (humpback whale, *Megaptera novaeangliae,* striped dolphin, *Stenella coeruleoalba* and bottlenose dolphin, *Tursiops truncatus*), who also have cortical minicolumns that are more discontinuous across cortical areas (Morgane et al., 1988; Manger, 2006; Hof and Van der Gucht, 2007). This specific feature of cortical organization in cetaceans is likely countered by integration of column activity occurring in their thick layer I which contains approximately 70% of the total cortical synapses (Glezer and Morgane, 1990). Strikingly, the cytoarchitectonic columns in the visual cortex of the striped dolphin and human contain roughly the same amount of synapses, indicating that any deficit or equivalence in cell column width is not necessarily indicative of the functional output of the cortical column for that species (Morgane et al., 1988), a point most strongly highlighted by the complex behavioral and cognitive abilities of cetaceans that compares with those of anthropoid primates (Marino, 2002; Marino et al., 2007, 2008).

Such species differences in minicolumn width for the visual cortex argue strongly in favor of further investigation into species-specific adaptations, especially as similar patterns may be expected for other regions of the cerebral cortex. Investigations of homologous areas of the primate cortex have indeed reported species-specific differences in minicolumn size and morphology. An example of this was reported in Wernicke's area homologue (area Tpt or area 22) of humans, macaque monkeys, and chimpanzees (Buxhoeveden and Casanova, 2000). Minicolumns in the left hemisphere of the human were 30% wider than those of the chimpanzee and macaque, with more neuropil space and less dense core areas (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001a). As indicated in **Figure 1**, minicolumn width measured in area 22 appears relatively uniform in macaque monkeys (36 μm), chimpanzees (35–36.5 μm), gorillas (33 μm), and orangutans (31.4 μm), but humans possess larger minicolumn sizes (50.4 μm). This difference would seemingly agree with restructuring in the human auditory cortex (Rilling et al., 2008) supposed to underlie human unique language abilities. In further support of this hypothesis, humans also display significantly larger minicolumn spacing in Broca's area (areas 44 and 45) than that observed for great apes (chimpanzee, bonobo, gorilla and orangutan) (Schenker et al., 2008).

A further argument in favor of recognizing the functional significance of diversity in the cortical minicolumn may be gleaned from studies of minicolumn widths among individuals of the same species and how these may relate to normal variation within a population. Casanova et al. (2007) recently reported significant differences in minicolumn width and mean cell spacing of a control group compared to that of three distinguished scientists. This study highlights the possibility for genetic and environmental effects on minicolumn phenotype, such as the number of founder cells, the duration of cell division cycle and selective cell death (see Rakic and Kornack, 2001). Minicolumn widths across a sample of the normal population shows continuous variation, suggesting that multiple independent variables may characterize its morphometry (Casanova, 2006). This is likely a reflection that, like other parts of the human organism, the cortical column may be (at least in part) prone to the effects of natural selection and adaptation.

Aside from size, minicolumns in different species may also show variability in their structural subcomponents (i.e., fibers and neurons). The following section is aimed at reviewing the evidence for phylogenetic differences indicative of diversity in the microcircuitry of mammalian cortical structure, with a focus on different subtypes of γ-aminobutyric acid (GABA)-containing interneurons.

GABAergic INTERNEURONS AND CORTICAL MINICOLUMNS

Inhibitory GABAergic interneurons are a heterogeneous group of cells that govern local cortical microcircuitry and are fundamental for intra- and intercolumnar processing (Hendry, 1987; DeFelipe, 2002; Casanova et al., 2003; Buzsaki et al., 2004; Ascoli et al., 2008). Whereas the morphological subtypes of interneurons that participate in cortical microcircuit regulation are highly conserved among mammals (Sherwood et al., 2009), there is a significant amount of variation among phyla in their diversity, density, distribution, and developmental patterns (e.g., Hof et al., 1999; Preuss and Coleman, 2002; Hof and Sherwood, 2005; Sherwood et al., 2007). For example, in rodents and other non-primate species, inhibitory interneurons comprise 15% or less of the cortical neuron population, whereas they may constitute more than 20% within the primate cortex (Hendry et al., 1987; Beaulieu et al., 1992; Gabbott and Bacon, 1996; Gabbott et al., 1997; DeFelipe et al., 1999, 2002). Additionally, the embryonic origin and migration of GABAergic cells has been demonstrated to differ between rodents and primates, with additional sites of neurogenesis in the lateral ventricular neuroepithelium in primates (Petanjek et al., 2009). The different origins of GABAergic interneuron subpopulations undoubtedly relate to species-specific distributions within the neocortex and likely

support differences in cognitive abilities. Inhibitory interneurons can be classified into subpopulations based on their immunoreactivity for the three calcium-binding proteins, CB, CR, and PV. Greater than 90% of all cortical GABAergic interneurons colocalize with one of these markers with little overlap among the separate populations (Hendry et al., 1989; Glezer et al., 1993; DeFelipe, 1997; Zaitsev et al., 2005). Different classes of interneurons interact with pyramidal cells to modulate cortical circuit processing, with CB- and CR-ir neurons involved mostly in intracolumnar communication and PV-ir interneurons involved in transcolumnar signaling **Figure 2** illustrates the major GABAergic cell types and **Figures 3 and 4** show examples of CB, CR, and PV immunostaining.

Cortical PV-ir GABAergic neurons are predominantly multipolar and include large basket and chandelier cells (Condé et al., 1994; Gabbott et al., 1997; Zaitsev et al., 2005; see **Figure 2**). The large basket cells have long-range axons that extend horizontally, targeting the perikaryon of pyramidal cells of different minicolumns (Lund and Lewis, 1993; DeFelipe, 1997; Somogyi et al., 1998). Chandelier cells, which are immunoreactive for PV, also provide lateral inhibition, making synaptic connections with the axon initial segments of pyramidal cells (DeFelipe, 1997; Wang et al., 2000a,b; Li et al., 2002). These various morphological classes of PV-ir cells regulate the rhythmic oscillations of pyramidal cell populations and have been identified as fast-spiking based on their brief action potentials and the absence of spike adaptation (Zaitsev et al., 2005; Sohal et al., 2009).

Cortical CR-ir interneurons have variable morphology and include bipolar, double bouquet, and Cajal-Retzius cells (DeFelipe, 1997). Bipolar and double bouquet CR-ir cells have axonal arbors that extend vertically, mostly targeting the dendrites of pyramidal cells in different layers of the cortex within a narrow column (DeFelipe et al., 1989; DeFelipe, 1997; del Rio and DeFelipe, 1997b; see **Figure 2**).

The characteristic phenotype of CB-ir interneurons in primate cortex is the double bouquet cell. As with the CR-ir double bouquet cells, CB-ir double bouquet axons provide vertical inhibition to pyramidal cells within the minicolumn (DeFelipe et al., 1989,

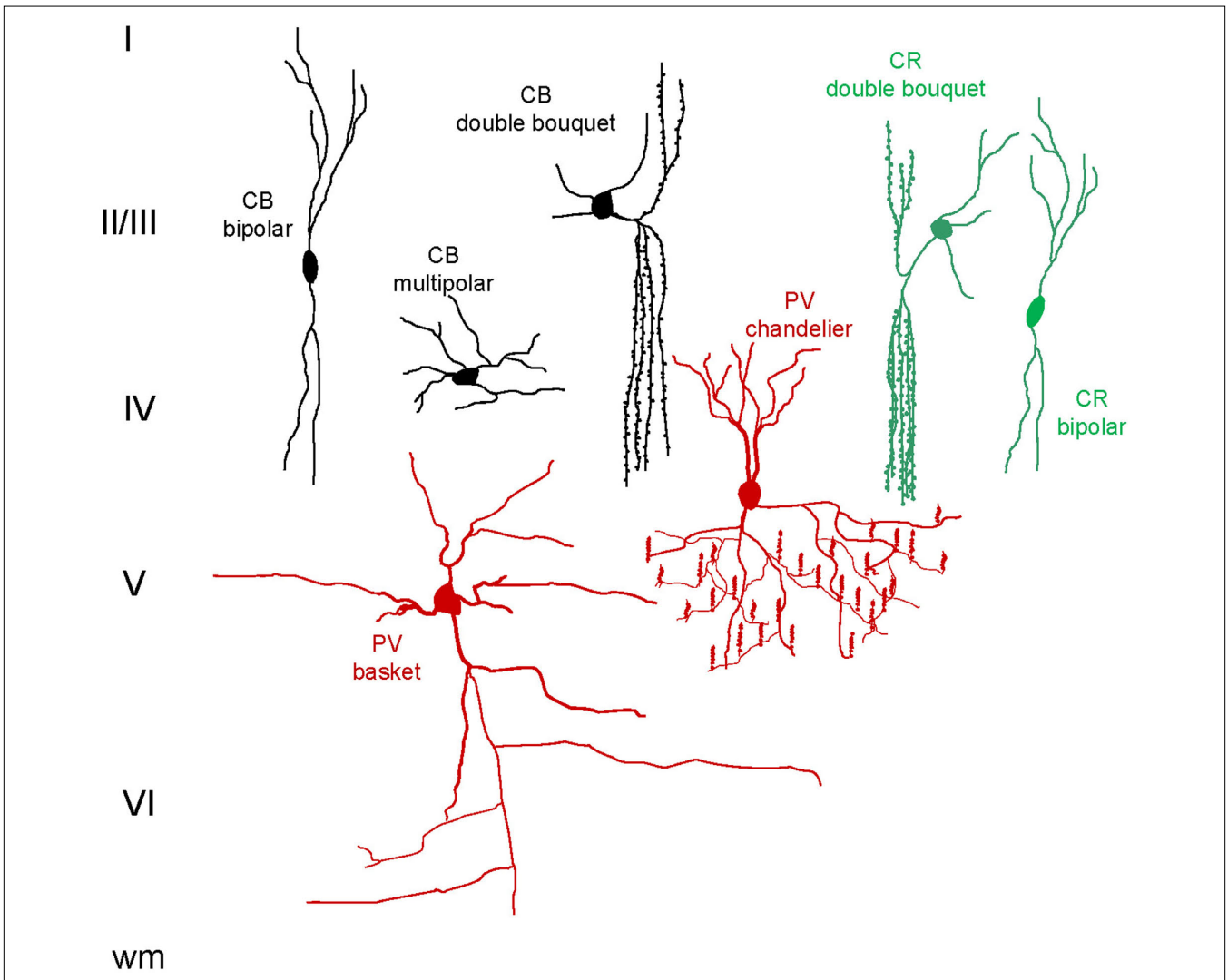

FIGURE 2 | Schematic representation of the major calcium-binding protein-ir interneuron subtypes. Roman numerals indicate cortical layers, wm = white matter.

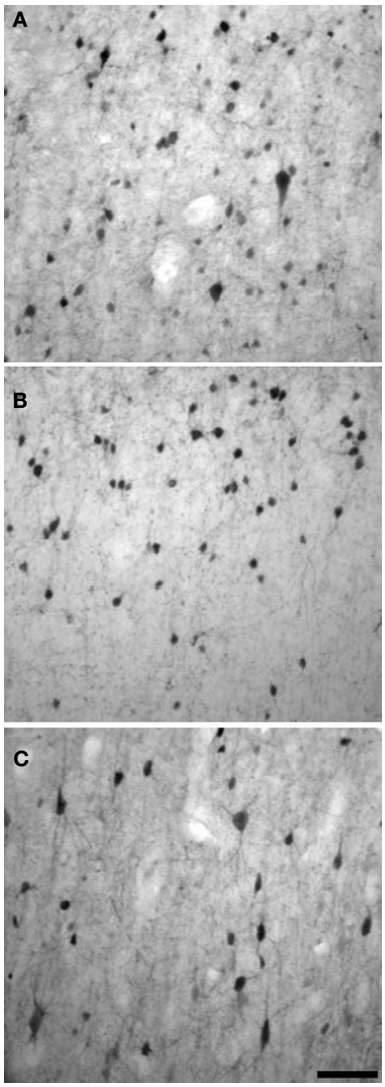

FIGURE 3 | Examples of CB (A), CR (B), and PV (C) immunostaining in layers II/III of chimpanzee motor cortex. Scale bar = 50 μm.

2006; Ballesteros-Yañez et al., 2005). In most non-primates except carnivores, CB-ir interneurons are mainly multipolar and bitufted, but lacking in the double bouquet morphotype (Ballesteros-Yañez et al., 2005; see **Figure 2**). Both CR- and CB-ir interneurons exhibit characteristics of non-fast-spiking cells in nonhuman primate cortex, with longer spike frequency and spike adaptation, but without distinctive differences between the two subpopulations (Zaitsev et al., 2005). Although CR- and CB-ir interneurons share similar physiological properties, the work of Zaitsev and colleagues suggests different pre- and postsynaptic connections, with CB-ir interneuron axons extending through cortical layers and accessing the cells of layer I, while CR-ir interneuron axons stop short of this layer.

GABAergic interneurons, particularly CB-ir double bouquet cells, contribute significantly to the morphology and distribution of minicolumns within the primate cortex (Buxhoeveden and Casanova, 2002b; Casanova et al., 2009). As a potential source for minicolumn diversity, we outline below the range of variation known for GABAergic neurons in mammals.

GABAergic NEURON VARIABILITY AMONG MAMMALS

The distributions of the calcium-binding protein-expressing interneurons have been reported for a variety of mammals, with a significant amount of diversity among species (Glezer et al., 1992, 1993, 1998; Hof et al., 1996, 1999; Hof and Sherwood, 2005; Zaitsev et al., 2005; Sherwood et al., 2007, 2009). Differences in GABAergic cell phenotype and density among species would be expected to represent alterations in local microcircuit processing, possibly supporting the expansion and differentiation of the cerebral cortex throughout mammalian evolution. Here we will highlight some of the major differences among mammals in terms of GABAergic cell populations (for review see Hof et al., 1999; Hof and Sherwood, 2005; Sherwood et al., 2009).

In primates, the three calcium-binding proteins are expressed in largely non-overlapping subpopulations of cortical interneurons (Gabbott and Bacon, 1996; DeFelipe et al., 1999; Zaitsev et al., 2005). In contrast, rodents show a greater degree of overlap, with colocalization of more than one calcium-binding protein expressed in the same cell (Kubota et al., 1994; Kawaguchi and Kubota, 1997). This difference in subpopulations in primates may support an enhanced,

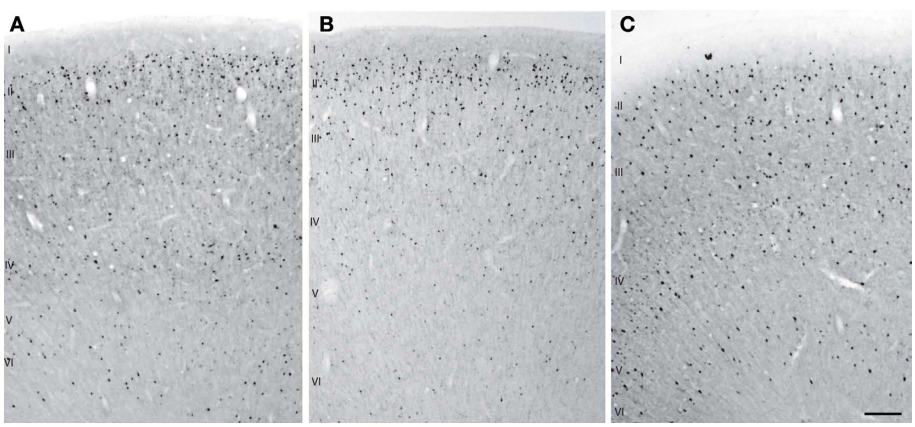

FIGURE 4 | Examples of CB (A), CR (B), and PV (C) immunostaining from layer I through layer VI in baboon parietal cortex. Scale bar = 500 μm.

or specialized, capability for specificity in local inhibitory control. In addition, the proportion of cortical interneurons varies among species, with primates having a higher overall percentage relative to rodents, afrotherians, and xenarthrans; though it is noteworthy that cetaceans have more than any of the terrestrial mammals that have been examined (Hof et al., 2000; DeFelipe et al., 2002; Sherwood et al., 2009).

In addition to variation in the relative concentration of cortical GABAergic cells, electrophysiological response properties can vary as well. A recent comparison of PV-ir fast-spiking basket cells in macaque monkeys and rats reported no significant morphological differences between species (Povysheva et al., 2008). However, Povysheva and colleagues did find a significant difference in excitability of PV-ir basket cell physiology, with neurons of macaque monkeys having a higher input resistance and lower firing threshold than those of rats. This finding coincides with differential firing of prefrontal cortex neurons during working memory task time delays, with lower frequency firing rates reported for rats (Povysheva et al., 2008). The actions of PV-ir interneurons appear to be fundamental for success in working memory tasks (Rao et al., 1999) and other cognitive functions (Constantinidis et al., 2002).

Phenotypic and morphological variation within interneuron subpopulations among species has also been reported. For example, chandelier cells express PV in primate primary motor and somatosensory cortex (DeFelipe et al., 1990), but not in canids (Hof et al., 1996). Further, CB-ir double bouquet cells are absent in rodents, lagomorphs, artiodactyls (Ballesteros-Yañez et al., 2005), xenarthrans, and afrotherians (Sherwood et al., 2009), but are present in the cortex of humans, macaque monkeys, and to a lesser extent and mostly restricted to visual cortex, in carnivores (Ballesteros-Yañez et al., 2005). These cells are found within layers II and III and are characterized by long descending bundles of axon collaterals that are columnar in structure and target pyramidal cells within a very narrow space. Such connectivity of double bouquet neurons has been proposed to represent a specialization of minicolumn inhibition within the primate order (del Rio and DeFelipe, 1997a; DeFelipe et al., 2002, 2006; Ballesteros-Yañez et al., 2005). Notably, the double bouquet axon bundles are associated with myelinated axons that comprise minicolumns in human cortex, although not all minicolumns were associated with CB-ir double bouquet cells (Ballesteros-Yañez et al., 2005).

While manifold differences have been reported between primates and nonprimates in terms of cortical GABAergic cell populations, our recent work revealed that there is actually a strong degree of conservation of inhibitory microcircuitry within several regions of the frontal cortex among human and nonhuman primates (Sherwood et al., 2009). Indeed, the density and distribution of interneurons expressing calcium-binding proteins across human and nonhuman primates closely adheres to general scaling rules without human-specific specializations in regions that are important for cognitive abilities such as language and mentalizing.

NEUROPATHOLOGIES, INTERNEURONS, AND MINICOLUMNS

Several neuropathological abnormalities in humans have been noted that involve minicolumns and interneurons. For example, decreased numbers of CB-ir interneurons have been reported consistently in the prefrontal cortex of patients with schizophrenia (Beasley and Reynolds, 1997; Reynolds et al., 2001; Beasley et al., 2002; Cotter et al., 2002; Eyles et al., 2002; Chance et al., 2005; Sakai et al., 2008) while CR-ir interneuron density is preserved (Woo et al., 1998; Reynolds and Beasley, 2001; Zhang and Reynolds, 2002). The relationship between PV-ir neuron distributions and schizophrenia is less clear, with reports of a decrease in density (Beasley and Reynolds, 1997) or no change in density (Woo et al., 1997; Cotter et al., 2002). Alterations of minicolumn width (i.e., neuropil space) are also well-documented in schizophrenia (Reynolds et al., 2004; Casanova et al., 2005, 2008; Chance et al., 2005; Di Rosa et al., 2009). These changes in minicolumn morphology appear to be consistent with a developmental abnormality rather than a progressive pathological process (Casanova et al., 2005, 2008).

Alzheimer's disease is also associated with a selective decrease of cortical CB-ir neurons in humans (Ferrer et al., 1993; Nishiyama et al., 1993; Beasley et al., 2002) and has also been reported in the canine expression of dementia of the Alzheimer's type (Pugliese et al., 2004) while both PV- and CR-ir neuronal subpopulations are spared (Ferrer et al., 1993; Hof et al., 1993; Pugliese et al., 2004). However, it should be noted that not all forms of dementia are associated with a reduction in cortical calcium-binding protein-containing interneurons (Hof et al., 1994; Gomez-Tortosa et al., 2001). The structure of minicolumns is selectively disrupted in Alzheimer's disease, and the loss of columnar organization was related to the number of neurofibrillary tangles (Buldyrev et al., 2000). Tangles cluster into columns and their numbers are positively correlated with degree of cognitive loss in Alzheimer's disease (Nagy et al., 1996). Minicolumn thinning was also noted in normal human aging and is a process that may be continuous with the increased risk of Alzheimer's disease over the age of 65 (Nagy et al., 1996).

It has been postulated that cortical minicolumn GABAergic inhibitory control is also compromised in autism (Casanova et al., 2003). However, while dysregulation of the calcium-binding protein-ir interneuron populations was recently demonstrated within the hippocampus of patients with autism (Lawrence et al., 2009), comparable data are not available for neocortical regions. Nonetheless, autism and Asperger's syndrome are associated with a narrowing of the minicolumns, specifically, the peripheral neuropil space (Casanova et al., 2002a,b,c, 2003). Because the peripheral neuropil space is dependent upon inhibitory interneuron populations, a deficit in GABAergic control is suspected. In particular, the modulation of minicolumnar activity would be altered for both local and long-range connectivity, resulting in collateral over-excitation among minicolumns (Casanova, 2008; Casanova and Trippe, 2009). Such a deficit is also suspected to contribute to the incidence of seizures in autistic individuals (Casanova et al., 2003). This relationship finds support in recent reports of deficits in both PV- and CR-ir interneurons with focal cortical dysplasias associated with epilepsy (Zamecnik et al., 2006; Barinka et al., 2009).

CONCLUSIONS

Interneuron subtypes are a major constituent of the peripheral neuropil space of minicolumns and the integrity of these local inhibitory circuits is critical for normal signal processing within the neocortex (Casanova et al., 2003). Highly conserved GABAergic neuron populations are targeted in certain

neuropathologies, perhaps owing to dysregulated cell migration in autism and schizophrenia, or a cholinergic-regulated decline in Alzheimer's disease. In both Alzheimer's disease and normal senescence, cholinergic cell bodies are lost, making a significant contribution to declining cognitive functions (Whitehouse, 1992; Mesulam, 1996; Mega, 2000). Notably, disruption of cortical acetylcholine has also been implicated in schizophrenia and autism (for review see Sarter and Parikh, 2005; Lam et al., 2006), demonstrating the importance of this neurotransmitter system for normal cognitive processing. Acetylcholine is involved in calcium regulation (Rathouz et al., 1996; Griguoli et al., 2009) and in modulating the function of GABAergic interneurons. Many of the interneurons receiving cholinergic innervation appear to be double bouquet cells (Xiang et al., 1998). The loss of cholinergic input coincides with the loss of CB-ir interneurons, dysregulation of calcium homeostasis, and the breakdown of minicolumn structure (Chance et al., 2006).

Comparative data indicate that there are differences among human and nonhuman primate species in the cholinergic innervation of the frontal cortex (Raghanti et al., 2008). Interestingly, the morphology of CB-containing interneurons has also been altered in primates, with a preponderance of double bouquet cells (Ballesteros-Yañez et al., 2005). Additionally, the major evolutionary alteration of minicolumn morphology is in minicolumn width. Humans deviate from other species in having a greater width of minicolumns in specific cortical areas, owing to constituents of the peripheral neuropil space, the most important predictor being the density of CB-ir double bouquet interneurons (Buxhoeveden et al., 2001b; Casanova et al., 2009).

Taken together, the unique prevalence of CB-ir double bouquet cells in the primate cortex, human-specific alterations in minicolumn width, disruption of cholinergic innervation and the loss of CB-ir interneurons in neuropathological processes in addition to their importance to the integrity of minicolumn structure point to a specialized function for these interneurons within the primate cortex.

Thus, variability not only in the connectivity of the minicolumn but also in the subtle subcomponents of the columnar organization such as composition of interneuron subtypes are a primary source of interspecific differences. Accordingly, both intra- and interspecific variation in cortical minicolumn morphology points in favor of diversity and an interpretation of the cortical minicolumn phenotype more strongly rooted in an evolutionary view. This perspective embraces complexity and the myriad morphological differences that biological species have evolved. More than 50 years after Mountcastle's observation of the cortical minicolumn and the 150 years since Darwin's (1859) highly influential evolutionary synthesis, it is perhaps fitting that we fuse the concept of the cortical minicolumn into an evolutionary perspective that acknowledges the eclectic nature of biology and the all-important contribution of variation to evolution.

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Does cell lineage in the developing cerebral cortex contribute to its columnar organization?

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Since the pioneer work of Lorente de Nó, Ramón y Cajal, Brodmann, Mountcastle, Hubel and Wiesel and others, the cerebral cortex has been seen as a jigsaw of anatomic and functional modules involved in the processing of different sets of information. In fact, a columnar distribution of neurons displaying similar functional properties throughout the cerebral cortex has been observed by many researchers. Although it has been suggested that much of the anatomical substrate for such organization would be already specified at early developmental stages, before activity-dependent mechanisms could take place, it is still unclear whether gene expression in the ventricular zone (VZ) could play a role in the development of discrete functional units, such as minicolumns or columns. Cell lineage experiments using replication-incompetent retroviral vectors have shown that the progeny of a single neuroepithelial/radial glial cell in the dorsal telencephalon is organized into discrete radial clusters of sibling excitatory neurons, which have a higher propensity for developing chemical synapses with each other rather than with neighboring non-siblings. Here, we will discuss the possibility that the cell lineage of single neuroepithelial/radial glia cells could contribute for the columnar organization of the neocortex by generating radial columns of sibling, interconnected neurons. Borrowing some concepts from the studies on cell–cell recognition and transcription factor networks, we will also touch upon the potential molecular mechanisms involved in the establishment of sibling-neuron circuits.

Keywords: cortical columns; sister neurons; cell lineage; transcription factors

ANATOMIC AND FUNCTIONAL ORGANIZATION OF THE MAMMALIAN CEREBRAL CORTEX

The cerebral cortex consists of distinct cytoarchitectonic areas, each serving a function ranging from sensory perception and motor control to symbolic thinking and language in humans. In fact, the anatomical observation of discontinuous architectural features of cerebral cortex uncovered very early by Ramón y Cajal and Lorente de Nó was followed by the realization that a functional architecture was also present probably as an emerging property of the underlying anatomical architecture. A striking feature of the neocortex, first unraveled in the work by Mountcastle (1957) describing the cortical representation of somatosensory perception, is its organization into functional columns.

The columnar organization of the cerebral cortex is a broadly documented principle of design preserved throughout mammalian evolution (Mountcastle, 1997), which has been proposed to be important to allow a large number of neurons to be connected without a significant increase in cortical volume. Indeed, it has been estimated that fusing 100 cortical columns would lead to a 10-fold increase in cortical volume (Mitchison, 1992). The explanation for such increase comes from the fact that neurons are locally connected within cortical columns and only restricted subsets of neurons are involved in long distance connections. Consequently, the length of axons that interconnect neurons is shortened, reducing also the cortical volume.

Within a given cortical column, discrete clusters of neurons project to a limited number of sites and tend to link columns of common functional properties (Mountcastle, 1997). The radial dispersion of these clusters is about 400 μm, similar to the spread of the dendritic arbor. Therefore, the cerebral cortex can be seen as a jigsaw of local neuronal microcircuits, which are interconnected by small subsets of neurons. Approaching the developmental building blocks of these microcircuits is an important step towards understanding the emergence of functional properties of cortical columns.

IS THE DEVELOPMENT OF CORTICAL COLUMNS INFLUENCED BY MOLECULAR CUES INTRINSIC TO THE DEVELOPING CEREBRAL CORTEX?

It has long been though that both development and plasticity of cortical columns rely exclusively on activity-dependent mechanisms. Indeed, the development of ocular dominance columns is highly dependent on visual experience, as clearly evidenced by the physiological and anatomical shifts caused by monocular eye closure during the critical period (Wiesel and Hubel, 1963, 1965; LeVay et al., 1978, 1980). However, there has also been accumulating evidence indicating that the initial establishment of cortical columns may take place before the critical period. For instance, it has been shown that the basic structure of segregated lateral geniculate nucleus (LGN) afferents in the primary visual cortex (V1) of macaque monkeys is formed before birth (Rakic, 1976).

Similarly, injection of anterograde tracers in the LGN of ferrets 2–3 weeks prior to the onset of the critical period reveals a clear ocular dominance segregation of the afferents (Crowley and Katz, 2000), indicating that molecular cues, intrinsic to the developing thalamocortical system, may be involved in the establishment of columns. Likewise, other systems which form discontinuous projections such as the interhemispheric cortico-cortical connections have also shown topographical precision in their innervation from the out start (Aggoun-Aouaoui et al., 1996; Hedin-Pereira et al., 1999). Moreover, laser-scanning photostimulation in brain slices combined with morphological analysis of axonal arbors has revealed that connections between layer 4 and layers 2/3 neurons develop with great specificity and without detectable pruning at the level of the cortical columns (Bureau et al., 2004).

These data prompt the question of which are the mechanisms governing this early columnar organization of neurons. As briefly noted above, cortical columns have been characterized by the existence of neurons sharing similar electrophysiological properties, involved in the processing of particular stimuli and distributed in discrete horizontal clusters along the cerebral cortex. Thus, the very first pre-requisite to link a given factor to the columnar organization of the cerebral cortex would be the capacity of this factor to organize neurons in discrete radial columns of neurons and to favor their interconnectivity.

Gap junctions have been recurrently implicated as players in the establishment of functional units (Yuste et al., 1992, 1995; Kandler and Katz, 1995). The coordinated calcium fluctuation patterns underlying gap junctional mediated communication were suggested to form the basis of functional cell assemblies in postnatal cerebral cortex. Blocking activity did not eliminate calcium functional domains suggesting that gap junctions may promote metabolic rather than activity related assemblies (Kandler and Katz, 1998). Recently, it has been shown that glial cells in layer IV of the somatosensory cortex form gap-junction coupled ensembles correlated to barrels (anatomical structures that display eletrophysiological responses to individual whiskers) showing that non neuronal cells may also be important players in the formation of cortical units (Houades et al., 2008).

It has been shown previously that since very early, during embryonic neurogenesis, cells in different stages of the cell cycle (mainly S and G2 but also G1) are gap junctionally coupled with radial glia forming columnar functional units with 15 to 20 cells (Bittman et al., 1997). It is possible that these cells belong to the same clone and their functional and metabolic coupling at this stage could be important for the later establishment of connections among themselves (see discussion about neuronal clones in the following section). It is also known that gap junctions regulate neuronal migration (Elias et al., 2007; Marins et al., 2009) and adhesive connections formed by gap junctions between migrating neurons and radial glia were shown to be important for gliophilic migration. Thus, gap junctions and other molecules that regulate migration may be important at later stages helping connect columnar neuronal networks whose identity may have been primed early within the VZ.

An important aspect of the formation of columnar structures is the radial axon-dendrite polarity established from the very beginning by neurons migrating attached to radial glia with their leading and trailing processes. During migration neurons derived from the cortical VZ grow an axon directed towards the white matter and later an apical dendrite that ramifies in layer I. The sterotypical polarity of pyramidal neurons, an important feature of columnar organization appears to partially derive from the phosphorylation of neurogenin 2 (NGN2), a proneural gene, which has a double function – as an important pyramidal neuron phenotype determinant and as a cytoskeleton organizer for the emergence of dendrites in the apical portion of these cells (Schuurmans et al., 2004; Hand et al., 2005). These functions appear to be independent. Molecules that guide migration and axon growth such as semaphorins may also be important to orient axon-dendrite polarization (Polleux et al., 2000) influencing each of these components in a differential manner (repulsing axons and attracting dendrites).

Recently, it has also been shown that the radial distribution of clonally related neurons in the developing cerebral cortex depends on the expression levels of Ephrin A receptors (EphA) and ephrin-As (Torii et al., 2009). By using loss- and gain-of-function strategies to manipulate ephrin-As and EphA7 expression, Torii et al. (2009) have provided compelling evidence indicating that EphAs and ephrin-As signaling controls lateral dispersion of cortical excitatory neurons, likely contributing to the generation of cortical columns. Interestingly, ephrins and Eph receptors have also been involved in the establishment of other maps in the cerebral cortex, such as retinotopy (Flanagan and Vanderhaeghen, 1998).

CLONES OF EXCITATORY NEURONS DISPERSE INTO INDIVIDUAL CORTICAL COLUMNS

The lateral dispersion of clonally related neurons could also contribute to the early specification of cortical columns by generating spatially restricted radial clusters of neurons, which would later receive afferent connections and become involved in specific tasks. According to this scenario, we could expect that neurons generated from the same progenitor would disperse into unique functional cortical columns. To the best of our knowledge, this possibility has not been directly tested. However, there is accumulating evidence indicating that sibling neurons keep spatial relationships and display connection preferences at least compatible with their tentative role in the organization of cortical columns.

In order to investigate the degree of neuronal dispersion during development, a technique has been used which allows the infection of few progenitor cells at early developmental stages using a replication incompetent retroviral vector carrying a reporter gene (Luskin et al., 1988, 1993; Price and Thurlow, 1988; Walsh and Cepko, 1988, 1992, 1993; Parnavelas et al., 1991; Luskin and McDermott, 1994; Mione et al., 1994, 1997; Kornack and Rakic, 1995; Reid et al., 1995; Gaiano et al., 1999; McCarthy et al., 2001; Reid and Walsh, 2002; Costa et al., 2009). Later, the progeny of those few infected progenitors can be identified by the expression of the reporter gene, allowing measuring the dispersion of neuronal siblings. These studies have been termed "cell lineage" or "clonal analysis" studies and we may use both terms interchangeably.

The first cell lineage studies have found controversial results regarding the radial distribution of sibling neurons (Luskin et al., 1988; Price and Thurlow, 1988; Walsh and Cepko, 1988). While Luskin and co-workers suggested that clonally related neurons occur in columns, the works by Price and Walsh suggested that clones of neurons do not form radial columns, but rather dis-

perse several hundreds of micrometers of cortex in the horizontal dimension. Several factors may have contributed for these divergent interpretations, such as the definition of clones based solely on the anatomical dispersion of cells (Luskin) or the expression of individual genetic tags by polymerase chain reaction (Walsh). For the reader interested in how these two variables may affect the conclusions of the cell lineage studies, we refer to a recent paper by Costa et al. (2009) where these issues were addressed.

Importantly, primary cell lineage studies were performed before the discovery of the massive tangential migration of GABAergic neurons in the developing brain (Marin and Rubenstein, 2001), what *per se* may have lead to misinterpretations about clonal relationship between cells. In fact, while the original population studies using tritiated thymidine to label post-mitotic cells have suggested that radial cell dispersion would be the primary mechanisms by which neurons could arrive to their final destination in the cerebral cortex (Angevine and Sidman, 1961; Rakic, 1974), it was only in the late 90s that tangential neuronal migration has been recognized as an important mechanism for settling GABAergic neurons in the cerebral cortex (Anderson et al., 1997). Nowadays, it is broadly accepted that, in the developing cerebral cortex of rodents, glutamatergic neurons migrate radially towards their final position in the cerebral cortex, whereas GABAergic neurons migrate tangentially (Marin and Rubenstein, 2003). Consequently, whilst radially migrating neurons could be distributed in arrays perpendicular to the pial surface, tangentially migrating ones disperse across different areas and no topographical relation has been detected between the ganglionic eminence VZ and the cortical destination of these cells. Rather the GE ventricular surface has been correlated to different types of interneurons (Fogarty et al., 2007; Xu et al., 2008). Therefore, it is possible that much of the tangential dispersion observed in early cell lineage studies was a consequence of tangential dispersion of GABAergic neurons (Walsh and Cepko, 1988, 1992, 1993; Tan et al., 1995).

At this point, it is important to cite that fate-mapping studies in the rodent cerebral cortex have provided compelling evidence indicating that glutamatergic and GABAergic neurons are indeed derived from separate pools of progenitors in the dorsal and ventral telencephalon, respectively. Using the Cre-lox system (Orban et al., 1992; Sauer, 1998), it has been shown that progenitors located in the dorsal telencephalon express the TF Emx1 and generate exclusively cortical excitatory neurons (Gorski et al., 2002), whereas those located in the ventral telencephalon express Nkx2.1, Lhx6, Gsh2 or Nkx6.2 and generate different subtypes of cortical interneurons (Fogarty et al., 2007; Xu et al., 2008). Thus, genetic fate-mapping studies indicate that the progeny of a single progenitor will comprise either glutamatergic or GABAergic neurons. Thus, these two types of neurons are very unlikely to be clonally related, at least in the rodent cerebral cortex.

Having that in mind, we have recently readdressed the issue of cell lineage in the developing cerebral cortex by using a combination of two silencing-resistant vectors carrying different reporter genes (Costa et al., 2009). We analyzed exclusively clones comprising spiny pyramidal neurons, so that the radial dispersion within clones of glutamatergic neurons, i.e. derived from dorsal telencephalic progenitors, could be accurately measured. Our results indicate that clonally related glutamatergic neurons generated from E13 progenitors do not disperse further than 280 μm in the adult cerebral cortex (**Figure 1**), what could very well be explained by the horizontal growth of the brain between the time of injection and analysis (Costa et al., 2009).

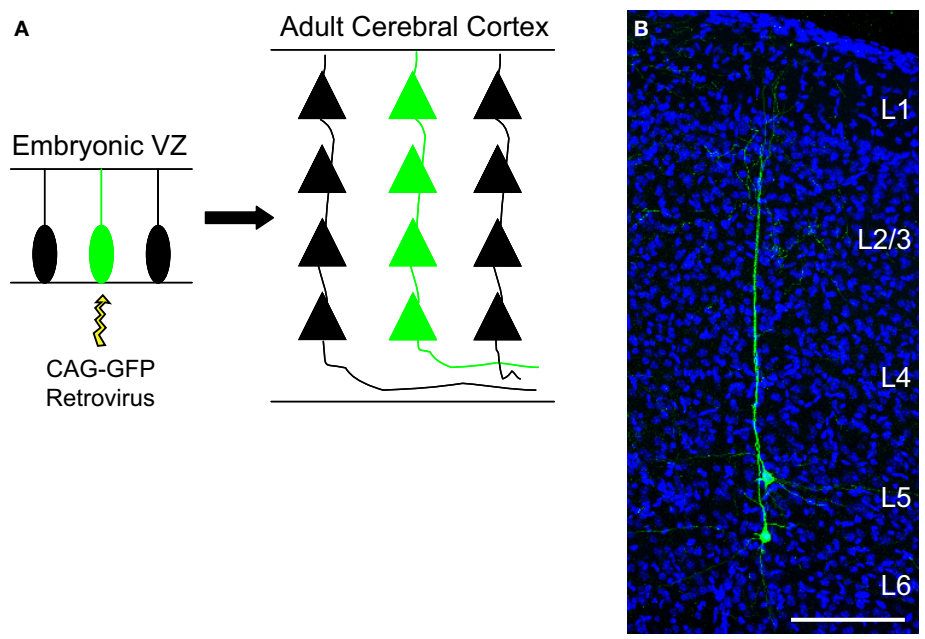

FIGURE 1 | Columnar distribution of sister neurons in the cerebral cortex. **(A)** Schematic representation of a single progenitor cell transfected by a retroviral vector and its subsequent progeny (green). **(B)** Coronal section of the adult mouse cerebral cortex labeled for GFP (green) and DAPI (blue) where two neuronal siblings can be observed. For this experiment, the retrovirus carrying the gene for the protein GFP was injected into the lateral ventricle of an E13 animal. Abbreviations: VZ, ventricular zone; CAG-GFP, green fluorescent protein encoding plasmid; L, layer. Calibration bar: 100 μm.

In fact, we found that most neuronal clones derived from E13 progenitors span 150–250 μm in the horizontal axis and contribute to all cortical layers generated after that embryonic stage, namely layers V, IV, and II/III. Mathematical extrapolations for injections performed at the onset of neurogenesis in the cerebral cortex (E10-11) suggest that neuronal siblings would not disperse more than 400–500 μm. Thus, both the radial and horizontal dispersion of excitatory neuronal clones fits well with the possibility that they could help to create a structural basis for the future specification of columns.

Concurrently, it has also been suggested that excitatory neurons generated from the same progenitor are more likely to establish synaptic connections than non-sibling neurons (Yu et al., 2009). By injecting EGFP-expressing retroviruses through the uterus into the lateral ventricle of mouse embryos at early neurogenesis, the authors were able to identify individual clones of pyramidal neurons, similar to the cells shown in **Figure 1**. Next, they performed simultaneous whole-cell recordings on two EGFP-expressing sister neurons and observed that these cells displayed unidirectional synaptic connections in 35% of pairs. In contrast, less than 7% of radially situated non-sister excitatory neurons were connected (Yu et al., 2009).

Taken together, these new lineage studies indicate that clonally related excitatory neurons not only keep a tight spatial relationship but are also capable of recognizing their siblings, either chemically or electrically, and establish functional synaptic connections.

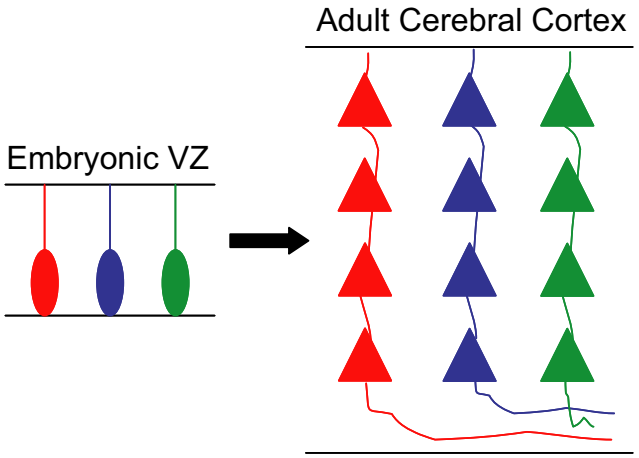

FIGURE 2 | Hypothetic model for the generation of functional units from individual progenitors. Schematic drawing showing three progenitor cells in the embryonic ventricular zone (VZ) expressing different sets/levels of transcription factors, labeled in red, blue, and green. Each of these cells generates a clone of pyramidal neurons that inherit analogous genetic information from the founder progenitor and are organized in discrete radial arrays in the adult cerebral cortex. The similar genetic pedigree of sibling neurons allows their recognition and establishment of synaptic connections, creating a microcircuit of clonally related glutamatergic neurons.

THE LINK BETWEEN PROGENITORS AND POST-MITOTIC NEURONS AS THE CELLULAR BASIS FOR THE GENERATION OF FUNCTIONAL CIRCUITS

Based on these findings, we would like to put forward a more tempting hypothesis, namely that transcriptional networks in cortical progenitors may help to establish functional units throughout the cerebral cortex by enabling these progenitors to generate neurons with similar electrochemical properties and high connectivity. According to this hypothesis, different levels and combinations of TFs expressed by discrete pools of progenitors would be responsible for the generation of individual microcircuits of sibling neurons, which would be able to recognize each other and establish synaptic connections. In other words, gene expression in individual cortical progenitors could influence the development of functional units throughout the cerebral cortex by generating small radial clusters of interconnected neurons (**Figure 2**), which in turn could be assembled together to generate functional minicolumns and columns.

The rationale behind our hypothesis is that neurons derived from the same progenitor are more likely to display similar chemical and physical properties, due to their genetic inheritance. Thus, sibling neurons would be more likely to recognize and respond stereotypically to the same molecular cues that could influence the early arrangement, metabolic coupling, and interconnectivity of those neurons within a single column. This ability is likely to rely on the expression of a similar set of surface molecules in sister neurons, which in turn could be controlled by TF networks operating in cortical progenitor cells.

But how molecules could contribute to specify the connection between sibling neurons? Sperry's theory (1963), known as chemoaffinity, proposed that molecules would be responsible for the wiring of neurons in the central nervous system. In fact, several groups have identified cell surface molecules involved in the patterning of neural circuits (Song and Poo, 2001). Yet, one important criticism to Sperry's theory has been the fact that the number of neurons and connections in the brain (about 10^{12} and 10^{15}, respectively, in humans) is far higher than the number of genes encompassed in the whole genome and, therefore, the number of different molecules would not suffice to specify all the neuronal connections. One possible solution envisioned by Sperry (1963) was the graded expression of cell surface molecules and their receptors, which has also been validated by recent findings (O'Leary et al., 1994). Such graded expression would lead to much higher combinatorial possibilities of cell responses than could be predicted by the number of signaling molecules and receptors. Indeed, it has been shown that a given ligand can elicit different responses in growing axons depending on the receptor complexes expressed in the target cell (Hong et al., 1999). Additional complexity is also added to the system when we consider the metabolic state of the axon. For example, the intracellular concentration of calcium can determine whether some axons are repelled or attracted by a given molecule (Hong et al., 2000). Thus, the number of genes in the cell genome clearly underestimates the repertoire of molecular combinations capable of dictating distinct cellular behaviors.

Furthermore, a large number of cell surface molecules can be generated from a limited number of genes in the nervous system through genetic rearrangements, such as the alternative splicing observed in the *Drosophila* gene *Dscam1* (*Down syndrome cell adhesion molecule*) (Schmucker et al., 2000; Wojtowicz et al.,

2004) and the mammal protocadherin family (Kohmura et al., 1998; Wu and Maniatis, 1999). Indeed, it has been proposed that *Dscam1* gene gives rise to 18,048 proteins that could control self-avoidance between neurites through isoform-specific homophilic binding (Wojtowicz et al., 2007), what clearly indicates that a limited number of genes may generate a far broader variety of molecular tags responsible for cell-to-cell specific recognition in the nervous system. Therefore, it is not entirely absurd to suggest that the connectivity preference between sister neurons may be mediated by the expression of a given set of proteins involved in cell–cell recognition.

But now, how could gene expression in progenitor cells influence that? One possibility is that different expression levels of TFs could control the expression of distinct sets of surface molecules allowing the recognition of sibling neurons. Although we are just beginning to understand this phenomenon, increasing evidence support the notion that (i) distinct expression levels of a given TF; (ii) combination of TFs; or (iii) interactions between TF and its cofactors in the same cell type can in fact lead to completely different biological outcomes, likely reflecting differential gene expression induced by the TF.

For instance, it has been shown that the expression level Pax6 in the developing cerebral cortex is essential for controlling the balance between proliferation and differentiation (Sansom et al., 2009). By using Pax6 gain- and loss-of-function strategies, Sansom and colleagues have shown that the Pax6-regulated networks operating in cortical progenitors are highly dosage sensitive, so that relative levels of Pax6 are key determinants for controlling whether VZ progenitors will self-renew, generate neurons or basal progenitors. Therefore, it is not entirely absurd to suggest that different levels of Pax6 (or any other TF) within cortical progenitors might also kick off individual genetic programs by their neuronal lineage, leading to the expression of molecules responsible for the recognition and connectivity of these neurons.

Another example of such diversity in cell-response to a single TF is the activation of specific target genes by REST (repressor element-1 silencing transcription factor) during neuronal subtype specification (Abrajano et al., 2009). In this study, the authors have shown by chromatin immunoprecipitation on chip (Chip-chip) that REST and its cofactor CoREST (corepressor for element-1 silencing transcription factor) modulate the expression of largely distinct gene profiles responsible for inducing and maintaining different neuronal subtype identities, such as cholinergic, GABAergic, glutamatergic, and medium spiny neurons. These data clearly indicate that the balance between a single TF and its corepressor can regulate complex and distinct gene networks underlying important cell behaviors, such as neuronal subtype specification.

Collectively, these data support the idea that TF networks could modulate the expression of genes encoding proteins involved in cell–cell recognition and, consequently, contribute for the capacity of sibling neurons to recognize each other and establish synaptic connections. In the future, it will be interesting to investigate which genes and molecules subsidize the high probability of connection between sister neurons. Notably, we have observed a similar phenomenon *in vitro*, further suggesting that the capacity of clonally related neurons to recognize each other is a cell-intrinsic property.

CONCLUDING REMARKS

It has been suggested that one important phenomenon for the increased cerebral complexity during evolution may be the multiplication of neuronal columns throughout the cerebral cortex (Rakic and Caviness, 1995). Here, we further refine this conjecture by suggesting that discrete alterations in the gene expression pattern during development may support this phenomenon by allowing a larger number of individual progenitor cells to generate individual and highly interconnected neuronal clones. In other terms, neuronal clones could be seen as fundamental blocks in the construction of brain circuits, upon which later influences brought by axonal growth, synaptogenesis and activity will act to establish the functional anatomy of the cerebral cortex (Sur and Rubenstein, 2005). These fundamental blocks could also be influenced by earlier factors, such as incoming afferent systems to cerebral cortex that have been shown to regulate the cell cycle length in the germinal zone and contribute to generate areal differences in the germinal zones (Dehay and Kennedy, 2007). Also in that direction, recent work has shown that horizontal interconnectivity between columns is important to stabilize columnar size (Kaschube et al., 2009). Therefore, although lineage relationship could be at the base of the columnar organization of the cortex, several environmental factors are able to regulate column size and determine the properties that will be processed by the functional unit.

As can be deduced from our previous discussion about the origin of glutamatergic and GABAergic neurons, our hypothesis apply exclusively to the generation of glutamatergic neuronal clones. In fact, there is no evidence supporting the notion that tangentially migrating GABAergic neurons would settle in the cerebral cortex in an orderly manner, reflecting their original position in the VZ of the ventral telencephalon. Further support to this notion comes also from recent transplantation experiments indicating that GABAergic neurons are rather plastic and may develop functional inhibitory circuits in the visual primary cortex despite their site of origin (Southwell et al., 2010).

Concluding, we suggest here that the development of individual clones of glutamatergic neurons is a fundamental step for the parcellation of the cerebral cortex. These individual clones could be seen as singular functional units, which will be assembled into more complex parcels, such as minicolumns or columns, under the influence of intrinsic and extrinsic signals. According to this view, the number of independent functional units throughout the cerebral cortex would be increased not only by the enlargement of progenitor pools (Caviness et al., 1995), but also by discrete changes in the combinatory levels of TFs expressed in the progenitor cells. Consequently, this transcription network would represent an important target in brain evolution.

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Dendritic bundles, minicolumns, columns, and cortical output units

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The search for the fundamental building block of the cerebral cortex has highlighted three structures, perpendicular to the cortical surface: (i) columns of neurons with radially invariant response properties, e.g., receptive field position, sensory modality, stimulus orientation or direction, frequency tuning etc., (ii) minicolumns of radially aligned cell bodies and (iii) bundles, constituted by the apical dendrites of pyramidal neurons with cell bodies in different layers. The latter were described in detail, and sometimes quantitatively, in several species and areas. It was recently suggested that the dendritic bundles consist of apical dendrites belonging to neurons projecting their axons to specific targets. We review the concept above and suggest that another structural and computational unit of cerebral cortex is the cortical output unit, i.e., an assembly of bundles of apical dendrites and their parent cell bodies including each of the outputs to distant cortical or subcortical structures, of a given cortical locus (area or part of an area). This somato-dendritic assembly receives inputs some of which are common to the whole assembly and determine its radially invariant response properties, others are specific to one or more dendritic bundles, and determine the specific response signature of neurons in the different cortical layers and projecting to different targets.

Keywords: pyramidal neuron, cerebral cortex, apical dendrite, projection

DENDRITIC BUNDLES: ORIGIN OF A CONCEPT

Although the radial arrangement of dendrites (Fifkova, 1970) and cell bodies (von Bonin and Mehler, 1971) had been previously noticed (quoted in Peters and Walsh, 1972) the first comprehensive descriptions of dendritic bundles in cerebral cortex appear to be those of Peters and Walsh (1972) and of Fleischhauer et al. (1972). The work in Peters' laboratory was motivated by the search of a morphological substrate for the cortical "columns" initially demonstrated by physiological methods by Mountcastle (1957) in the primary somatosensory cortex, by Hubel and Wiesel (1963) in the primary visual cortex, and by Abeles and Goldstein Jr. (1970) in the primary auditory cortex. The study was carried out in tangential sections of the primary somatosensory cortex (area 3) of the rat, and later extended to the visual cortex (see below), and led to the following seminal concepts. (i) "Clusters" of apical dendrites are clearly visible in tangential sections through layers IV and III; (ii) the number of dendrites in a cluster varies between 1 and 14, the distance between clusters between 50 and 150 μm; (iii) the clusters correspond to dendritic bundles originating in layer V, amplified by the addition of dendrites from more superficial neurons; (iv) the dendritic bundles correspond to aggregates of cell bodies visible in Nissl preparations. The work in Fleischhauer's laboratory was carried out in the sensory-motor cortex of rabbit and cat. The observations were compatible with what we summarized above, but with some emphasis on inter-area and interspecies comparisons and differences (reviewed in Fleischhauer and Detzer, 1975).

The serial section reconstructions of Massing and Fleischhauer (1973) revealed some complications in the topographical organization of dendrites within a bundle. Individual dendrites changed their neighborhood relations along a bundle; superficial dendrites could be added between the dendrites from deeper layers; and individual dendrites could bifurcate to neighboring bundles. Subsequent work, reviewed in Peters (1997) and Rockland and Ichinohe (2004) refined some of the concepts above and extended them to a number of different species (next section). In addition to the bundles organized around the apical dendrites of layer V neurons, separate bundles of layer VI dendrites were described (Sakai, 1985; Escobar et al., 1986). Although dendritic bundles are most easily seen in layers III and IV, with appropriate methods they can also be identified in layer II (Miyashita et al., 2009). In the visual cortex the mean spacing between modules was found to be 60 μm in the rat, 56 μm in the cat and 23 μm in the rhesus monkey (Peters, 1997). And the total number of bundles in the visual cortex was calculated to be 2.5 to 3.4 10^3 in the rat, 1.6 10^5 in the cat and 2.9 10^6 in the monkey. The physiological significance of the modules remained elusive, although their dimension would fit that of Mountcastle's (1997) minicolumns, and that of the orientation columns, at least in the monkey (see Section Functional Correlates of the Dendritic Bundles).

It was later found that myelinated axons are also organized in bundles; these bundles course close to those of the dendrites and at least some of them originate from neurons whose apical dendrites

are in a bundle (Peters and Sethares, 1996). This is a first indication that neurons in a dendritic bundle might send their axons to the same target as it will be shown below.

DENDRITIC BUNDLES ARE PRESENT BOTH ACROSS PHYLOGENESIS AND ONTOGENESIS

In the cerebral cortex of mammals, dendritic bundles can be found across cortical areas, including area 17 (rat, Peters and Kara, 1987; cat, Peters and Yilmaz, 1993), somatosensory (rat, Peters and Walsh, 1972; mouse, White and Peters, 1993), motor (mouse, Lev and White, 1997; rabbit, Fleischhauer et al., 1972) and prelimbic (rat, Gabbott and Bacon, 1996) cortex, with quantitative features possibly reflecting differences in the density of neurons (rat, Skoglund et al., 1996) or locale. For example, in the barrelfield of rodent somatosensory cortex, dendritic bundles are mostly located in the barrel walls and septa, avoiding hollows (mouse, Escobar et al., 1986).

The appearance of vertically oriented dendritic bundles seems to be closely related to the evolution of the mammalian neocortex as a multilayered structure. Dendritic bundles have been found in the cerebral cortex of different mammalian species, such as rodents (mouse, Detzer, 1976; Escobar et al., 1986; rat, Peters and Walsh, 1972; Winkelmann et al., 1975; Peters and Kara, 1987; Gabbott and Bacon, 1996), lagomorphs (rabbit, Fleischhauer et al., 1972; Schmolke and Viebahn, 1986; Schmolke, 1996), carnivores (cat, Fleischhauer, 1974; Ikeda et al., 1989; Peters and Yilmaz, 1993) and primates (Peters and Sethares, 1991), including humans (von Bonin and Mehler, 1971). In the opossum they were reported as unpublished data by Peters and Feldman (1973). In the lesser hedgehog tenrec, a mammal bearing one of the lowest neocorticalization indices, dendritic bundles can be found in all cortical areas as well, including paleocortex (entorhinal cortex) and archicortex (hippocampus) (Schmolke and Künzle, 1997). On the contrary, vertically arranged dendritic bundles are not found in the primitive cortex of turtles (Schmolke and Künzle, 1997).

Dendritic bundles are described as early as E16, when the cortical plate forms in the rat parietal cortex (Hirst et al., 1991) and throughout development (Peters and Feldman, 1973; Schmolke, 1989; Hirst et al., 1991). According to Peters and Feldman (1973), in Nissl preparations, the cell bodies tend to align in vertical rows separated from each other by bundles (clusters) of vertically oriented processes, i.e., developing dendrites in the upper layers and axons, in lower layers. This is particularly evident at the top of the plate, where the lateral separation is wider than more deeply. The arrangement in vertical clusters is apparent at E19-E21. Vertical dendritic bundles (clusters) are masked in the mature cortex by the extensive proliferation of the neuropile.

The dendritic bundling, from neurons in layer II, was enhanced in the barrelfield by the over expression of the neurotrophin NT-2, an effect paralleled by an increased dendritic branching in layer I (Miyashita et al., 2009).

METHODOLOGICAL AND DIMENSIONAL ISSUES

The definition of a dendritic bundle raises a number of methodological issues. The histological methods used include material prepared for electron microscopy and viewed in thin or semithin sections, the Golgi technique, staining with microtubule associated protein 2 (MAP2), a selective somato-dendritic marker, retrograde transport of lipophilic tracers and intracellular injection in slice preparations. Depending on the sensitivity and resolution of the technique, bundles can vary in the number and size of the constituent dendrites, and be complicated by the addition of oblique and basal dendrites. Indeed, the diameter and number of dendrites in a bundle can vary in different reports from the same author. This stresses the objective difficulty of defining the limits of the bundle, which, depending also on tangential location and depth, can be more or less sharp. In several papers the distance between bundles is mentioned but not the statistical size of the sample, nor if corrections were introduced for the tissue shrinkage.

In our study (Vercelli et al., 2004), we intended to use the retrograde transport of lipophilic tracers, but because only a few dendrites are labeled from each site of tracer application we needed to define strict criteria to include apical dendrites in a bundle. To this end we preliminarily quantified aspects of dendritic bundles in the rat visual cortex, based on MAP2 immunostained material. To identify apical dendrites in tangential sections, we measured the size of apical dendrites in layer III in coronal sections, where they could be traced from the cell body and therefore could be distinguished from the oblique branches. Having obtained an average size of 1.1–1.44 μm (depending on age) for apical dendrites, we drew maps of apical dendrites in tangential, MAP2-immunoreacted sections through layer III.

We transformed these maps into sets of points, one for each dendrite, whose coordinates were used to (i) eliminate as outliers all dendrites which were further than the maximal nearest neighbor distance (NND); this was set at 5 or 6 μm (depending on age) since 90% of measured NNDs between two dendrites in the same bundle fell in the range of 1–5 or 1–6 μm (depending on age); (ii) generate Dirichlet tessellation/Voronoi polygons for apical dendrites, to obtain objective, quantifiable criteria to consider them clustered (**Figure 1**). Briefly, a polygon was assigned to each point, corresponding to an apical dendrite, by joining the midpoints of the segments connecting the apical dendrite to its neighbors. High values (>64%) in the coefficient of variation for Voronoi polygons are suggestive of a clustered distribution of points, and this was the case for apical dendrites; (iii) in the next step, we assigned a point to each dendritic bundle, to generate Voronoi polygons to analyze their tangential spatial distribution. This procedure allowed quantifying density, NNDs, CV, average diameter, average center-to-center distance, and number of dendrites/bundle. The coefficient of variation of Voronoi polygons for dendritic bundles was very low (<36%), thus indicating a regular spatial distribution. The same procedure was used at all ages considered, with consistent results. By comparing data obtained at different ages, we observed that dendrites display a moderate increase in diameter between P3 and P30. Between those ages there is also a small increase in diameter of the bundles which seems more related to the increased dendritic size than to the number of dendrites in a bundle. There is a consistent increase in the NNDs between bundles, and a parallel decrease in the density of dendrites and dendritic bundles, probably reflecting the increase in the neuropil and glia.

This procedure produced smaller bundles than those observed by Peters and Kara (1987) in the same area and species, bearing 6–6.4 dendrites instead of 8, at a higher density and with a smaller

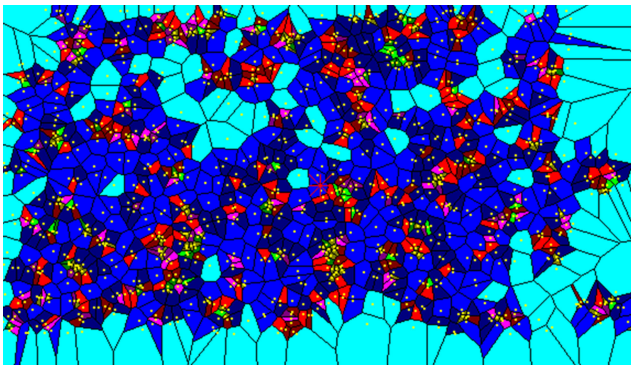

FIGURE 1 | Voronoi polygons drawn from tangential maps of MAP2-positive apical dendrites. Each polygon area delineates the territory of the map which is closer to the point than to any other point of the map. Yellow dots correspond to apical dendrites. Colors of polygons are related to their size (green the smallest, light and dark blue the largest). The clusterization of dendrites is obvious.

NND. However, the two sets of results are only partially comparable, since (i) we considered dendrites of smaller size (1.1 instead of 2 μm), (ii) we drew our maps in layer III instead of layer IV, (iii) we considered bundles consisting of at least two apical dendrites instead of three, and (iv) we did not consider layer V apical dendrites to be essential in forming a bundle. Interestingly, our data are very similar to those obtained by other authors in area 17 and 18 of the monkey (Peters and Sethares, 1996; Peters et al., 1997), and in motor and somatosensory cortex of mice (Lev and White, 1997).

SHIFTING CONCEPTS: DENDRITIC BUNDLES AS ASSEMBLIES OF TARGET-DEFINED NEURONS

The meaning of the dendritic bundles underwent a potential fundamental shift with the work of Lev and White (1997). They showed that, in the mouse area MsI, following injection of horseradish peroxidase in the contralateral hemisphere, all dendrites in a labeled bundle belonged to callosally projecting neurons, thus suggesting that dendritic bundles are target-specific. The concept that different dendritic bundles may comprise neurons with different outputs is in keeping with the observed heterogeneity of dendritic bundles: bundles differ in the size of the constituent dendrites and that not all apical dendrites from layer V enter into the composition of dendritic bundles, as stressed by Rockland and Ichinohe (2004).

Because in the course of development some callosally projecting neurons lose their callosal axon and establish connections in the ipsilateral hemisphere (Innocenti et al., 1986; Clarke and Innocenti, 1990; reviewed in Innocenti and Price, 2005) it seemed likely that callosally and ipsilaterally projecting neurons would participate in the same dendritic bundle. Alternatively, the composition of the dendritic bundles might change in development.

Therefore we decided to identify, at different ages, pyramidal neurons in the visual cortex of the rat projecting to different targets by retrograde axonal tracers (Vercelli et al., 2004). We used DiI and DiA as tracers in the developing animal, and fluoro-emerald (FE) and fluoro-ruby (FR) in adults, labeling callosally projecting neurons with one dye and neurons projecting either to (i) the ipsilateral cortex, (ii) the superior colliculus, (iii) the pons, (iv) the lateral geniculate nucleus, (v) the striatum with the other dye. Moreover, in some animals we labeled corticopontine and corticostriatal neurons with the two dyes, respectively. Lipophilic dyes such as DiI and DiA are used to trace connections in vitro in fixed material thus allowing a precise placement of the dye even in deep subcortical targets and in small brains. On the other hand, these dyes hardly diffuse in adult material, so we were obliged to use *in vivo* tracing with FE and FR in adult rats. To maximize the chance of detecting double labeling in a bundle we analyzed regions with the highest density of neurons labeled with each tracer.

Compared with MAP2-immunostaining, retrograde tracing visualizes only a small number of neurons projecting to a target, proportional to the size of the crystal of lipophilic dyes or to the injection size for FE and FR. Also, retrograde tracing is not as efficient as MAP2 immunostaining in visualizing apical dendrites. Therefore, it was likely that not all the apical dendrites in the same bundle could be labeled retrogradely, implying that they could be at higher NND than in MAP2-immunostained sections. For this reason, we used the maximal diameter of MAP2-immunostained bundles (28 μm) as cut-off distance to consider apical dendrites of neurons retrogradely labeled from different structures as participating in the same bundle.

Apical dendrites of neurons projecting to either corpus callosum, ipsilateral cortex or striatum as well as those projecting to pons and striatum were at a NND below this value indicating that they belonged to the same bundle. Of the 433 bundles, 30% containing apical dendrites of pyramidal neurons projecting to either ipsilateral or contralateral cortex contained both types of dendrites (**Figures 2A,C, and 3**), while 34% contained only dendrites of neurons projecting to ipsilateral cortex and 30% neurons projecting to contralateral cortex. In contrast, the distance between apical dendrites of neurons projecting to cortical targets, and of those projecting to subcortical targets, i.e., either superior colliculus, lateral geniculate or pons was never less than 30 μm, and peaked at 60 and 110 μm suggesting that they belonged to different bundles. Of 551 bundles in brains in which callosally projecting and corticocollicular neurons were labeled, 81 and 19% projected to either target, but none contained dendrites from both types of neurons (**Figures 2B,D, and 3**).

Our results are summarized in **Figure 4**. They strongly support the concept that dendritic bundles are target-specific. Moreover, the composition of dendritic bundles does not seem to depend on the age of the animal and is already established at P3.

FUNCTIONAL CORRELATE OF THE DENDRITIC BUNDLES: THE CORTICAL OUTPUT UNIT HYPOTHESIS

The search for the functional correlate of the dendritic bundles is of fundamental importance and has been the object of many speculations. One important preliminary question is that of the relation between the columns of radially aligned cell bodies, stained for example with the Nissl method, which have become known as minicolumns (Buxoeveden and Casanova, 2002), and the dendritic bundles. Dendritic bundles and minicolumns of cell bodies are closely related although not identical entities. The distance and the transverse diameter of both is roughly the same, i.e., about 50 μm in most areas and species. In reconstructions from a limited series of tangential sections the dendritic bundles can be mapped onto

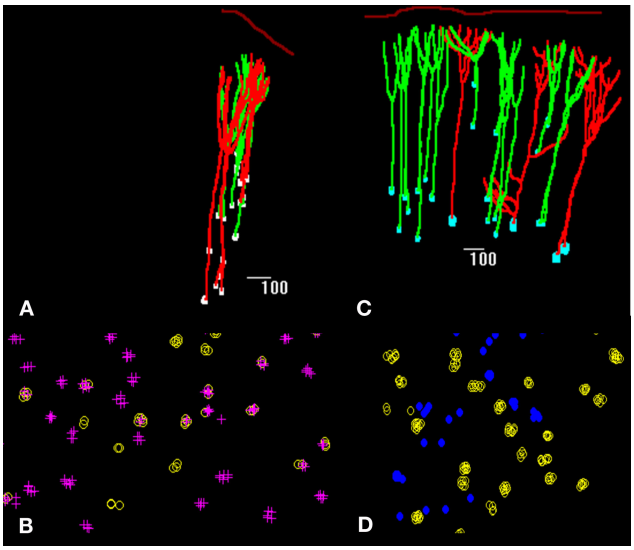

FIGURE 2 | Spatial distribution of retrogradely labeled pyramidal neurons in the rat visual cortex (A,C) and tangential maps of apical dendrites (B,D) drawn with Neurolucida software. In **(A)**, neurons projecting to the corpus callosum in red and neurons projecting to the ipsilateral cortex in green; in **(B)** apical dendrites of callosally projecting neurons are identified by crosses, those of ipsilaterally projecting corticocortical neurons by yellow circles. In **(C)**, neurons projecting to the corpus callosum in green and neurons projecting to the superior colliculus in red; in **(D)** apical dendrites of callosally projecting neurons are identified by yellow circles, those of corticocollicular neurons are identified by blue dots.

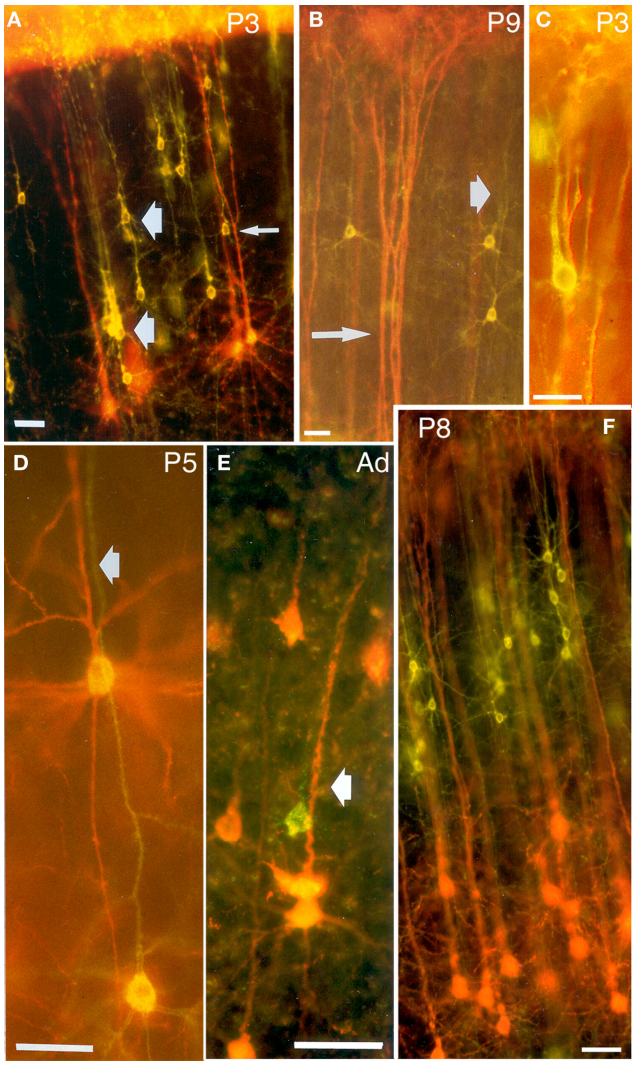

FIGURE 3 | Double exposures photomicrographs of coronal sections of double-labeled brains. In **(A,B)**, callosally projecting (DiA, yellow-green) and corticocollicular (DiI, orange-red) neurons form separate bundles in the visual cortex of P3 and P9 rats. **(A)** Bundles are indicated by thick arrows, whereas the thin arrow points to a callosally projecting neuron whose apical dendrite, at higher magnification, was found to be separated from the corticocollicular ones on the *z*-axis. **(B)** The thin arrow points to a corticocollicular dendritic bundle, and the thick one to a callosally projecting bundle. **(C)** A bundle comprising apical dendrites of callosally projecting (DiI, orange-red) and corticostriatal neurons (DiA, yellow-green), in a P3 rat. **(D,E)** callosally projecting (DiI and fluoro-ruby, respectively, orange-red) and ipsilaterally projecting corticocortical (DiA and fluoro-emerald, respectively, yellow-green) neurons in the visual cortex of P5 and adult rats establish common bundles (thick arrows). **(F)** Callosally projecting (DiA, yellow-green) and corticogeniculate (DiI, orange-red) neurons in the visual cortex of P8 rats. Scale bars, 50 μm (from Vercelli et al., 2004).

the underlying cell bodies of the minicolumn (Peters and Walsh, 1972; Peters and Kara, 1987). This shows that already in layer V and more so in layer III the dendritic bundles lie between the columns of cell bodies (see Figures 3, 7 and 11B in Peters and Kara, 1987). Indeed, as schematized in **Figure 5A**, cell bodies of neurons in a minicolumn can be seen to orient obliquely to engage their apical dendrite into the neighboring dendritic bundles already in layer V and more so in layer III (Peters and Walsh, 1972; Peters and Kara, 1987; Gabbott, 2003). Neurons in a minicolumn can send their apical dendrite to different bundles (Peters and Kara, 1987), some of them through bifurcating apical dendrites (Massing and Fleischhauer, 1973; Fleischhauer and Detzer, 1975), and neurons in separate minicolumns can send their dendrites to the same bundle (see Figure 3 in Peters and Kara, 1987). The progressive addition of dendrites to the bundle from depth to surface in cortex ("like onions held by their stem"; Peters and Kara, 1987) also indicates that the bundles collect dendrites from more than one minicolumn of cell bodies.

The evidence above is particularly relevant in view of our observation that the dendritic bundles consist of neurons with specific targets. At least nine types of target-specific bundles seem to exist (**Figure 4**) each of which, based on the MAP2 analysis contains 2–70 dendrites (6.5–8 on average, Peters and Kara, 1987; Vercelli et al., 2004). Since the minicolumns contain 80–100 cell bodies, out of which probably 60–80 are pyramidal neurons (Buxoeveden and Casanova, 2002) it would be in theory possible that one minicolumn gives rise to a whole set of 9, target specific bundles. However, the evidence quoted above suggests that this is not the case, and that, instead, the bundles are formed both by dendrites diverging from neurons to more than one bundle as well as by convergence of dendrites whose parent cell bodies lie in separate minicolumns. The dendritic bundles, therefore, appear to select neuronal subsets within one minicolumn of cell bodies and integrate them with those of neighboring minicolumns.

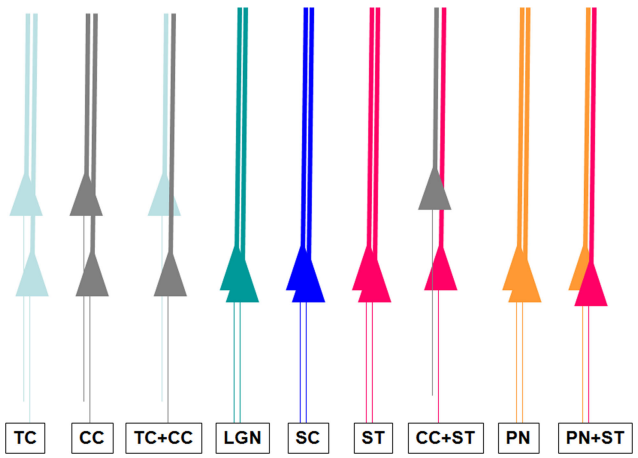

FIGURE 4 | Summary of the different types of dendritic bundles in the visual cortex, related to the axonal targets of their cell body. TC: neurons projecting to the ipsilateral cortex; CC: callosally projecting neurons; LGN: corticothalamic neurons; SC: corticocollicular neurons; ST: corticostriatal neurons; PN: corticopontine neurons.

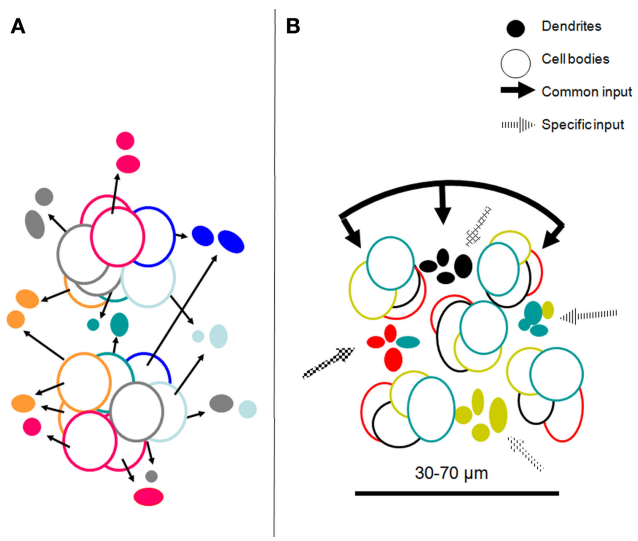

FIGURE 5 | (A) Topographical relations between minicolumns and dendritic bundles summarizing information from Massing and Fleischhauer (1973), Peters and Kara (1987; inspired by their Figure 3) and Vercelli et al. (2004). Dendrites are shown as "clusters" as they appear in transverse sections; the cell body outlines belong to neurons in minicolumns adjacent to the bundles; they are in different layers but are projected on the plane of the dendrites. The colors refer to specific targets or combinations of targets for each dendritic bundle and their parent somata (see **Figure 4**). **(B)** Model of a COU in layer III of V1 with bundles of apical dendrites of neurons projecting to four different targets (colors). The COU is defined as an assembly of apical dendritic bundles, large enough to include each of the outputs to distant cortical or subcortical structures, of a given cortical locale (area or part of an area), their parent somata and basal dendrites, and the portion of the neuropil which pertains to them. It receives a common input to the apical and basal (not shown) dendrites as well as specific inputs to the different dendritic bundles. The distance between the dendritic bundles and between minicolumns 30–70 µm is similar and corresponds to the minimal transverse diameter of an orientation column in the visual cortex, estimated electrophysiologically.

The search for the functional correlate of the dendritic bundles is constrained by the following evidence.

From the time of their discovery, the dimension of the dendritic bundles, about 50 µm (or less in the visual cortex of the monkey; see above) excluded that they might correspond to the large cortical columns, i.e., Hubel and Wiesel's ocular dominance columns or Mountcastle's receptive-field and tactile modality columns which average about 500 µm in diameter (reviewed in Mountcastle, 1997). The possibility was raised that they might correspond to narrower assemblies of neurons with radially invariant activation/response properties such as orientation (and direction) of stimulus motion, in the visual, motor and auditory areas.

A microelectrode driven into the cortex perpendicularly to the cortical surface will, as a rule, record activity from neurons at 50–100 µm from its tip, therefore collecting responses of neurons which belong to the same minicolumn of cell bodies as well as to different minicolumns. Even when a microelectrode records simultaneously from nearby neurons, these very often share some response properties but differ for others (Creutzfeldt et al., 1974; Molotchnikoff et al., 2007; Sato et al., 2007; Dahl et al., 2009), suggesting that they receive a common input, but each of them also distinct ones. Nearby neurons have largely overlapping basal dendritic arbors which could receive common input but their apical dendrites can segregate to different bundles (Krieger et al., 2007) which might receive distinct inputs.

Although some response properties of cortical neurons are radially invariant, and therefore must be determined by an input which, directly or indirectly, reaches neurons in different layers, many are not. In particular, receptive field structure and size in the visual cortex vary with cortical depth (Hubel and Wiesel, 1962; Gilbert, 1977) and are different in neurons projecting to different targets (Swadlow and Weyand, 1987; Niida et al., 1997). This could be easily achieved by a differential distribution of inputs to dendritic bundles containing different sets of output neurons.

Finally, the evidence that the radially invariant properties of cortical neurons are the result of intracolumnar computation is lacking. In fact at least one of the response properties used to define a cortical column, i.e., orientation specificity is preserved in spite of the deletion of the deep cortical layers V and VI (Innocenti et al., 1993). Orientation specificity most probably originates from spatially organized excitatory input reaching several output neurons, possibly sharpened by inhibition (Hubel and Wiesel, 1962; Wörgötter and Koch, 1991; Crook et al., 1998; Ferster and Miller, 2000).

We propose that neurons in the different layers of one minicolumn, projecting to different targets, send their apical dendrites to separate dendritic bundles where they join apical dendrites of neurons from neighboring minicolumns, projecting to the same target or combination of targets. An assembly of apical dendritic bundles, which includes each of the outputs to distant cortical or subcortical structures, of a given cortical locale (area or part of an area), their parent somata and basal dendrites, and the portion of the neuropil which pertains to them, constitutes a cortical output unit (COU). We assume that the COU receives excitatory and inhibitory afferents some of which common to all its constituent neurons in particular those reaching the dendritic tufts in layer I or the largely overlapping basal dendrites (Krieger et al., 2007). Other inputs are probably specific for a given bundle and therefore reach

specific sets of output neurons in the same or in different layers, determining their specific response properties (above). The dendritic bundling seems to offer two important advantages. It might minimize the length of the axonal arbors which contact specific neuronal classes and, in development it might simplify the axonal search and recognition of targets.

This view is summarized in the model shown in **Figure 5B**. The properties of the COU, match several aspects of Mountcastle (1997) definition of a cortical column: *"A cortical column is a complex processing and distributing unit that links a number of inputs to a number of outputs via overlapping internal processing chains. Cortical efferent neurons with different extrinsic targets are partially segregated; those of layers II/III project to other cortical areas, those of layers V/VI to subcortical structures. This suggests that the intracolumnar processing operations leading to those different output channels may differ in some fundamental way"*.

Unfortunately, the internal connectivity of the COU is incompletely known. Unlike what might have been expected, neurons of the same bundle are not more interconnected than neurons of different bundles (Krieger et al., 2007). Instead, there are preferential connections between clonally related neurons, presumably belonging to the same minicolumn (Yu et al., 2009). There are also preferential connections between certain output neurons, interestingly between corticocortical and cortico-tectal neurons, whose apical dendrites, as we have described, lie in separate dendritic bundles (mice, Brown and Hestrin, 2009).

MINICOLUMNS OF CELL BODIES, DENDRITIC BUNDLES, CLONES AND GENES

One strong appeal of the hypothesis that minicolumns might be the fundamental computational unit in the cerebral cortex is that it appears to link cortical morphology and function to development, since the minicolumns of cell bodies seemed to be the likely counterpart of Rakic's ontogenetic radial units (Rakic, 1988; discussed in Buxoeveden and Casanova, 2002). Thus, there might be a direct path from the genetic make-up of clonally related neurons, at least the pyramidal neurons, to their connectivity and response properties although these are further refined by activity. The evidence for lateral dispersion leading to clonal intermingling among radially aligned neurons (Torii et al., 2009) complicates this perspective. However, a more direct link between cortical morphology, function and development, applies to dendritic bundles. Newly generated neurons reach their final position in cortex by ascending with their apical dendrites in close contact to the radial glia (Rakic, 1988). It would not be surprising if neurons which select the same radial glia processes because of some genetically determined membrane signaling properties, would also, in adulthood, participate in the same dendritic bundle whereby receiving the same input, while with their axons they participate in the same axonal bundle, proceeding to specific pathways and targets.

PERSPECTIVES

The hypothesis that assemblies of dendritic bundles, and minicolumns of cell bodies constitute COUs, and that these in turn might represent computational building blocks of neocortex requires several structural and physiological refinements. The retrograde tracing experiments mentioned in this paper could not adequately describe the frequency and spatial arrangements of the different target-specific dendritic bundles and the related minicolumns. Therefore, the model shown in **Figure 5B** is tentative and is meant to illustrate a concept the details of which are still fuzzy. Moreover, the arrangements might be area-specific, and might differ e.g. between the primary areas and/or between primary and secondary-association areas. The type and origin of the inputs to the different components of the COU needs specifying. The relations between COUs and the classical cortical macro-columns needs clarifying as does the clonal origin and the genetic make up of the neurons participating in the bundles.

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Alterations in apical dendrite bundling in the somatosensory cortex of 5-HT_{3A} receptor knockout mice

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In various species and areas of the cerebral cortex, apical dendrites of pyramidal neurons form clusters which extend through several layers of the cortex also known as dendritic bundles. Previously, it has been shown that 5-HT_{3A} receptor knockout mice show hypercomplex apical dendrites of cortical layer 2/3 pyramidal neurons, together with a reduction in reelin levels, a glycoprotein involved in cortical development. Other studies showed that in the mouse presubicular cortex, reelin is involved in the formation of modular structures. Here, we compare apical dendrite bundling in the somatosensory cortex of wildtype and 5-HT_{3A} receptor knockout mice. Using a microtubule associated protein-2 immunostaining to visualize apical dendrites of pyramidal neurons, we compared dendritic bundle properties of wildtype and 5-HT_{3A} receptor knockout mice in tangential sections of the somatosensory cortex. A Voronoi tessellation was performed on immunostained tangential sections to determine the spatial organization of dendrites and to define dendritic bundles. In 5-HT_{3A} receptor knockout mice, dendritic bundle surface was larger compared to wildtype mice, while the number and distribution of reelin-secreting Cajal–Retzius cells was similar for both groups. Together with previously observed differences in dendritic complexity of cortical layer 2/3 pyramidal neurons and cortical reelin levels, these results suggest an important role for the 5-HT_3 receptor in determining the spatial organization of cortical connectivity in the mouse somatosensory cortex.

Keywords: column, neocortex, development, serotonin, Cajal–Retzius, reelin

INTRODUCTION

In various species and areas of the cerebral cortex, ascending apical dendrites of pyramidal neurons are organized in clusters also referred to as dendritic bundles (Fleischhauer et al., 1972; Peters and Walsh, 1972; Escobar et al., 1986; Peters and Kara, 1987; White and Peters, 1993; Lev and White, 1997; Ichinohe et al., 2003a; Vercelli et al., 2004). Also in the mouse somatosensory cortex, dendritic bundles of ascending apical dendrites of pyramidal neurons have been observed through several layers of the cortex and their properties described (Escobar et al., 1986; White and Peters, 1993). These dendritic bundles could form the basis of small functional units of vertically interconnected pyramidal and non-pyramidal neurons called cortical modules, yet so far functional evidence is lacking for this hypothesis (Peters and Sethares, 1996; Lev and White, 1997; Rockland and Ichinohe, 2004).

Recently, our group found that Cajal–Retzius cells, a population of cells which play an important role in cortical development by secreting the glycoprotein reelin (D'Arcangelo et al., 1995), express the 5-HT_3 receptor and that serotonin is the main excitatory drive for these cells (Chameau et al., 2009). Moreover, we showed that in the postnatal cortex, the serotonin 5-HT_3 receptor plays a pivotal role in the regulation of apical dendrite arborization of cortical layer 2/3 pyramidal neurons via a reelin-dependent pathway (Chameau et al., 2009). In mice lacking the 5-HT_{3A} receptor, we found a reduction in reelin levels and a hypercomplex dendritic tree of apical dendrites of layer 2/3 pyramidal neurons in the somatosensory cortex (Chameau et al., 2009).

To date, a number of factors have been implicated to play a role in dendritic bundle formation such as neurotrophins, cell adhesion molecules, gap junctions, and cytoskeletal changes (Ichinohe et al., 2003b; Miyashita et al., 2010). As will be discussed later, the formation of dendritic bundles in several areas of the cortex most likely results from a complex interplay between these factors. Interestingly, in the mouse presubicular cortex, also reelin is involved in the formation of modular structures (Nishikawa et al., 2002; Janusonis et al., 2004). In neonatal mice of which the serotonergic innervation to Cajal–Retzius cells was disrupted, reelin levels were decreased, and cortical column organization was also disrupted (Janusonis et al., 2004). Given this observation, we hypothesized that together with the alterations in reelin levels and dendritic complexity of cortical pyramidal neurons, mice lacking the 5-HT_{3A} receptor show alterations in the organization of dendritic bundles in the somatosensory cortex.

In the current study, we investigated the organization of dendritic bundles of ascending apical dendrites of pyramidal neurons in the somatosensory cortex of 5-HT_{3A} receptor knockout mice and compared them with wildtype mice. The properties of the dendritic bundles were compared in microtubule associated protein (MAP)-2 immunostained tangential sections from layer 3 of the somatosensory cortex. In addition, we investigated the number and distribution of reelin-secreting Cajal–Retzius cells in both groups.

MATERIALS AND METHODS

ANIMALS

Both male and female C57BL/6J wildtype and 5-HT_{3A} knockout mice (Zeitz et al., 2002) were used. In this study, 5-HT_{3A} knockout mice were maintained on the C57BL/6J background and back-crossed for at least 35 generations. From weaning (postnatal day 21) onward, offspring was group-housed (four per cage), with access to food and water *ad libitum* on a 12/12-h light dark cycle according to the guidelines of the animal welfare committee of the University of Amsterdam.

IMMUNOHISTOCHEMISTRY

At postnatal day (P) 4 and 14 and at 4 months of age, 3–8 mice per group were deeply anesthetized with a lethal i.p. dose of euthasol and perfused with 0.1 M PBS, pH = 7.4, followed by 4% paraformaldehyde in PBS. Brains were dissected and after 1 h of postfixation, hemispheres were separated and one hemisphere was flattened between two plastic foil-covered glass slides. After 24 h of postfixation, both intact and flattened brains were kept in 0.25% paraformaldehyde in PBS. Forty micrometer thick coronal and tangential slices were cut on a vibroslicer (Leica VT1000S) and collected in PBS.

For the MAP-2 immunostaining, both coronal and tangential slices from adult mice were rinsed with PBS and endogenous peroxidases were removed with 3% H_2O_2 in PBS for 30 min, then slices were incubated in 0.1% triton-X and 5% NGS in PBS for 1 h. Subsequently, slices were incubated overnight at 4˚C with 1:1500 MAP-2 HM-2 anti-mouse primary antibody (Sigma) in 0.1% triton-X and 5% NGS in PBS. The next day, slices were rinsed with PBS and incubated with 1:200 biotinylated sheep anti-mouse secondary antibody (Amersham) in 0.1% triton-X and 5% NGS in PBS for 1 h, rinsed again with PBS, incubated with ABC (Vector labs UK) for 2 h and visualized with a DAB (Invitrogen USA) reaction. After 3 min, reaction was stopped and slices were mounted with moviol. The next day, images of the somatosensory cortex were captured using a Zeiss FS2 microscope with a 20× objective: dry Plan Neofluor 20×/0.50 and with Image Pro software.

For the reelin staining, tangential slices from P4 and P14 mice were rinsed with PBS and incubated in 0.25% triton-X and 10% NGS in PBS for 1 h. Subsequently, slices were incubated overnight at 4˚C with 1:1000 G-10 anti-mouse primary antibody (Abcam) in 0.25% triton-X and 5% NGS in PBS. The next day, slices were rinsed with PBS and incubated with 1:250 Alexa 488-conjugated goat anti-mouse (Molecular Probes) in 0.25% triton-X and 5% NGS in PBS for 2 h. Again, slices were rinsed and mounted on glass slides with Vectashield (Vector labs UK). Images were scanned on a confocal microscope (Zeiss LSM 510). Objective: dry Plan Neofluor 20×/0.75.

DENDRITIC BUNDLE ANALYSIS

Tangential maps of 0.244 mm^2 through layer 3 (between 250 and 350 μm from the pial surface) were taken from sections immunostained for MAP-2. The mean dendrite diameter was determined as a mean of 20 apical dendrites per animal in layer 3 of the primary somatosensory cortex (in all sections barrels were visible in layer 4) and all apical dendrites were located (x–y-coordinates). This analysis was performed using ImageJ software (**Figure 1A**). The location data were further analyzed with custom-made software written in MATLAB (MathWorks version 2007b).

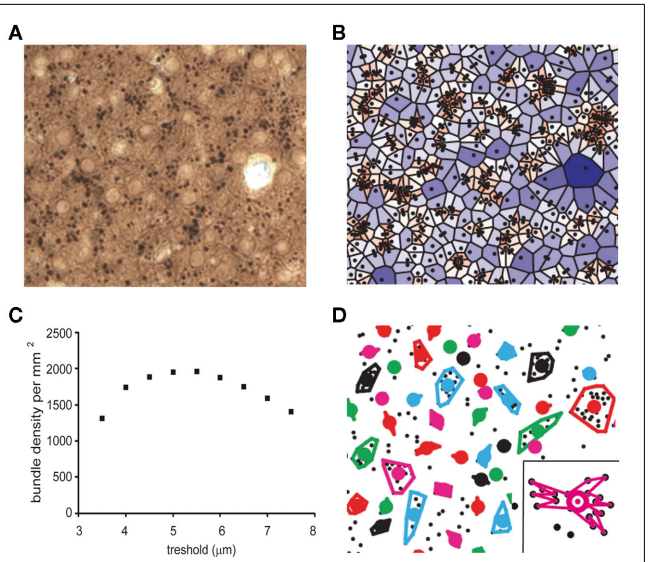

FIGURE 1 | Quantitative analysis of dendritic bundles in the mouse somatosensory cortex. (A) Typical example of a MAP-2 immunostained tangential section showing clusters of ascending apical dendrites of pyramidal neurons (black dots). **(B)** Surface polygons as determined by the Voronoi tessellation (borders excluded), are marked by a color scale that indicates surface. Values range from small (red) to large (blue). **(C)** The mean dendritic bundle density per mm^2 in layer 3 of the somatosensory cortex of wildtype mice remains similar when the nearest neighbor distance threshold varies from 4.5 to 6 μm. **(D)** Using a nearest neighbor distance threshold of 5.5 μm and a minimum of three dendrites per bundle, bundles are now determined and bundle surface can be calculated in two different ways: (1) as the surface of the polygon with the smallest circumference that includes all dendrites (as shown in figure) or (2) as the surface of the polygon that connects all dendrites continuously (see inset). Colored circles represent the location of the bundles (see Materials and Methods).

In order to analyze the spatial distribution pattern of dendrites and in particular to determine if they are clustered in bundles, we defined the "local dendritic density" by uniquely attributing each point in space to the closest dendrite. The mathematical procedure to accomplish this, is called a Voronoi Tessellation and it has been used before on neuronal structures (Duyckaerts and Godefroy, 2000). A Voronoi tessellation or Voronoi diagram partitions a plane with n points into n convex polygons such that each polygon contains exactly one generating point and every point in a given polygon is closer to its generating point than to any other. In the case of our tangential sections of the cortex, "points" are the cross-sectioned apical dendrites (**Figure 1B**) and each dendrite is uniquely linked to a fraction of the total surface, which defines the local dendritic density. The coefficient of variation (CV) for the complete tessellation is given by inverse ratio between the average polygon area and its SD. A (Monte-Carlo style) study by Duyckaerts et al. (1994) demonstrated that the CV value of a polygon surface is indicative for the nature of the spatial organization of the dendrites: a CV value larger than 0.64 implies that the dendrites are clustered while a value less than 0.36 indicates a

regularly distributed spatial organization of the dendrites, for CV equal to 0 the organization is completely regular. CV values that lie between 0.36 and 0.64 represent a randomly distributed spatial organization of the dendrites.

A second application of the Voronoi tessellation is that it links each dendrite to a unique set of neighbors, which implies that we can uniquely define and calculate the inter-neighbor distances. Using a simple threshold criterion in the inter-dendrite distance we can now determine which dendrites belong to the same bundle. If the threshold value is set too small, there will be no bundles and if it is set too high, they will all belong to the same bundle. The relation between threshold (range 3.5–7.5 μm) and calculated bundle density (**Figure 1C**) shows an optimum between 4.5 and 6 μm. This relation was similar for wildtype and 5-HT$_{3A}$ receptor knockout mice and for the rest of the study we choose a fixed threshold of 5.5 μm to define bundles. To prevent that single dendrites or pairs show up as bundles, we also required that a bundle needed to consist of at least three dendrites in order to qualify as such (**Figure 1D**). Around 75% of the dendrites were located in bundles, a substantial number of the excluded dendrites were located at the border of the investigated region.

Once a bundle was defined as consisting of n dendrites, its location (x_b, y_b) was calculated as its center of gravity: $x_b = (\Sigma x_i)/n$ and $y_b = (\Sigma y_i)/n$. Next bundle surface was calculated in two different ways: (1) as the area of the polygon that connects all dendrites continuously (see inset **Figure 1D**) or (2) as the area of the polygon with the smallest circumference that includes all dendrites (see other bundles **Figure 1D**). The first measure was systematically about 0.69 of the second one. We therefore present here only the second measure (see **Figure 5**). With these definitions a set of parameters was calculated that characterizes the bundles and their organization. The spatial aspect of the dendritic bundles was then assessed by a second Voronoi tessellation now performed using the bundle locations (x_b, y_b) as the starting points.

ANALYSIS OF THE DISTRIBUTION OF REELIN-POSITIVE CAJAL–RETZIUS CELLS

In tangential sections of 0.21 mm^2 through layer 1 immunostained for reelin, the location of all reelin-positive Cajal–Retzius cells was determined, comparable to the determination of dendrite location, using ImageJ software. On these coordinates a Voronoi tessellation was performed in order to determine the spatial organization of the Cajal–Retzius cells.

STATISTICAL ANALYSIS

All data are expressed as mean ± SE of the mean (SEM). Unless otherwise mentioned, values were compared with Student's *t*-test. $p < 0.05$ was used to indicate a significant difference (in graphs indicated as *).

RESULTS

In both wildtype and 5-HT$_{3A}$ receptor knockout mice, ascending apical dendrites of pyramidal neurons extend toward the pial surface in MAP-2 immunostained coronal sections of the somatosensory cortex (**Figure 2**). In these coronal sections of the somatosensory cortex, apical dendrites of pyramidal neurons from upper layers adjoin apical dendrites of pyramidal neurons from deeper layers to form clusters also known as dendritic bundles. A repetitive pattern of dendritic bundles of ascending apical dendrites through several layers of the cortex was visible, allowing quantification of the spatial organization of dendrites in MAP-2 immunostained tangential sections from layer 3 (located between 250 and 350 μm from the pial surface) of the somatosensory cortex (**Figure 3A**).

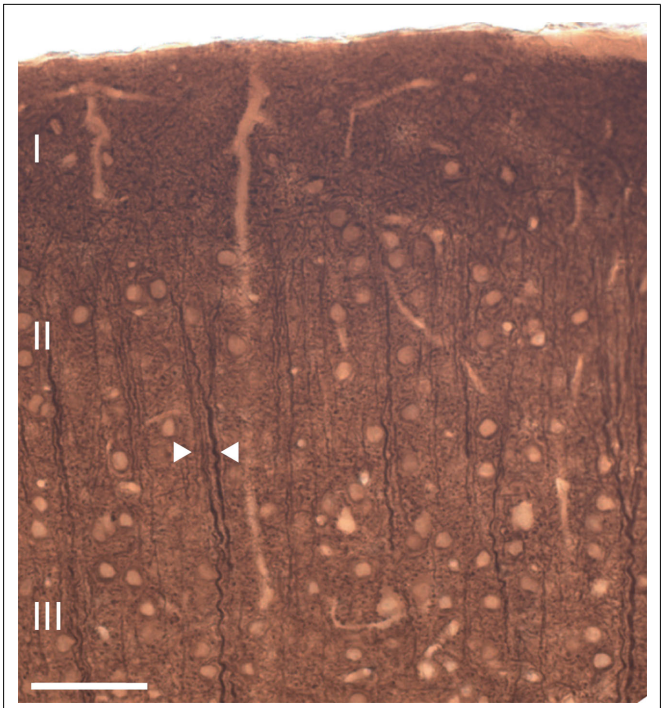

FIGURE 2 | Ascending apical dendrites of cortical pyramidal neurons through several layers of the mouse somatosensory cortex form dendritic bundles. A typical example of a coronal section of the mouse somatosensory cortex showing MAP-2 immunostained dendritic bundles of ascending apical dendrites. Arrows indicate an example of a dendritic bundle. Scale bar 50 μm.

A Voronoi tessellation was performed on the collected dendritic coordinates to analyze the spatial organization of the apical dendrites in wildtype and 5-HT$_{3A}$ receptor knockout mice (**Figure 3B**). The calculated polygon CV indicated that the distribution of apical dendrites in layer 3 of the somatosensory cortex of both wildtype and 5-HT$_{3A}$ receptor knockout mice was clustered (WT; 0.67 ± 0.02, $n = 8$, KO; 0.64 ± 0.02, $n = 5$, n.s.) with no indication that there were differences between the groups.

Subsequently, dendritic bundles were defined using a nearest neighbor distance threshold of 5.5 μm and a minimum number of three dendrites in a bundle (**Figure 3C**). To determine the properties of the spatial distribution of the dendritic bundles, a second Voronoi tessellation was performed on the location coordinates (x_b, y_b) of the above defined bundles (**Figure 3D**).

For wildtype and 5-HT$_{3A}$ receptor knockout mice, the polygon CV was 0.36 ± 0.01 ($n = 8$) and 0.34 ± 0.01 ($n = 5$) respectively, which for both situations is less or equal to 0.36 leading to the conclusion that the dendritic bundles are regularly organized

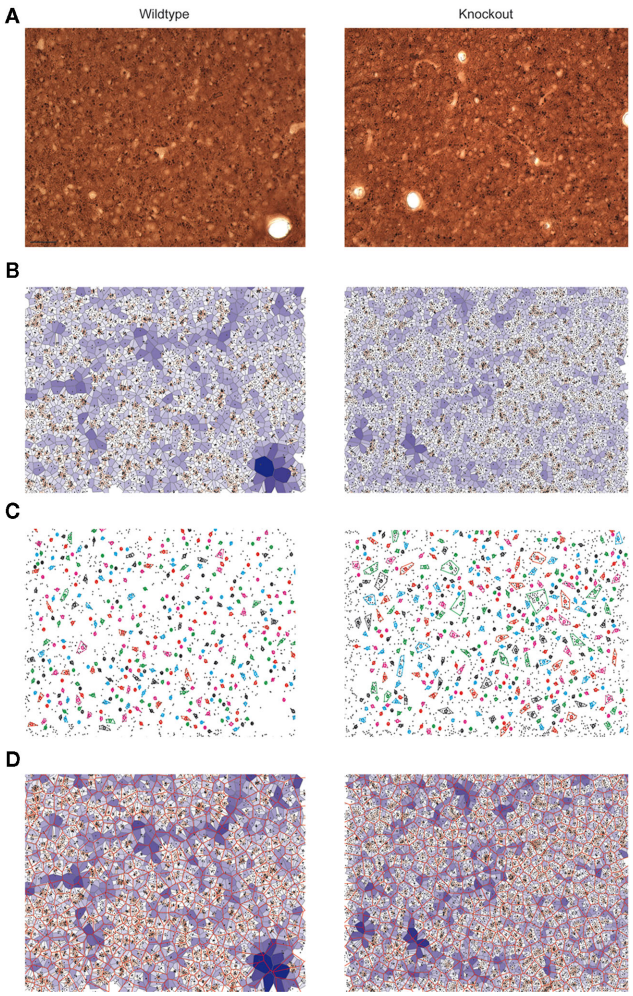

FIGURE 3 | Quantitative analysis of dendritic bundles in the mouse somatosensory cortex of wildtype (left) and 5-HT$_{3A}$ receptor knockout mice (right). Typical examples of **(A)** MAP-2 immunostained tangential sections show clusters of ascending apical dendrites of pyramidal neurons (seen from above as small black circles). **(B)** The polygons obtained using the Voronoi tessellation where the surface associated with each dendrite is indicated with a color scale from small (red) to large (blue). **(C)** Dendritic bundles as defined using a nearest neighbor threshold of 5.5 μm and a minimum of three dendrites in a bundle. Colored circles indicate the center of the bundles, drawn colored polygon indicates the outer circumference of the bundle and thus its size **(D)** Bundle polygons (red honeycomb structure) superimposed on the dendrite polygons. Scale bar 50 μm.

Table 1 | Quantitative analysis of dendritic bundle properties in tangential sections from layer 3 of the somatosensory cortex of wildtype and 5-HT$_{3A}$ receptor knockout mice.

	WT, $N = 8$	KO, $N = 5$
Average diameter dendrites (μm)	1.5 ± 0.01	1.5 ± 0.02
CV dendrites	0.67 ± 0.02	0.64 ± 0.02
Dendritic density (per mm^2)	14232 ± 881	16504 ± 1233
CV dendritic bundles	0.36 ± 0.01	0.34 ± 0.01
Bundle density (per mm^2)	1977 ± 103	1947 ± 76
Average dendritic bundle surface (μm^2)	31 ± 4	56 ± 9*
Average center-to-center distance (μm)	25.3 ± 0.6	25.6 ± 0.5
Number of dendrites per bundle	5.4 ± 0.2	6.7 ± 0.5

Analysis was performed on 0.244 mm^2 tangential sections from layer 3 immunostained for MAP-2 of eight wildtype and five knockout mice (mean ± SEM). CV, coefficient of variation. ∗Indicates a significant difference between wildtype and KO group ($p < 0.05$). Number of dendrites per bundle was tested using a Mann–Whitney test for non-parametric data.

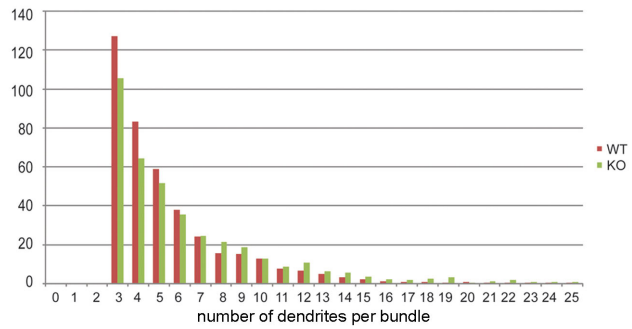

FIGURE 4 | Histogram showing the distribution of the number of dendrites per bundle in tangential sections of the somatosensory cortex of wildtype and 5-HT$_{3A}$ receptor knockout mice.

(Duyckaerts et al., 1994). In addition, dendritic bundles are not differently organized in both groups of animals. Numerical properties of the dendritic bundles defined above for wildtype and 5-HT$_{3A}$ receptor knockout mice are given in **Table 1**.

Within the optical resolution the mean dendritic diameter was the same in both groups. Also the number of dendrites per mm^2, the number of dendritic bundles per mm^2, and mean center-to-center distance between neighboring bundles were not different in wildtype and 5-HT$_{3A}$ receptor knockout mice.

To analyze the distribution of the number of dendrites per bundle a histogram was made (**Figure 4**). Although a tendency toward an increase in the number of dendrites per bundle in 5-HT$_{3A}$ receptor knockout mice was observed, the difference did not reach statistical significance. The analysis of the mean bundle surface of wildtype and 5-HT$_{3A}$ receptor knockout mice, calculated as described in the Section "Materials and Methods" (**Figure 5A**), showed that bundle surface was almost twice as large in 5-HT$_{3A}$ receptor knockout mice than in wildtype mice (WT; 31 ± 4 μm^2, $n = 8$, KO; 57 ± 9 μm^2, $n = 5$, $p < 0.05$, **Figure 5B**).

To investigate the spatial organization of reelin-positive Cajal–Retzius cells, we performed a reelin staining to visualize Cajal–Retzius cells. At P4 and at P14, a similar number of reelin-positive Cajal–Retzius cells could be observed in tangential sections from layer 1 of the somatosensory cortex of wildtype and 5-HT$_{3A}$ receptor knockout mice (**Figures 6A,B**). A typical decrease in the number of reelin-positive Cajal–Retzius cells between P4 and P14 was found in wildtype (46%) as well as in 5-HT$_{3A}$ receptor knockout mice (38%; **Table 2**).

A Voronoi tessellation was performed on the location coordinates of Cajal–Retzius cells and the polygon CV was calculated as described above for dendrites and bundles. Analysis showed that

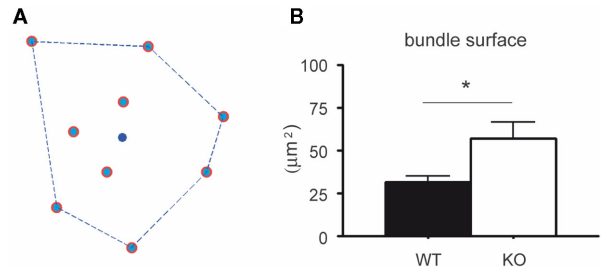

FIGURE 5 | Surface of the dendritic bundles in layer 3 of the somatosensory cortex of wildtype and 5-HT$_{3A}$ receptor knockout mice. **(A)** Bundle surface is determined by the polygon that connects dendrites in the bundle so that its circumference is the smallest one possible, while all dendrites in the bundle are localized within that polygon. The blue dot in the center represents the center of gravity (x_b, y_b). **(B)** The mean surface of layer 3 dendritic bundles in the somatosensory cortex is larger in 5-HT$_{3A}$ receptor knockout mice than in wildtype mice ($p < 0.05$).

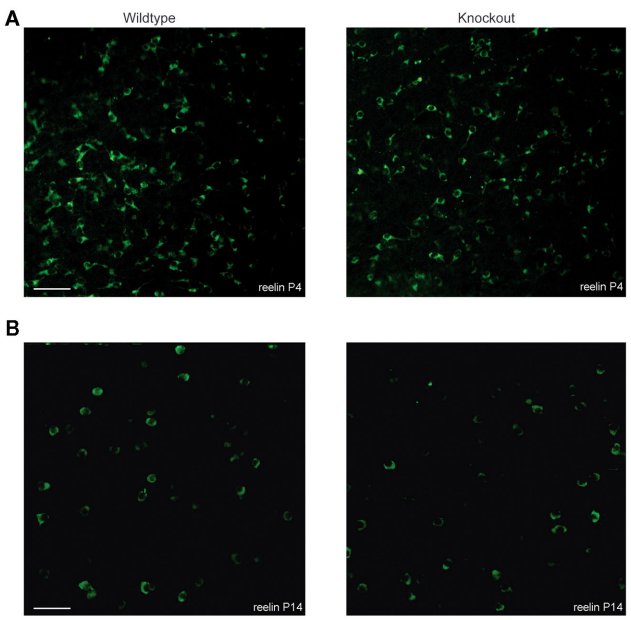

FIGURE 6 | Tangential sections showing the distribution of reelin-positive Cajal–Retzius cells in the somatosensory cortex of both wildtype and 5-HT$_{3A}$ receptor knockout mice. Typical examples of reelin-positive Cajal–Retzius cells in P4 **(A)** and P14 **(B)** tangential sections of the somatosensory cortex of both wildtype and 5-HT$_{3A}$ receptor KO mice. Scale bar 50 µm.

for both P4 and P14 wildtype and 5-HT$_{3A}$ receptor knockout mice, the distribution of reelin-positive Cajal–Retzius cells was random, not clustered, and not different between the two genotypes (**Table 2**).

Table 2 | Quantitative analysis of reelin-positive immunostained Cajal–Retzius cells in tangential sections of 0.21 mm^2 from layer 1 of the somatosensory cortex of wildtype and 5-HT$_{3A}$ receptor KO mice.

	WT, $N = 3$	KO, $N = 4$
Age	**P4**	**P4**
CV Cajal–Retzius cells	0.40 ± 0.01	0.38 ± 0.02
Cajal–Retzius cell density (per mm^2)	598 ± 54	656 ± 7
Age	**P14**	**P14**
CV Cajal–Retzius cells	0.40 ± 0.02	0.35 ± 0.04
Cajal–Retzius cell density (per mm^2)	275 ± 9	251 ± 21

Analysis was performed on reelin layer 1 P4 and P14 tangential sections of three wildtype and four knockout mice. CV, coefficient of variation.

DISCUSSION

In the current study, we quantified the spatial organization of ascending apical dendrites of pyramidal neurons which are organized in dendritic bundles in the somatosensory cortex of both wildtype and 5-HT$_{3A}$ receptor knockout mice. In layer 3 tangential sections of the somatosensory cortex of 5-HT$_{3A}$ receptor knockout mice, the average bundle surface is larger than in wildtype mice. To investigate dendritic bundle organization of both wildtype and 5-HT$_{3A}$ receptor knockout mice we used a similar approach as Vercelli et al. (2004) to show that in both groups the distribution of layer 3 apical dendrites was clustered while the distribution of the dendritic bundles was regular. In concordance with a study of White and Peters (1993), who reported a bundle density of 1918 bundles per mm^2 and an average center-to-center distance between 22 and 25 µm in the mouse somatosensory cortex, here it was shown that dendritic bundles which in the current study consisted of at least three dendrites, have a bundle density of 1978 bundles per mm^2 and mean center-to-center distance of 25 µm in wildtype mice. In line with previous studies, we also observed single dendrites that did not belong to a dendritic bundle. Earlier work from Peters and Kara (1987) and White and Peters (1993) have traced these dendrites and found that these dendrites were either from layer 4 neurons or layer 5 neurons that did not participate in bundles. Since we only analyzed layer 3 of the somatosensory cortex we assume that these single dendrites have the same origin as described earlier.

It has been reported that in the postnatal cortex serotonin is the main excitatory drive for 5-HT$_3$ receptor-expressing Cajal–Retzius cells and that reelin controls dendritic maturation of cortical pyramidal neurons (Chameau et al., 2009). During postnatal development, transient patches of serotonergic innervation have been observed in the rat somatosensory and visual cortex, suggesting a role for serotonin in orchestrating cortical cytoarchitecture (D'Amato et al., 1987; Nakazawa et al., 1992). Interestingly, in neonatal mice of which the serotonergic innervation to layer 1 Cajal–Retzius cells was depleted, reelin levels were decreased and cortical column organization was disrupted (Janusonis et al., 2004). Based on the current observation that dendritic bundle surface is larger in 5-HT$_{3A}$ receptor knockout mice, we suggest a relation between dendritic maturation and dendritic bundle formation in the somatosensory cortex and a role for reelin in regulating these events.

According to the hypothesis of Nishikawa et al. (2002), the distribution of Cajal–Retzius cells determines where dendritic bundles develop by forming reelin-rich cylindrical zones in which

migrating neurons and their dendritic extensions do not settle. By showing a similar number and distribution of Cajal–Retzius cells at P4 and P14 in layer 1 tangential sections of both wildtype and 5-HT$_{3A}$ receptor knockout mice, we ruled out the possibility that the observed differences in dendritic bundle surface were a mere consequence of a change in the number and distribution of reelin-positive Cajal–Retzius cells. Nevertheless, additional studies should be performed at several stages of development and in particular during the first postnatal days when the Cajal–Retzius cell density is the highest, to examine whether a relation between the distribution of Cajal–Retzius cells and the position of dendritic bundles exists or not. In these studies the spatial organization of Cajal–Retzius cells and dendritic bundles needs to be compared and the average distance between Cajal–Retzius cells and dendritic bundles needs to be determined.

It has to be mentioned that also a number of other factors have been implicated to play a role in dendritic bundle formation such as neurotrophins, cell adhesion molecules, gap junctions, and cytoskeletal changes (Ichinohe et al., 2003b; Miyashita et al., 2010). Additionally, it has been proposed that already during early development, sibling cells originating from a single radial glia cell, form the basis of radial columns of interconnected cells (Costa and Hedin-Pereira, 2010). In another study, it was shown that postmitotic pyramidal precursors that migrate into the medial limbic cortex during the first postnatal week, develop dendritic bundles in layer 1 (Zgraggen et al., 2011). Most likely, the formation of dendritic bundles in several areas of the cortex results from a complex interplay between these factors. However, whether one of these other factors has contributed to the changes in dendritic bundle surface in the somatosensory cortex of 5-HT$_3$ receptor knockout mice remains elusive.

In the cortex, information processing occurs through local cortical microcircuits which show both interlaminar and intralaminar connections (Thomson and Bannister, 2003). It has been suggested that dendritic bundles of ascending apical dendrites of cortical layer 5 pyramidal neurons form the center of cortical modules of vertically interconnected neurons which share functional properties (Peters and Sethares, 1996; Mountcastle, 1997). Labeling studies in both the visual and motor cortex suggested that pyramidal neurons from the same bundle project to the same target, thereby supporting the idea that dendritic bundles are functionally related (Lev and White, 1997; Vercelli et al., 2004; Innocenti and Vercelli, 2010). However, in another study it was shown that synaptic connectivity is independent of apical dendrite bundling (Krieger et al., 2007). Only when vertically aligned pyramidal neurons originate from the same radial glia cell and are thus siblings, they prefer to form synaptic connections (Yu et al., 2009). Although investigation about the functional relevance of dendritic bundles in the cortex is still ongoing, it remains interesting to speculate about the functional consequences of alterations in apical dendrite bundling as observed in the current study in 5-HT$_{3A}$ receptor knockout mice. The fact that in 5-HT$_{3A}$ receptor knockout mice the surface of these bundles is increased, could imply that connectivity between neurons has changed which could lead to alterations in information processing in the cortex of these mice. However, if indeed alterations in information processing in 5-HT$_{3A}$ receptor knockout mice would be observed, they might also be a consequence of the previously observed alterations in dendritic complexity of cortical layer 2/3 pyramidal neurons (Chameau et al., 2009).

In conclusion, the results from the current study show that in the somatosensory cortex of 5-HT$_{3A}$ receptor knockout mice, dendritic bundle size is different from wildtype mice. This finding, together with previously observed differences in dendritic complexity of cortical layer 2/3 pyramidal neurons and cortical reelin levels, suggests an important role for the 5-HT$_3$ receptor in determining the spatial organization of cortical connectivity in the mouse somatosensory cortex.

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Neocortical layer 6, a review

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This review attempts to summarise some of the major areas of neocortical research as it pertains to neocortical layer 6. After a brief summary of the development of this intriguing layer, the major pyramidal cell classes to be found in layer 6 are described and compared. The connections made and received by these different classes of neurones are then discussed and the possible functions of these connections, with particular reference to the shaping of responses in visual cortex and thalamus. Inhibition in layer 6 is discussed where appropriate, but not in great detail. Many types of interneurones are to be found in each cortical layer and layer 6 is no exception, but the functions of each type remain to be elucidated (Gonchar et al., 2007).

Keywords: cortex, layer 6, anatomy, histology, electrophysiology, pharmacology, development, review

INTRODUCTION

This review of neocortical layer 6 focuses on primary sensory regions, largely because layer 6 has been more thoroughly studied in these regions than in motor, or association areas. This is not to say that layer 6 has been comprehensively investigated in any region, or that it is possible to define all aspects of its structure and function, in any region. In fact, for many reasons, layer 6 has been studied in rather less detail than layers 3, 4 and 5 and it has been necessary here to correlate information from a range of different types of study, different cortical regions and different species, in an attempt to place the knowledge we have in something approaching a functional context. These correlations have been hampered by the fundamental limitations of each technique. For example, in many *in vivo* extracellular recording studies, the type of neurones recorded could not be identified. This limits the conclusions that can be drawn about the response properties of the several sub-types of layer 6 neurones and any structure-function relationships that might pertain. A number of elegant anatomical studies form the essential platform upon which much of the discussion here resides, but all too few functional studies have even attempted to place their findings in this context.

Layer 6 remains something of an enigma. Some of the cells in this layer receive direct thalamo-cortical input, placing layer 6 with layer 4 as a sensory input layer. It is also, however, an important output layer, from which large descending projections to many thalamic nuclei arise. Moreover, the several subclasses of corticothalamic neurones constitute only some 30–50% of the pyramidal cells in layer 6. Layer 6 corticocortical (CC) cells form another large group of pyramidal cells that send long horizontal axons which form connections across cortical columns and cortical areas, eg. somatosensory and motor. The fourth major class of pyramidal cells projects to the claustrum in addition to sending long horizontal axons through the deep cortical layers. At the end of each section is a summary in italics.

Abbreviations: A1, primary auditory cortex; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; bHLH, basic helix-loop-helix [a transcription factor]; CASK, calcium/calmodulin-dependent serine protein kinase 3 [multidomain scaffolding protein with a role in synaptic transmembrane protein anchoring]; CC, corticocortical; CHL1, neural adhesion molecule close homolog of L1; CT, corticothalamic; EPSP, excitatory postsynaptic potential; GABA, γ-aminobutyric acid; GluR, glutamate receptor; mGluR, metabotropic glutamate receptor; neuroD2, neurogenic differentiation 2 [a neurogenic bHLH protein]; Npn1, Neuropilin1 [a semiphorin receptor]; NR2B, N-methyl-D-aspartate receptor type 2B; nRT, *nucleus reticularis thalami* or reticular nucleus of the thalamus; Pom, posterior thalamic group; S1/Sm1, primary somatosensory cortex; Tbr1, a neurone-specific T-box transcription factor; V1, primary visual cortex; VB, ventrobasal nucleus of the thalamus; VPm, ventral posteromedial nucleus of the thalamus

A NOTE ON NOMENCLATURE

In the literature, discussion of different regions of thalamus uses the terms primary sensory, or 'specific' to describe the thalamic nuclei or regions that receive direct excitatory input from the periphery, eg. from the retina, or from the trigeminal nucleus. Regions that receive sensory input indirectly, via the cortex, have often been rather loosely termed 'non-specific' or association regions. In this review, the term 'primary sensory' is used to describe those thalamic regions that receive sensory input directly from the periphery. Similarly, to assist those less familiar with the cytoarchitectonically identifiable regions of sensory and association cortex, the term primary sensory cortex is used broadly here to describe those regions that receive thalamo-cortical input from primary sensory regions of thalamus, eg. V1 (primary visual cortex, Brodmann's area 17), SmI (or SI, somatosensory, areas 1–3), or AI (auditory, areas 41, 42). Secondary sensory refers to those cortical regions that receive sensory information directly from primary regions and association regions of cortex, rather loosely to define cortical regions that receive sensory information via cortex and 'non-specific', or association thalamus.

DEVELOPMENT OF LAYER 6

EARLY DEVELOPMENTAL DIFFERENTIATION OF CORTICOCORTICAL AND CORTICOTHALAMIC PYRAMIDAL CELLS IN LAYER 6

Early data from spontaneous mouse mutants indicated that the basic neuronal phenotype reflects the birth-date of a neurone, relatively independently of its subsequent laminar position. In a review

of these data, Caviness and Rakic (1978) concluded that neurones *attract* appropriate thalamic input rather than being equipotent on their birthday and then specified, as one type of cell or another later, by the type(s) of input they receive. Layer 6 is the first neocortical layer to develop (Rakic, 2009, for an excellent review of cortical development) and the cortico-thalamic (CT) pyramidal cells of layer 6 may be the earliest pyramidal cells to populate the developing neocortex (for distinctive features of CT and CC cells in adult cortex see below and **Figure 1**).

Pyramidal and spiny stellate cells are born in the ventricular zone and to reach their destination in the cortex, they are first guided to the cortical plate. Here, later born cells destined for more superficial layers, must pass the earlier born neurones of the deep layers as they migrate radially to their final positions. The finding that the cortex develops 'inside out' in mice lacking the Reeler gene (reln) prompted a large body of work on the development of cortical lamination and the involvement of the secreted signal, Reelin, its receptors and their downstream signalling pathways that control/promote both the migration and termination of migration (Huang, 2009; Rakic, 2009, for reviews). Unlike pyramidal cells, cortical interneurones are born in the medial and caudal ganglionic eminence (MGE and CGE) in the ventral forebrain and, in primates, in the subventricular zone. From these regions they migrate tangentially through the cortical marginal zone and along the subventricular/intermediate zone, to reach their final positions by radial migration within the cortex.

The selective expression by the L6 CC pyramidal cells, of latexin (a carboxypeptidase A inhibitor: Arimatsu et al., 1999a, expressed predominantly by glutamatergic neurones, Arimatsu et al., 1999b) allows two major pyramidal cell classes, CC and CT, to be distinguished during embryonic and postnatal development. The CT cells of the second somatosensory cortex of the rat are born earlier (on or before embryonic day 14, E14) than the CC cells (on or after E15) (Arimatsu and Ishida, 2002). Regional target preferences of these two cell classes have also been explored in co-culture studies in which the latexin-positive, CC cell axons readily invaded and innervated explants of other cortical regions, but failed even to invade dorsal thalamic explants. These thalamic explants were, however, readily innervated by co-cultured, latexin-negative CT cell axons (Arimatsu and Ishida, 2002). Latexin-positive CC cells are reported to contribute to so called 'feed-back' connections from sensory association cortical areas to primary sensory cortical regions, but rarely to 'feed-forward' callosal or intra-hemispheric connections (Bai et al., 2004).

It therefore appears that whether the axon of a layer 6 pyramidal cell will, as the cell develops, project subcortically and innervate the thalamus, or whether it will remain confined to the cortex and form long horizontal corticocortical connections, is determined at- and even possibly by- the time of the neurone's birth, before it migrates to the primordial cortex.

THALAMOCORTICAL PATHWAY DEVELOPMENT

Many of the first neurones of the embryonic cortex are born in the subplate. They are the first cortical neurones to receive input from the thalamus, long before layer 4 develops, but most of them will disappear as the cortex matures (Friauf et al., 1990). Transient connections between afferent axons and subplate neurones help to guide subsequent innervation patterns, with the subplate acting as a substrate for competition, segregation, and growth of these afferents, those from the brain stem arriving first, then those from the basal forebrain, the thalamus and finally from the ipsi- and contra-lateral cerebral hemispheres. As these fibres enter the cortical plate, the subplate zone disappears, leaving just a few cells of subplate origin scattered within the subcortical white matter (Kostovic and Rakic, 1990).

How the ordered, topographically precise targeting of thalamic fibres, from primary sensory thalamic nuclei to appropriate primary sensory regions of the neocortex, is controlled, is still a subject of intense interest. A wide range of cell-recognition and guidance molecules, such as the semaphorins, are fundamentally important here. Semaphorins are proteins, some membrane bound, some secreted, that inhibit the growth of axons that bear the appropriate receptor, deflecting them from regions rich in these proteins. For example, although thalamocortical pathways subserving other modalities appear to organise normally, axons from the LGN fail to innervate the developing visual cortex, at the appropriate time, in the absence of Semaphorin-6A. Visual cortex becomes innervated by somatosensory thalamic afferents instead. However, axons from the LGN do eventually reach the visual cortex, albeit via unconventional routes and successfully compete with those from somatosensory regions for postsynaptic targets (Little et al., 2009). This implies that final connectivity patterns are determined before and, to an extent, independently of, subplate sorting. It is, however, clear that the time at which each events occurs, in relation to others, is also critically important in the appropriate maturation of the brain and subplate sorting may play critical roles here.

Projections from the ventrobasal nucleus of the thalamus (VB) to the somatosensory cortex also utilize semaphorins. This pathway is disrupted by deletion of the Semaphorin 3A receptor Npn1 (Neuropilin1), or a protein associated with Npn1-triggered growth cone collapse (CHL1, or neural adhesion molecule close homolog of L1). In Npn1 knockouts, axons from VB shifted caudally and innervated the visual cortex (Wright et al., 2007).

Early topographic sorting of thalamocortical axons, as they grow towards the cortex through the ventral telencephalon (future basal ganglia), requires Ephrins and their receptors. This molecular recognition system uses thalamic axon receptor- and cortical neurone ligand-expression and contributes to interareal topographic mapping of thalamocortical axons within the developing cortex (Dufour et al., 2003).

Subplate neurones are the first immature cortical neurones to appear and they form some of the earliest connections with subcortical structures, receiving, for example, the first synaptic inputs from the developing thalamus. Some controversy appears to surround precisely how important the subplate is in controlling the orderly arrangement of incoming and outgoing axons, since there is evidence that even originally misdirected axons can eventually reach their preordained targets. Whether all aspects of maturation proceed normally when this input is delayed, however, is unclear. Axon guidance molecules, particularly members of the semaphorin family are associated with the orderly arrangements of thalamic afferents as they invade the developing cortex, deflecting growth cones from inappropriate paths.

CORTICOTHALAMIC CELL PATHWAY DEVELOPMENT

The subplate also appears to play a role in guiding cortical efferents, some subplate neurones acting as pioneers for pathways such as the corticothalamic pathway (McConnell et al., 1989). However, the numbers of subplate and layer 6 neurones retrogradely labelled from the thalamus are very low at E43–44 in the ferret, when a powerful projection from layer 5 is already invading the thalamus. The subplate and upper layer 6 neurones have migrated to their final positions by E36 (a few days before birth, gestation lasts 38–44 days in the domestic ferret), but wait for another 2–3 weeks before projecting beyond these regions. Over the next few days to weeks, these cells innervate their final target regions and eventually, the descending projection from layer 6 CT cells overtakes the layer 5 innervation of the thalamus (Clascá et al., 1995). The projections from these two layers are, of course, also differently distributed in the mature cortex (see below and **Figure 4**).

Within the cortex, the local axonal arbours of the layer 6 CT cells also continue to develop slowly, barely invading layer 5 by P13–15 in the ferret and continuing to branch in layer 4 up to P35 (Callaway and Lieber, 1996). For those more familiar with kitten/cat development, the critical period, which lasts from week 4 to 6 in the kitten visual cortex, peaks later in the ferret, between postnatal weeks 5 and 8 (Issa et al., 1999). The sublayer-specific ramifications of layer 6 CT cell axons in layer 4 (Wiser and Callaway, 1997, see also **Figure 3**) therefore approach mature distributions during the critical period.

In mice, CT cell axons reach and invade the thalamus between E14 and 15, with subplate neurones being the first to arrive, a day or two later than the thalamic fibres reach the cortex. By E20, ie. at birth, thalamocortical relay cell axons have branched extensively in the deep cortical layers (Auladell et al., 2000). In mouse somatosensory cortex, a Ca^{2+}-regulated basic helix-loop-helix (bHLH) transcription factor, Neurogenic Differentiation 2 (neuroD2), is required for the appropriate segregation of the terminal arbours of layer 6 CT cell axons within layer 4 barrels. In neuroD2-null mice, barrel organization is disrupted and synaptic transmission is defective (Ince-Dunn et al., 2006).

A range of transcription factors are also involved in regulating the descending projections from CT cells. It appears that the transcription factor SOX5 is required for the down-regulation, in the subplate and in layer 6, of Fezf2 and Bcl11b. These zinc finger genes are transiently expressed in all newly postmigratory early-born neurones and their down-regulation is necessary for the maturation of layer 6 neurones and for establishing their mature axonal projection patterns. Amongst other abnormalities, layer 6 neurones remain immature without SOX5 and the axons of both subplate neurones and layer 6 CT cells become mis-directed to the hypothalamus (Kwan et al., 2008). Fezf2 and Ctip2, the transcription factor that is its major down-stream effector, regulate the projections, whether corticocortical or subcortical, of deep layer neurones (Shimizu and Hibi, 2009). Tbr1 (a transcription factor that interacts with CASK and regulates reelin expression) is highly expressed in early-born glutamatergic cortical neurones, like those of layer 6. Mice that are deficient in Tbr1, in addition to decreased expression of Reelin and a reeler-like cortical migration disorder, demonstrate errors in the thalamocortical, corticothalamic, and callosal projections (Hevner et al., 2001).

Although neurones have arrived in the subplate and in layer 6 before layer 5 begins to develop, at first the axons from layer 5 innervate the developing thalamus more densely, being overtaken by layer 6 CT cell axons only later. The local axonal arbours of layer 6 cells are also relatively slow to develop, reaching fully mature ramifications and sublayer specificity during the postnatal critical period. A number of transcription factors have been shown to regulate the maturation of layer 6 neurones and the organisation of their cortical and subcortical axonal projections. Without one or other of these factors, or their effectors, misdirection of pyramidal axons to inappropriate targets occurs.

IDENTIFICATION OF MATURE LAYER 6 PYRAMIDAL CELL CLASSES

STRUCTURAL CHARACTERISTICS OF THREE MAJOR CLASSES OF LAYER 6 PYRAMIDAL CELLS

A detailed comparison of the layer 6 pyramidal cells that project to the thalamus (CT cells) and those that provide only corticocortical projections (CC cells) in rat somatosensory cortex, demonstrated that there are striking structural differences between these two broad cell classes. Put simply, CT cells are fairly short, upright pyramids with narrow local axonal arbours that project up towards more superficial layers, while CC cells include a range of atypical dendritic morphologies: inverted pyramids and bipolar cells as well as short upright pyramids. Unlike CT cells, CC cells have long, horizontally oriented axons that remain confined to the deep layers. From somatosensory cortex, for example, these branches project to the second somatosensory or motor cortices, or to the corpus callosum. **Figure 1** summarises the main distinguishing features of the different classes of layer 6 pyramidal cells.

Layer 6 CT cells can be further sub-divided. For example, in rodent somatosensory cortex, those that project only to the primary sensory, ventral posteromedial nucleus of the thalamus (VPm) are found in upper layer 6 (Bourassa et al., 1995) and have a well developed apical dendritic tuft and a narrow, vertically oriented axonal arbour, with drumstick-like appendages, both of which terminate in layer 4 (Zhang and Deschênes, 1997). CT cells that project to the posterior thalamic group (Pom), which does not receive primary sensory input, as well as to the VPm are found in deep layer 6. These are shorter upright pyramids with both their axons and their apical dendrites ramifying and terminating in layer 5 (Zhang and Deschênes, 1997). This sublayer selectivity in CT morphology and target selection, is seen in a wide range of species and in all primary sensory cortical regions. Simplistically, upper layer 6 CT cells selectively and therefore reciprocally innervate the region of primary sensory thalamus from which they receive direct input. These CT cells also innervate the GABAergic, inhibitory *nucleus reticularis thalami* (nRT, or reticular nucleus of the thalamus) (Bourassa and Deschênes, 1995), while the shorter, lower layer 6 CT cells innervate interlaminar nuclei and/or regions of association thalamus affiliated with the primary sensory modality, in addition to providing reciprocal innervation to primary sensory thalamic nuclei (Deschênes et al., 1998; Llano and Sherman, 2008, see also **Figure 4**). These shorter CT cells do not, however, innervate nRT. In cat visual cortex, some of these shorter CT cells with complex receptive fields, project to the supra-granular layers, ie.

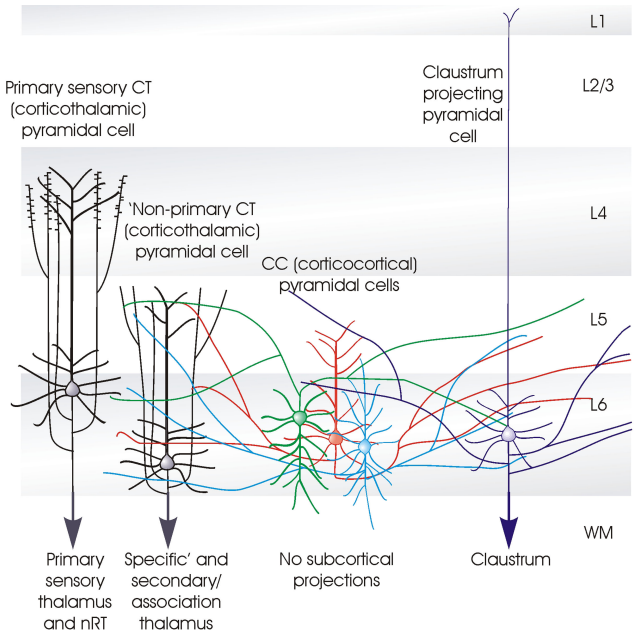

FIGURE 1 | Cartoon summarising some of the distinctive features of the different classes of layer 6 pyramidal cells. The term 'specific' is used here to identify the CT (corticothalamic) cells that project exclusively to primary sensory, or 'specific' thalamic nuclei (or regions) and to the (nRT). Both the apical dendrites of these 'specific' CT cells, and their axons ramify in layer 4. The axons of 'non-specific' CT cells ramify in layers 5 and 6 and sometimes in layers 2/3. These shorter CT cells innervate both 'specific' and 'non-specific' or association regions of thalamus, but not the nRT. CC (cortico-cortical) pyramidal cells do not project below the cortex. Both their dendrites, which display a range of morphologies and their long horizontally oriented axonal arbours are confined to layers 5 and 6. The axons of claustrum projecting pyramidal cells are also horizontally oriented and confined to the deep layers, but these cells have long slender apical dendrites that reach layer 1 with little branching.

layers 2 and 3, which, like layer 5, are rich in complex cells (Hirsch et al., 1998). They also project to ventral, rather than dorsal LGN and to the konicellular layers of the geniculate nucleus and to extra-geniculate thalamus (for reviews, see Lund, 1988; Fitzpatrick, 1996; Callaway, 1998). Another feature that distinguishes CT cells in primate V1 from their near neighbours is a significantly more dense innervation of their somata by inhibitory synapses (Lund et al., 2001)

A third group of layer 6 pyramidal cells that project to the visual claustrum was first identified in cat (similar morphologies have since been described in rat) by retrograde labelling with fluorescent latex microspheres and were found to differ from both CT and CC cells in their dendritic and axonal arborizations. Claustrum-projecting cell axons resembled those of CC cells, with long, fine, horizontal projections largely confined to layer 6 and lower layer 5. However, unlike other layer 6 cells, claustrum projecting pyramidal cells have very long, slender apical dendrites that reach layer 1, with little if any branching in layer 4 and at best a meagre apical tuft (Katz, 1987) (for morphometric analysis of Golgi-labelled layer 6 neurones see: Chen et al., 2009).

These structural differences have allowed the three major classes of layer 6 pyramids, CT, CC and claustrum-projecting cells, to be identified following dye-labelling during *in vitro* recordings, at least tentatively. Since major differences in the physiology of the cells and their synaptic connections have been identified, this differentiation has proved extremely valuable.

There are three major classes of layer 6 pyramidal cells that can be distinguished by the shapes of their axonal and dendritic arbours; the upright corticothalamic or CT cells with vertically oriented axons that ascend to layer 4 or 5 and a well developed apical dendritic tuft in layer 4 or 5; the CC cells which come in a range of different shapes from short upright pyramids, to bipolar cells with axons that do not leave the cortex, or, typically, ascend above layer 5, but extend for long distances horizontally and the claustrum projecting pyramids with a long slender apical dendrite and long horizontally oriented axons confined to the deep layers.

THE ELECTROPHYSIOLOGICAL CHARACTERISTICS OF LAYER 6 PYRAMIDAL CELLS CORRELATE WITH THEIR STRUCTURAL SUBTYPE.

The electrophysiological properties of CC and CT cells differ sufficiently to allow them to be distinguished in healthy mature preparations. CC cells have a strongly phasic response to square-wave depolarizing current injection. One to three, short interval spikes are elicited at the start of a depolarizing pulse, but additional current elicits no further firing. For those more familiar with the hippocampus, these responses somewhat resemble those of the granule cells of the dentate gyrus. A large, systematic study correlating physiology and morphology has not yet been performed and it is possible that more subtle differences between for example, bipolar, inverted and upright CC cells may emerge. However, as a broad class, almost all CC cells and indeed all claustrum projecting cells subsequently identified morphologically, displayed this very strongly adapting electrophysiological signature.

In striking contrast, CT cells have a near tonic firing pattern. They display some spike frequency adaptation in response to prolonged depolarization, but typically, do not cease firing while the cell is above firing threshold. Interestingly, the firing properties of these two broad classes of layer 6 pyramidal cells and the short term dynamics of their synaptic outputs could combine to ensure a powerfully phasic output from CC cells, but a maintained, or even, at some outputs, an augmenting response at CT cell outputs (see below and Mercer et al., 2005).

Electrophysiologically, both CC cells and claustrum projecting cells display powerful spike frequency adaptation in response to maintained depolarization. In contrast, CT cells display a weakly adapting firing pattern, maintaining near tonic firing. In mature preparations, these characteristics allow neurones to be tentatively identified during intracellular recordings.

INPUTS TO LAYER 6

In a retrograde labelling study, the most profuse, longer distance, cortical inputs to layer 6 of the whisker barrel field (part of primary somatosensory cortex in rodents) came from motor cortex, with sparser inputs provided by other cortical somatosensory regions, thalamic afferents (from VPm, Pom and the intralaminar nuclei) and the claustrum (Zhang and Deschênes, 1998).

LOCAL CIRCUIT CONNECTIONS TO LAYER 6 NEURONES

In rat thalamo-cortical slices, electrical stimulation in the thalamus elicited short and long latency monosynaptic EPSPs (excitatory post-synaptic potentials) in layer 6 pyramidal cells and interneurones. The

EPSPs elicited in interneurones being larger than those in pyramidal cells. The short latency events, concluded to be due to the thicker, more rapidly conducting thalamocortical inputs, exhibited paired pulse depression, while the presumed local circuit inputs, from antidromically activated CT cells, exhibited paired pulse facilitation. A small population of EPSPs, studied using paired recordings in layer 6, all exhibited paired pulse depression, leading the authors to conclude that local connections made by CT and CC cells may exhibit different short term synaptic dynamics (Beierlein and Connors, 2002).

This difference between CC and CT outputs was confirmed with dual intracellular recordings in adult rat and cat layer 6, in which the pre- and post-synaptic cells were labelled with biocytin/HRP and therefore identifiable (Mercer et al., 2005; West et al., 2006). All connections made by the axons of presynaptic CT pyramidal cells, whether onto another pyramid, or onto an interneurone, exhibited facilitation. This is highly unusual for pyramidal outputs. In all other layers, pyramid to pyramid connections 'depress' (eg. Thomson et al., 1993; Thomson, 1997; Thomson, Bannister, 1999) as do pyramidal inputs onto several classes of interneurones, including many of those that are immuno-positive for parvalbumin or CCK (eg. Thomson et al., 2002; Ali et al., 2007; Bannister and Thomson, 2007) and those that are immuno-positive for VIP (vasoactive intestinal polypeptide) (Porter et al., 1998). More typically, the only strongly facilitating outputs of pyramidal cells are those onto specific subclasses of interneurones (Deuchars and Thomson, 1995; Thomson et al., 1995; Markram et al., 1998), such as those that are immuno-positive for somatostatin (Kawaguchi and Kubota, 1996). In contrast, the local circuit outputs of layer 6 CC pyramidal cells onto other pyramidal cells and the two connections onto interneurones recorded, as well as the outputs of claustrum projecting pyramids, were all depressing (Mercer et al., 2005). Fluctuation analysis demonstrated that both the facilitation and depression observed were of presynaptic origin, ie. due to a change in release probability '*p*' (West et al., 2006).

It was also possible to demonstrate that a binomial model of release described these connections well and to compare the binomial parameters, '*p*', '*q*' (quantal amplitude) and '*n*' (number of release sites) obtained from fits of the model to experimental data, for different types of cortical connections (Brémaud et al., 2007). Pyramid to pyramid connections in layer 6 made by CC and CT axons, had very similar quantal amplitudes (0.37 mV ± 11 mV *vs* 0.37 ± 0.18 mV), which were smaller than those in layer 3 and layer 5, but larger than those in layer 4 in rat. However, CT and CC outputs produced significantly different estimates for '*n*' (CC 9.9 ± 12.6 mV *vs*. CT 2.7 ± 1.3) and '*p*' (CC 0.61 ± 0.14 *vs*. CT 0.28 ± 0.03).

In summary, CC pyramids innervate other pyramids much more frequently (>4×) than CT cells do. The connections made by CC cell axons with their near neighbours involve larger numbers of release sites that, at low frequencies, release transmitter with a higher probability and therefore display paired pulse and brief train depression. The outputs of CT cells onto other pyramids typically utilise fewer release sites and, with a lower release probability at low frequencies, exhibit facilitation.

Another striking difference between the connections made by CC and CT cell axons was their target preference. CT cells rarely innervated other pyramidal cells, while CC cells rarely innervated interneurones. Claustrum projecting pyramidal cells appeared to resemble CC cells in these respects, preferentially innervating pyramidal cells. The differences were not due simply to the amount of axon in layer 6, despite the profound differences in axonal arbour shape and the differences in the total length of axon in layer 6 for these cells classes. The total length of axon within the virtual sphere in which the cell pairs were recorded was not significantly different between the classes (Mercer et al., 2005). This degree of selectivity in target choice has not previously been described for intra-laminar connections in other layers. It has been reported for inter-laminar connections. For example, layer 3 pyramidal cells innervate layer 4 interneurones almost as commonly as layer 4 spiny cells do, but layer 3 pyramidal cells rarely, if ever, innervate layer 4 spiny cells (pyramidal and spiny stellate cells) (Thomson et al., 2002; Bannister and Thomson, 2007). A similar pattern is apparent in connections between layer 3 and 5 (see Thomson and Bannister, 1998; Thomson and Lamy, 2007, for review).

Layer 6 CC cells and claustrum projecting cells innervate layer 6 pyramidal cells of all classes with a relatively high 'hit rate'. They very rarely innervate layer 6 interneurones, however. The opposite is true for layer 6 CT cells which rarely innervate other layer 6 pyramids, but frequently innervate inhibitory interneurones.

CONNECTIONS TO LAYER 6 FROM OTHER CORTICAL LAYERS

Unlike layers 3, 4 or 5, layer 6 does not receive elaborate, focussed axonal arbours from spiny, excitatory cells in other layers. Instead, the descending axons of many layer 3 and layer 4 pyramidal cells traverse layer 6 with little or no branching, while the sparse, but very long, horizontally oriented axons of deep layer pyramids provide substantial opportunities for cross-columnar and inter-areal connections within the deep layers. The simple predictions that might be drawn from the above, ie. that layers 2, 3 and 4, would provide only a small and narrowly focussed excitatory input to layer 6, while layers 5 and 6 would provide a more powerful and possibly laterally extensive input, are, to a large extent, borne out by functional studies (see **Figure 2**).

In a caged glutamate study in which the inputs to single dye-filled layer 6 neurones were activated, Zarrinpar and Callaway (2006) documented the laminar profiles of inputs to layer 6. Pyramidal cells whose dendritic arbours resembled those of upper layer 6 CT cells, ie. the pyramidal cells that project to primary sensory thalamic regions, received inputs from layers 4, 5B and 6. Short, tufted, upright pyramids (ie. CT cells that may also project to thalamic regions that do not receive primary sensory input) received the majority of their excitatory input from within layer 6, with only a minor component from 5B. CC cells also appeared to receive almost all their inputs from the deep layers: inverted pyramids near exclusively from layer 6, bipolar cells from layer 5A, 5B and 6, with a very small input from layer 4, while non-tufted putative upright CC cells received minor inputs from layer 2/3, a very small input from layer 4, but again, their major excitatory inputs came from layers 5 and 6. Layer 6 multipolar interneurones also received most of their excitatory input from within layer 6, but some input, indeed more than to any other layer 6 cells, came from layer 2/3. Although the layer, or even the sub-layer of origin of cortical inputs to identified target neurones can be relatively clearly distinguished with this approach, the class(es) of presynaptic cell(s) involved

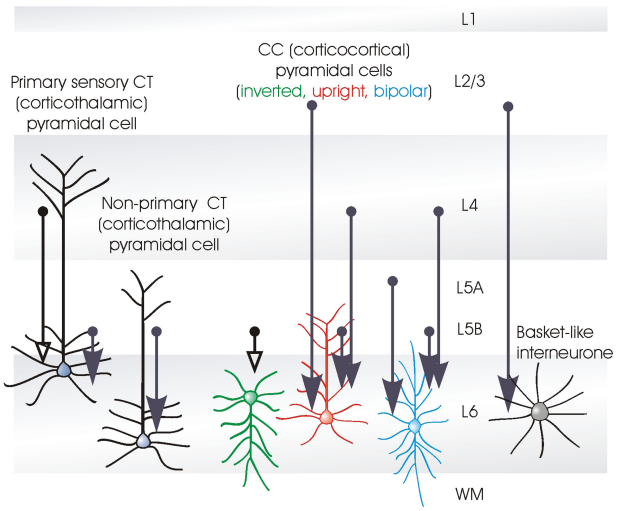

FIGURE 2 | Interlaminar connections that contribute to activation of layer 6 pyramidal neurones. The thickness of the arrows indicates the relative strength of each input (largely based on Zarrinpar and Callaway, 2006). The types of connections that have been confirmed with dual intra-cellular recordings are indicated by open arrowheads. The majority of intracortical excitatory input to layer 6 comes from the deep layers (5 and 6), some involving long, cross-columnar and interareal horizontal axons. In addition to inhibition from layer 6 interneurones, fast, proximally targeting, descending inhibition originates in some large layer 4 basket cells, while slower, dendritic inhibition may come from layer 4 double bouquet cells. The preferred cellular targets of these axons remain to be identified.

cannot be unambiguously identified. In some cases, probable cellular source(s) of a given input can be surmised by correlating with other studies, but unless both neurones are unambiguously identified, uncertainty remains.

Unambiguous identification requires dye-filling and full reconstruction. This has only rarely been achieved for inter-laminar connections involving layer 6 and the only published examples are for inputs to layer 6 from layer 5 (Mercer et al., 2005). In that study, two layer 5 pyramid to layer 6 pyramid pairs were described (one in cat, one in rat). The postsynaptic cells were both inverted CC-like pyramidal cells in layer 6 and the presynaptic layer 5 cells were a small adapting pyramidal cell (in cat) and a large tufted, intrinsically burst firing pyramidal cell (in rat). The sample was too small to allow 'hit rates' to be estimated, but the impression gained was that this is a relatively common type of connection – possibly as common as intralaminar layer 6 pyramid-pyramid connections. Clearly, however, these existing studies need to be extended considerably to explore the relative strengths of inputs from different types of layer 5 cells to different types of layer 6 cells.

The long receptive fields, that are typical of a proportion of pyramidal cells in layer 6 of primary visual cortex (area V1) (eg. McGuire et al., 1984), have been proposed to result from the properties of presynaptic layer 5 cells and their projections to layer 6. When layer 5 was blocked very locally with GABA application, layer 6 cells lost the component of their receptive fields that corresponded in visual space with the inactivated region of layer 5. This effect was maintained with horizontal displacements of up to 3 mm (Bolz and Gilbert, 1989). Cross-correlation studies suggested that layer 6 cells and co-oriented, coaxially aligned standard complex layer 5 cells, some displaced several millimetres laterally, receive common input, though the origin of that input was unknown. It was proposed that if direct connections from these layer 5 cells also exist, this topographic arrangement and the length summation properties of the layer 5 cells could be well suited to generate the long receptive fields typical of some layer 6 cells (Schwarz and Bolz, 1991). Clearly, layer 5 does provide significant input to layer 6. Whether the targets in layer 6 of the laterally displaced layer 5 cells are CC or CT cells, or indeed whether it is the CC, or the CT cells, or both, that have long receptive fields remains unclear.

The position of layer 6 cells in the cortical circuit

A number of interesting uncertainties arise from these previous studies and by analogy with other cortical layers. The demonstrable local connectivity associated with layer 4 pyramidal and spiny stellate cells results in a simple proposal. Layer 4 spiny cells are directly thalamo-recipient and their response properties are similar in many ways to those of their presynaptic thalamic relay cells. Given this, it is perhaps not surprising that layer 4 cells do not receive a significant and potentially 'contaminating' input from layer 3 pyramidal cells, since layer 3 cells have very different response properties, requiring more complex stimuli for a brisk response. The simplicity of first order, layer 4 cell response properties appears to be preserved by the channelling of sensory information in one direction, from layer 4 to layer 3 and from layer 3 to layer 5 and not back again. From layer 5, a highly processed signal is then sent out to subcortical structures.

What then of layer 6 CT cells? They, like layer 4 spiny cells, are first order, thalamo-recipient cells. Again, like V1 layer 4 cells, many upper layer 6 CT cells appear to be simple cells. But they are also output neurones. Their position in the local circuit is similarly ambiguous. They do not receive the equivalent of the powerful, unidirectional 'columnar' input from a single other layer – like that from layer 4 to 3, or from layer 3 to 5. Their inputs from all layers but 5 and 6 appear to be relatively weak, while their input from layer 5 appears to be spatially diffuse. As far as we understand it, therefore, their place in the cortical circuit predicts that layer 6 CT cells integrate already highly processed information, from layer 5 pyramids and from layer 6 CC cells, with the direct input they receive from the thalamus.

INHIBITORY INPUTS TO AND FROM LAYER 6

In contrast to the relatively weak excitatory input from layer 4 to 6, there appears to be a relatively strong inhibitory input. Just as some of the large layer 3 interneurones send an axon collateral to layer 5, where a secondary arbour forms, large layer 4 basket cells relatively frequently have an axonal arbour in layer 6, in addition to their arbour(s) in layer 4. In some cases the axon ramifies in layer 5 *en route*, in others, the arborisation is much stronger in layer 6 (see also Lund and Yoshioka, 1991). These interneurones include large layer 4 basket cells with a fast spiking firing pattern, a large myelinated descending axon and somatic or proximal dendritic targets. Layer 4 double bouquet cells (Somogyi and Cowey, 1981) with an adapting firing pattern, a bundle of fine descending unmyelintated axons and more distal dendritic targets, also innervate layer 6.

These inhibitory connections are reciprocal, layer 5 interneurones projecting to layer 3, while layer 6 interneurones project to layer 4 (Lund et al., 1988). Large basket cells are, again, one of the major classes of layer 6 interneurones that project to layer 4, with layer 6 Martinotti cells probably forming the major class of more distal dendrite-targeting interneurone with an ascending axonal arbour and innervating all layers from 6 to 1. Interneurones that innervate both layers 6 and 5 are also common.

We are far from understanding the roles played by the many different classes of inhibitory interneurones in the neocortex and detailed discussion of this issue is beyond the scope of this review. It is however interesting that the two thalamo-recipient layers, 4 and 6, like the two layers (3 and 5) that are rich in complex cells (in V1), are linked by the axonal arbours of large basket cells. The near simultaneous, fast and powerful inhibition in layers 4 and 6, provided by these multi-laminar large basket cells, will promote synchrony between these thalamo-recipient layers; a synchrony that will be strongly influenced by thalamo-cortical input, since these parvalbumin-containing layer 4 interneurones are a major class of inhibitory cells innervated by thalamic afferents (Staiger et al., 1996b). It should also be remembered that the inhibition provided by layer 6 interneurones, including those that link layers 4 and 6, is very much more strongly influenced by first order CT cells than by potentially higher order CC cells. This influence, will not, however, activate these interneurones rapidly, since these layer 6 CT cell to interneurone connections are facilitating. It might, however, prolong the responses of these interneuones if they also, like those in layer 4, are directly thalamo-recipient and receive depressing inputs from these axons.

INTRACORTICAL OUTPUTS OF LAYER 6

CONNECTIONS FROM LAYER 6 TO 4

Although a number of anatomical studies have described the – often quite dense – axonal arbours of layer 6 CT cells in layer 4, very few have documented the properties of the connections formed there, or even the relative numbers of different types of postsynaptic targets in layer 4. In a caged glutamate study in slices of rat somatosensory cortex, layer 4 pyramidal cells were shown to receive excitatory inputs from layers 4, 5 and 6, although the inputs from the deep layers were substantially weaker than those from within layer 4. Layer 4 spiny stellate cells, moreover, received intra-columnar excitatory input almost exclusively from layer 4 (Schubert et al., 2003).

One dual intracellular recording study in slices of cat visual cortex described seven layer 6 to 4 connections involving a presynaptic CT cell and a postsynaptic pyramidal or spiny stellate cell. The EPSPs generated were smaller (around 0.2 mV in average amplitude) than those of connections between layer 4 spiny cells (0.9 mV) and, unlike layer 4 intralaminar connections, but like the other outputs of CT pyramids, exhibited paired pulse facilitation (Tarczy-Hornoch et al., 1999). Interestingly these layer 4 synapses share another property of synapses made by CT cell axons that was first described in thalamus (see below), the activation of postsynaptic group 1 metabotropic receptors (mGluR). In slices of auditory and somatosensory cortex, electrical- or photo-stimulation of layer 6 elicited a prolonged depolarization mediated by these receptors (Lee and Sherman, 2009).

LAYER 4 TARGETS OF PRESYNAPTIC LAYER 6 CT CELLS

It is unclear whether the apparent functional weakness of the projection from layer 6 to 4 results from a true functional weakness, or from axons being cut during slicing. In none of the published pairs were both neurones recovered histologically, so that the relative position of the postsynaptic neurone within the presynaptic axonal arbour is unknown. It is also possible that layer 4 interneurones constitute a major target for layer 6 CT axons. This was strongly suggested by an ultrastructural analysis of the postsynaptic targets of HRP-filled layer 6 pyramidal cells in cat striate cortex. The majority of targets were dendritic shafts belonging to smooth or sparsely spiny layer 4 cells, suggesting that inhibitory interneurones contribute a significant proportion of the postsynaptic population. The smooth dendritic targets included both beaded and non-beaded profiles and the majority of synapses were made by *bouton terminaux* from the side twigs typical of these axons (McGuire et al., 1984). In contrast, however, in an ultrastructural study of rat somatosensory CT cell axons that had been retrogradely labelled from the primary sensory thalamic VPm nucleus, only a minority (14%) of the targets in layers 4 and 5 were immuno-positive for GABA (Staiger et al., 1996a). The other targets were GABA-negative dendritic spines (55%) and shafts (31%) (see also: Anderson et al., 1994). Whether this difference is due to species, regional, or methodological differences remains to be determined.

The role that layer 6 plays in shaping the responses of layer 4 cells to sensory input continues to be controversial (see length-tuning, below). That layer 6 CT cells provide a significant input to layer 4 is clear. It is also clear that this input will not generate rapid layer 4 cell activation (or indeed, inhibition) at the very beginning of a response to a novel stimulus, since all inputs mediated by layer 6 CT cells are low probability at low frequencies, facilitating on repetitive activation. It is possible that layer 6 mediates, or modulates, some of the later components of layer 4 responses to novel sensory stimuli, since layer 4 responses to thalamic input can be powerful and fast, but depress strongly.

LAYER 6 TO LAYER 5

Like the reverse connection from layer 5 to 6, layer 6 CC cells appear to provide a significant excitatory input to layer 5 pyramidal cells. In a paired recording study (Mercer et al., 2005) two such connections were recorded, one to a small layer 5 pyramid in rat and one to a large layer 5 pyramid in cat. Both were relatively powerful (>1 mV average amplitude) and exhibited presynaptically mediated paired pulse and brief train depression.

INPUTS TO LAYER 6 CELLS FROM THE THALAMUS

RECEPTIVE FIELD PROPERTIES

When compared with studies of layer 4 and more superficial layers, relatively few studies have recorded responses of layer 6 cells to sensory input. Fewer still have been able to identify the neurones recorded. Visual cortical neurones described as simple cells, ie. those in which excitatory and inhibitory components of the receptive field do not overlap in visual space, are reported to reside almost exclusively in thalamo-recipient layer 4 and upper layer 6. In lower layer 6 and layers 2, 3 and 5, complex cells were more common (Martinez et al., 2005). Since simple cells are most commonly found in the thalamo-recipient layers and their response properties are closer to

those of 'specific' thalamic relay cells than are those of other cortical neurones, many layer 4 and upper layer 6 spiny cells can be assumed to be in receipt of direct primary sensory thalamo-cortical input. Lower layer 6, CT cells and perhaps all CC cells, like pyramidal cells in layers 2, 3 and 5, are more likely to receive less direct, integrated sensory information via both local cortical circuits and via thalamic cells that do not themselves receive direct peripheral input. This may correlate with the outputs of the different classes of layer 6 cells, with complex short CT and CC cells targeting layer 5 and in some cases, layers 2 and 3, layers that are rich in complex cells, while simple CT cells that receive direct primary sensory input from thalamus, target layer 4, which is rich in simple cells (see above).

INPUTS TO LAYER 6 FROM THE THALAMUS

Thalamo-cortical inputs from primary sensory thalamic regions, like the VPm barreloids, to whisker barrels in the somatosensory barrel cortex, remain discrete. They do not enter the septa between barrels, nor do they innervate barrels associated with other whiskers and they terminate quite selectively in 'thalamo-recipient' layer 4, lower layer 3 and layer 6. In contrast, inputs from Pom (an association thalamic nucleus), terminate in inter-barrel septa and in all layers from upper L5 to L1. Moreover, these axons branch and innervate widely separated cortical regions (eg. sensorimotor or Sm cortex and frontal cortex). In primary sensory regions, layers that are not typically considered to be 'thalamo-recipient' (layers 1, 2, upper layer 3 and layer 5) receive input from regions of thalamus variously described as non-specific, secondary or association regions ie. regions that do not receive direct peripheral sensory input. In some cases entire nuclei can be defined either as primary sensory or not. In others, different sub-divisions of a single nucleus, eg. the LGN, play these different roles, the calbindin immuno-reactive cells (in primate) contributing to secondary or association thalamus, or matrix, and the parvalbumin immuno-reactive cells contributing to the primary sensory, or core regions (Jones, 1998). Thus, the more proximal dendrites of layer 6 neurones, within L6, are positioned to receive direct, focussed input from a topographically appropriate region of primary sensory thalamus, eg. the appropriate VPm barreloid. Whether their more distal dendrites in layer 5 receive input from secondary or association regions, such as Pom, whose relay cell axons terminate in layer 5 (Deschênes et al., 1998), and/or whether CC or claustrum-projecting cells receive this input, remains to be explored. The excitatory inputs to the distal dendritic tufts, in layer 4, of primary sensory CT cells also remain to be fully elucidated, but it is likely to originate with primary sensory thalamic afferents and/or thalamo-recipient layer 4 spiny cells.

The existence of parallel information-processing streams (Nassi and Callaway, 2009) is perhaps more apparent in layer 6, than in any other layer. The major pyramidal cell classes : CC cells, CT cells associated with primary sensory thalamic nuclei, CT cells associated with secondary or association thalamic regions, and claustrum projecting cells, have different relationships with a range of thalamo-cortical and cortical inputs, as well as different outputs within the local circuit, across different regions of cortex and in subcortical regions. The extent to which these streams interact functionally is not known in detail, but interactions are apparent, for example, in the relatively dense innervation of all layer 6 pyramidal cells by layer 6 CC cells and by layer 5 pyramidal cells.

CORTICOTHALAMIC OUTPUTS

To make any sense of the possible function(s) of layer 6 CT cells, some understanding of its complex interactions with the thalamus is necessary. This is, however, a complex system that is difficult to study and the influences that layer 6 has on thalamic function have become somewhat controversial.

PROPERTIES OF CORTICOTHALAMIC SYNAPSES IN SPECIFIC SENSORY THALAMIC NUCLEI

LGN relay cells receive large, fast, secure AMPA-receptor- and NMDA-receptor-mediated EPSPs from the retina, via large, proximally located synapses (Jones and Powell, 1969), some of which are glomerular (Mason et al., 1984). These connections exhibit paired pulse depression. In contrast, the more distally activated (Sherman and Guillery, 1996), smaller, slower EPSPs generated by CT cells in primary sensory thalamic nuclei (Landisman and Connors, 2007), involve a large NR2B-mediated component with slower kinetics, possibly a kainate receptor (GluR5) component (Miyata and Imoto, 2006) and an mGluR1-mediated component, in adult (Turner and Salt, 1998) and in juvenile rat LGN slices (Hughes et al., 2004; Reichova and Sherman, 2004). Activation associated with GluR5 may also result from disinhibition via GluR5 receptors located on GABAergic nRT axon terminals (Godwin et al., 1996; Binns et al., 2003). Presynaptic mGluR2 receptors activated during repetitive CT firing appear to reduce facilitating relay cell responses to trains of cortico-thalamic spikes (Alexander and Godwin, 2005). These inputs from cortex display paired pulse facilitation in juvenile rat LGN thalamo-cortical slices (Granseth et al., 2002) and adult mouse VB (ventrobasal thalamic nucleus) slices (Castro-Alamancos, 2002). Although paired pulse and brief train facilitation were apparent in both, they were more pronounced in cortico-thalamic EPSPs in ferret LGN relay cells than in the visual division of the inhibitory nRT nucleus, the perigeniculate (Alexander et al., 2006), while the cortico-thalamic inputs to nRT cells in juvenile mice were substantially more powerful, involving 3–4 times the number of receptors (Golshani et al., 2001). All the connections from CT cell axons studied to date have, unusually for cortical pyramidal cells, generated facilitating EPSPs, whatever the class of target cell involved, though with differences in degree and expression of augmentation (Tarczy-Hornoch et al., 1999; West et al., 2006). How the properties and distribution of the cortico-thalamic inputs may influence the impact of cortical 'feedback' to the thalamus is explored in a multi-compartmental model in Emri et al. (2003).

PATTERNS OF CORTICOTHALAMIC INNERVATION

Although L6 CT cells are not the only cortical pyramidal cells to innervate the thalamus, it is the taller, upper L6 CT cell axons that innervate the nRT as well as primary sensory regions, such as the LGN. Here they make rod-like terminal arbours, synapsing with the distal dendrites of relay cells and providing what has been termed 'modulatory input' (Sherman and Guillery, 1998). These upper layer 6 pyramidal cell axons do not send branches to other thalamic nuclei, or to other subcortical structures. In contrast, lower L6 CT cell axons innervate both primary sensory thalamus, with distal, rod-like terminations and secondary or association thalamic regions, while L5 pyramidal cells project only to intralaminar, or association areas of the thalamus (see **Figure 4**). These cortical pro-

jections to secondary or association areas often form larger boutons and complex glomerular synapses with the proximal dendrites of relay cells (Usrey and Fitzpatrick, 1996; Murphy et al., 2000) (for properties of layer 5B CT synapses see Groh et al., 2008). Primary auditory cortico-thalamic connections exhibit similar differences between the projections from layers 5 and upper and lower layer 6 to those seen in the visual system (Ojima, 1994; Llano and Sherman, 2008). Unlike the axons of layer 6 cells, layer 5 cell axons that innervate the thalamus also innervate other subcortical structures, leading to the suggestion that secondary sensory and motor thalamus also receive a parallel readout of motor instructions from the cortex (Guillery, 2005; Sherman, 2005).

TARGETS IN THALAMUS CORRELATE WITH CORTICAL PROJECTIONS

Thus, primary sensory thalamus receives tightly coupled excitatory 'feed-back' from the appropriate primary sensory region of the cortex, via relatively slow, facilitating connections onto the distal dendrites of relay cells. These cells also receive disynaptic inhibition via cortico-thalamic activation of the nRT. In primary sensory thalamic regions, the more powerful, fast, proximally located, depressing excitatory inputs come from the periphery (see **Figure 4**). Whether the terminations of somatosensory cortico-thalamic axons display a sub-modality selectivity equivalent to that displayed by the peripheral inputs remains to be determined (for discussion see Diamond et al., 1969; Murphy and Sillito, 1996; Deschênes et al., 1998). There is evidence for such selectivity in macaque visual cortex, with some segregation between the lower layer 6 projections to the magnocellular and upper layer 6 projections to parvocellular compartments of the LGN. Cells in the middle of the layer did not appear to project to the LGN (Fitzpatrick et al., 1994; and in tree shrew, Fitzpatrick, 1996). In a retrograde labelling study also in macacque V1, the neurones in upper layer 6 that projected to parvocellular LGN regions, also projected to lower 4C, which receives P cell input. In contrast, cells in lower layer 6 that projected to magnocellular regions of LGN, projected to upper layer 4, which receives M cell input. Mid layer 4C the zone of M and P combination, received from the middle of layer 6 (which did not project to the LGN), while P cell-recipient layer 4A received projections from both upper and lower layer 6 (Yoshioka et al., 1994) (see **Figure 3**). These findings support an earlier proposal, that layer 6 pyramidal neurones target both specific sub-populations of LGN relay cells and the specific cells in layer 4 that are postsynaptic to those LGN cells (Lund and Boothe, 1975).

The major extra-areal input to layer 4 of the second visual area, V2, is provided by CC projections from V1, ie. these inputs occupy the territory that is occupied by thalamic afferents in V1. The output synapses from V2 CT cells to the pulvinar complex, a large region of association thalamus, occupy proximal dendritic locations and involve large glomerular synapses, similar to the types of synapses that relay information from the periphery to primary sensory thalamic nuclei. In contrast to this V2 projection, which comes largely from layer 6, the projection from V1 to the pulvinar arises from layer 5B. Thus pulvinar receives highly processed information from more than one region of cortex, several synapses away from the LGN inputs to V1. In turn, the pulvinar provides the major subcortical input to V2 via lower layer 3, innervating both thick and thin stripes of high cytochrome oxidase activity (Levitt et al., 1995).

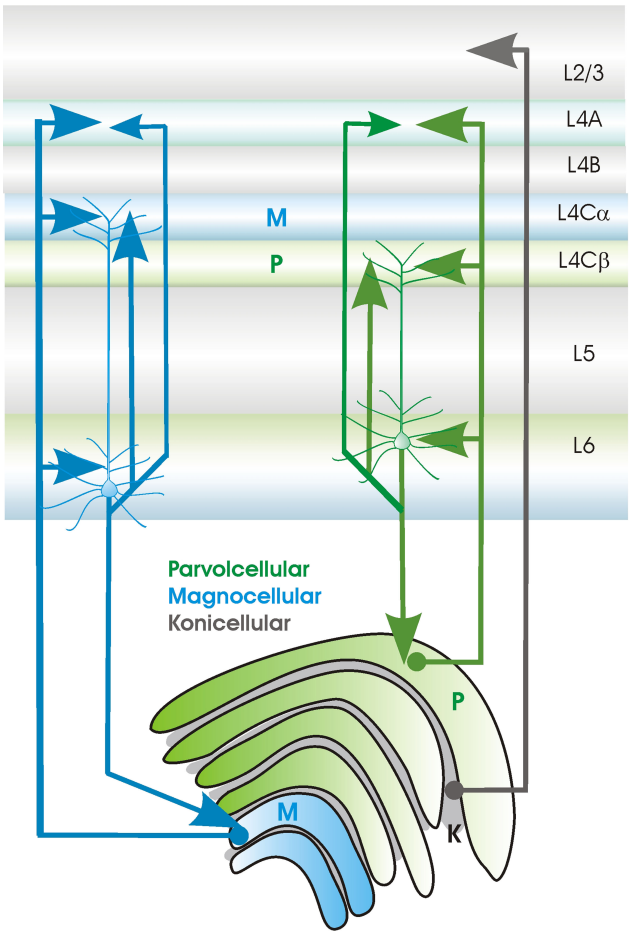

FIGURE 3 | Sublayer specificity of connections between primary sensory thalamus (in this case between the LGN, lateral geniculate nucleus and primary sensory cortex (in this case V1, primary visual cortex) in the primate (see text for source references). Relay cells in the Magnocellular (green) and Parvocellular (blue) LGN layers project to different sublayers in V1. CT cells that project to these different compartments of the LGN also project to different subdivisions of layer 4 and their apical dendritic tufts in layer 4 colocalise with these axonal arbours.

Similarly, in the somatosensory system, primary sensory cortical layer 5 projections to the secondary thalamic posterior group, Po, form clusters of large boutons, which are confined to the dorsal part of the nucleus. This part of Po, also receives input from layer 6 of the primary and second somatosensory areas and from the motor and insular cortices (Veinante et al., 2000). The relay cells in the head of the barreloids in primary sensory VPm also receive input from layer 6 of the vibrissa motor cortex. Unlike other regions of the barreloids, each of which is primarily responsive to a single whisker, these relay cells receive input from multi-whisker-responsive trigeminal neurones. Urbain and Deschênes (2007) propose that this pathway may relay information about the phase of whisker motion during free 'whisking'.

The interactions between cortex and thalamus are thus complex, but orderly. Primary sensory regions of thalamus that receive strong, proximal input from the periphery, project specifically and with a highly organised topography, to layers 4 and 6 (and in some

cases to lower layer 3) of primary sensory cortex and receive modulatory input from layer 6 of precisely the same region. These inputs and outputs are further segregated in some modalities according to tightly matched layer 4 and thalamic nucleus subdivisions (see **Figure 3**). Secondary or association regions of thalamus receive their strong proximal inputs from another subclass of CT cells that do not project solely to primary sensory thalamus and from layer 5 cells in primary sensory cortex. Their cortico-thalamic modulatory inputs from secondary sensory cortex. These regions project to non-thalamo-recipient layers 1, 2, upper 3 and 5 of primary sensory cortex, but to layer 4 of secondary sensory cortex (see **Figure 4**).

Corticothalamic synapses in primary sensory thalamic regions differ significantly from those provided by afferent sensory axons. They are smaller and more distally located on relay cell dendrites, utilise NR2B and mGluR1 receptors in addition to the AMPA receptors that dominate afferent inputs and facilitate, rather than depress on repetitive activation. These inputs are described as modulatory. Primary sensory CT cells, typically found in upper layer 6, with axonal and dendritic arbours in layer 4, innervate primary sensory thalamic regions reciprocally and have axon collaterals that innervate nRT. In primate visual cortex, sublayer specificity is apparent in the regions of layer 4 and the layers in LGN that are supplied by these CT cell axons and, moreover, their distal dendritic tufts in layer 4 exhibit the same sublayer preferences. The other class of CT cells, typically found in deep layer 6 are shorter upright pyramids with axonal and dendritic arbours that terminate in layer 5 (some have axons that ramify in layer 2/3). They innervate primary sensory thalamus reciprocally, with modulatory synapses, but, like layer 5 pyramids, they also innervate secondary and association regions of thalamus with large more proximal boutons. They do not, however, innervate nRT.

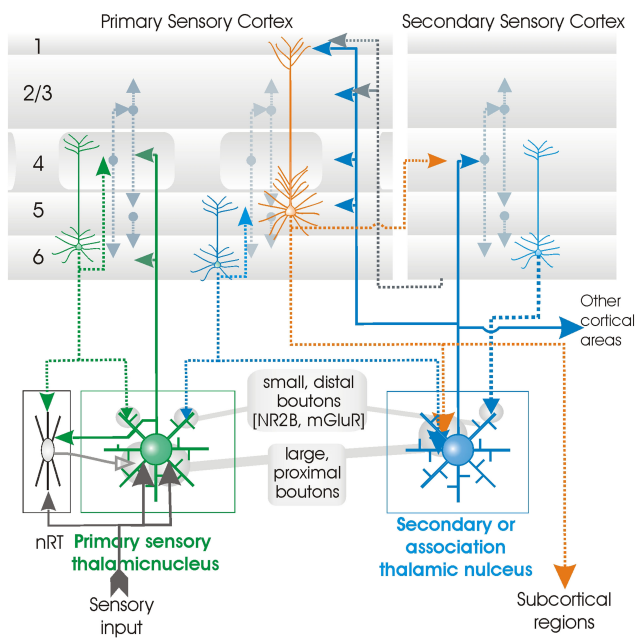

FIGURE 4 | Cartoon depicting some of the major connections between the neocortex and thalamus. The axons of cortical neurones are indicated by dotted lines, axons from thalamic cells by solid lines. The relationships summarised here appear broadly similar in somatosensory, visual and auditory systems. Layer 6 CT cells innervate the distal dendrites of relay cells in specific thalamic regions (eg. LGN, VPm, MGBv), with small boutons that seem to play a modulatory rle. In these nuclei, sensory input from the periphery provides the more powerful, faster input via large, proximal synapses. In association thalamic areas, the larger, more proximal synapses are provided by layer 6 CT cells and layer 5 corticofugal cells in primary cortex, while the smaller, more distal inputs come from association cortex. This is analogous to the powerful input to layer 4 in primary sensory cortex which comes from 'specific' thalamus, while layer 4 of the secondary cortical region receives information about the outside world from the appropriate primary sensory region of cortex.

FUNCTION(S) OF CORTICOTHALAMIC CONNECTIONS

This section focusses on the visual system where so called 'thalamo-cortical feed-back' has been most studied. LGN cells have lower background and stimulus-driven firing rates than their retinal inputs. Their responses increase more steeply with contrast, their centre-surround antagonism is greater and their spatial properties are more complex than those of their retinal inputs (Kaplan et al., 1987, see also discussion in Raiguel et al., 2006). These differences have, in part, been ascribed to the properties of the retinal input synapses and to local inhibitory circuitry (see also Wang et al., 2007), but cortico-thalamic input also plays an important role. Indeed, it is difficult to imagine a projection as powerful as the cortico-thalamic projection serving no significant function. The role of cortico-thalamic 'feedback' in co-ordinating oscillatory activity in the thalamocortical system is reviewed in Destexhe (2000).

ORIENTATION AND DIRECTION PREFERENCES

The elongate axonal arbours of some CT cells, which extend well beyond the dendritic trees of single relay cells, are proposed to innervate rows of LGN cells (whose receptive fields represent bars or lines in visual space), promoting their simultaneous activation. Coincident activity in rows of LGN relay cells aligned with the stimulus orientation would enhance orientation sensitivity, while activation of rows with different orientations might enhance sensitivity to direction (Murphy et al., 1999). In the LGN, the axonal arbours of CT cells are retinotopically organised, as are their effects on LGN cell receptive fields. Interestingly, these effects are reversed in phase relative to their ON and OFF zones (Wang et al., 2006).

LENGTH TUNING

Despite relatively concentric receptive fields, length-tuning is apparent in the LGN A laminae (Jones and Sillito, 1991), ie. increasing the length of the stimulus results in a decrease in the LGN response. The inhibitory perigeniculate nucleus (PGN, a subdivision of nRT) clearly contributes to this tuning, since pairs of receptive field-matched LGN and PGN cells exhibit mirror image firing (Funke and Eysel, 1998). Some components of PGN- and thus LGN- length tuning may result from subcortically derived PGN receptive field structure, since PGN responses to long bars were not influenced by silencing the cortex (Jones and Sillito, 1994), The cortex does, nevertheless, refine the transfer of information from the thalamus in a manner that is dependent on the stimulus context (Cudeiro and Sillito, 1996). Layer 6 primary sensory CT cells are good candidates for cells that can activate centre-surround antagonism in the thalamus. They provide reciprocal innervation of primary sensory nuclei with parallel innervation of the nRT (including the PGN) and some layer 6 cells have extremely long receptive fields (approximately 17% of layer 6 cells in V1 had fields greater than

8°, Grieve and Sillito, 1991a). Of particular relevance to their influence on thalamic responses, layer 6 cell responses increase when stimuli (eg. drifting gratings) that cover the centre and surround components of their receptive fields respectively, are aligned and have the preferred orientation. LGN cells, on the other hand, are minimally responsive under these conditions (Sillito et al., 1993). Moreover, suppression of LGN cell receptive field centre responses by activation of the surround, and the impact, in the LGN, of centre-surround alignment, were lost when cortico-thalamic inputs to the LGN were silenced (Cudeiro and Sillito, 1996).

Inactivating layer 6 decreased end-inhibition in layer 4 and in cells in layers 2 and 3 that receive their major inputs from layer 4. This effect was specific to end-inhibition as orientation and direction selectivity were unaffected (Bolz and Gilbert, 1986). However, the extent to which layer 6 cell activation of layer 4 interneurones contributes to end zone inhibition in layer 4 continues to be controversial. For example, in another series of experiments in cat visual cortex, pharmacological blockade of layer 6 decreased the response to the optimal length in layer 2–4 hypercomplex end-stopped cells, rather than increasing the response to longer stimuli. From this result, Grieve and Sillito (1991b) concluded that the predominant effect of layer 6 on layer 4 is mediated via excitatory input from layer 6 to 4, rather than via the recruitment, by layer 6 cells, of inhibition in layer 4.

GAIN CONTROL

A role for cortico-thalamic connections in LGN response gain control has also been proposed. In responses to moving, oriented stimuli (drifting gratings), cortico-thalamic input promotes correlated firing, in the LGN, of cells whose alignment within the receptive field matches that of the stimulus (Sillito et al., 1994). When correlated LGN cell firing was used to construct orientation-tuning curves, these were more sharply tuned when cortico-thalamic input was intact (Andolina et al., 2007). Since coincident presynaptic spikes and the resultant EPSPs are more likely to sum to firing threshold than widely separated EPSPs in thalamo-recipient cortical cells (Pinto et al., 2000; Bruno and Sakmann, 2006) and since supra-linear summation of these inputs has been reported (eg. Usrey et al., 2000; Roy and Alloway, 2001), the synchronisation of thalamic inputs to the cortex, by cortico-thalamic connections, may have important influences on cortical responsiveness to sensory input.

The mGluR1 component of synaptic activation in the thalamus that is specific to cortico-thalamic inputs, has been exploited to study CT cell influences on LGN receptive field structure. By manipulating these receptors pharmacologically in adult cat, cortico-thalamic, mGluR1- mediated input was found to enhance the excitatory centre of LGN receptive fields selectively. Moreover, the effect of manipulating mGluR1 activation was maximal with stimuli that drive cortical cells most effectively (Rivadulla et al., 2002).

The influences that layer 6 has on the responses of other cortical and thalamic neurones to sensory input, clearly remain controversial. That cortex influences thalamus is not in dispute. Orientation and direction tuning, length tuning and gain control in the thalamus all appear to be influenced by the cortex, ie. by layer 6 CT cells. It is the extent of this influence, in comparison with that of local thalamic mechanisms, and the precise conditions under which it is generated, that remain controversial. The relative paucity of compelling evidence results largely from the complexities of the system and the many cell classes and types of connections that shape these responses in thalamus and cortex. It is extremely difficult to manipulate and monitor a single type of neurone, or connection, in an entirely controlled way, to investigate its effect on such a system, without influencing the activity of others. The selective pharmacology approach has been used successfully in some such studies and could be exploited further. It is also possible that small rodents in which receptors have been modified in selected neuronal populations, or in which light-responsive ion channels are expressed in specific cell types, may provide useful tools.

Does layer 6 provide 'feed-back'?

Despite the powerful reciprocal connections between primary sensory thalamic nuclei and layer 6 of primary sensory cortical regions, the terms 'feed-forward' and 'feed-back' have been purposely avoided in this review. In an active brain these terms are nonsensical. They imply that one limb of a circuit sits there idly doing nothing and playing no part in circuit behaviour until, or unless, the other limb has activated it. Types of neurones and even complete circuits have, misguidedly, been defined in these terms, but where in the cortex, or thalamus, are there cell types that receive only one input, or that fire only in response to one type of signal, to be found? Layer 6 CT neurones may receive direct input from thalamic relay cells and send excitatory input to those same neurones (and/or their close neighbours), but they also receive strong inputs from within the cortex. These inputs, possibly from a neighbouring topographic region, could as easily cause a group of CT cells to modify the response of a thalamic relay cell to a novel incoming input, as a thalamo-cortical input could, via layer 6, modify the response of its cells of origin to a repetition of the same stimulus. It may be more useful to consider pathways such as those from cortex to thalamus and from regions of association cortex to primary sensory regions, as inputs that prime these lower order regions to transmit behaviourally relevant information within the current context; cognitive feed-forward perhaps, as much as sensory feed-back.

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On the fractal nature of nervous cell system

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In a detailed study entitled "Morphological development of thick – tufted layer V pyramidal cells in the rat somatosensory cortex," an international team of scientists (Romand et al., 2011) reported a series of results pertaining to an analytical investigation of the morphological development of thick-tufted layer V pyramidal cells (also called the principal cells) in the rat somatosensory cortex. At the end of the Introduction Section, the Authors stated "all compartments of a TTL5 cell undergo different developmental changes, supporting the notion that multiple functional compartments receive different inputs and may integrate distinct signal transduction systems." Following on a careful reading of this stimulating report a main question rose which concerned the epistemic view adopted by the Authors and in turn the analytical procedure chosen for investigating neural cells from an highly organized system, privileging in fact the recourse to "conventional" morphometry. These morphometric approaches are usually termed conventional because being based on single *scale* measuring which may suite well for evaluating biological objects assumed to be or arbitrary approximated to regular Euclidean structures, but inappropriate to quantitatively describe the morphology of thick-tufted layer V pyramidal cells, characterized by complex functional properties and irregular morphological features. Therefore an objective estimation could be reached only by applying the principles and rules of the Fractal geometry proposed by the mathematician Mandelbrot (1982) in the early 1980s. The Authors specified that most neural parameters, including lengths and diameters of individual segments, surface area, branch angles, and other cellular elements were "subjectively classified" and thereafter analyzed either from reconstructed figures or obtained from unrealistic representations. Another incongruous sentence was found in the Somatic Development Section: "Somata were subjectively classified into three formats according to shape: triangular, round, and oval. Although three shapes were found at all ages, somata of TTL5 neurons appeared to be mostly triangular or round at P7 and predominantly triangular thereafter." It is by far evident from Figures 1 and 3 of Romand et al. (2011) that somata, dendrites and axons are neither round or triangular bodies, nor linear segments, but appeared as irregularly shaped anatomical entities susceptible to be adequately investigated by the "non-conventional" fractal morphometry. Suffices it to mention that, during the last two decades, several studies have been performed on brain tissue and nervous system cells by adopting fractal concepts and methods, which has enabled to quantitatively elucidate most developmental, morphological, and spatial pattern avoiding arbitrary approximation or smoothing of cellular shapes and structures. (Smith and Bejar, 1994; Smith et al., 1996; Bernard et al., 2001; Grizzi and Chiriva-Internati, 2005; Milosevic and Ristanovic, 2006; Ristanovic et al., 2006; Di Ieva et al., 2007; Jelinek et al., 2008; Di Ieva, 2011). Therefore, it may not be surprising that the Authors, despite a huge investigative effort, were obliged to recognize a frank blank, honestly admitted, when they were trying to interpret the data in the light of Methodological considerations (Page 20), with the words: "Variations in results across different studies can be due to many methodological factors such as differences in the staining procedure, the section thickness, the measuring, and analyzing method, the cell selection criterion, the sample size, and the cortical area. These differences make it difficult to directly compare results between different studies." Proper considerations indeed, but not unpublished, because they evoked considerations much similar reported as far as thirty years ago by a Swiss Group (Paumgartner et al., 1981) in a pioneer study which clearly demonstrated the influence of resolution scale, i.e., objective magnification, on the estimates of geometric irregular features of liver cell membranes, or in other words the role of resolution scale at which the measurements were performed. The large observed discrepancy was consistently annulled while the variations reported by different investigators could be explained by taking into account the "resolution effect" according to the concepts of the Fractal geometry, such as the irregularity, the statistical self-similarity, the scale invariance of form, the occurrence of repetitive morphologic determinants and the fractal, i.e., non-integer dimension, rather than the trivial methodological factors called upon to explain estimate variations across different studies. Biologic structures with irregular shape and complex morphology should not be approximated to ideal geometric objects, since far from the real pictures, while a single scale of measurements should not be adopted *a priori* if an objective morphological description of complex objects has to be achieved (Losa and Nonnenmacher, 1996). It should be pointed out that fractal and conventional morphometric approaches, built up on distinct epistemological principles, may set the understanding of the biologic reality at different level. The former describes the morphological complexity within an experimental interval of observation scales that obviously encompasses the Euclidean dimension, while the latter proceeds at a primary level, i.e., by reducing cellular shapes and tissue structures to monotone elements which could be described by means of deterministic rules. Nevertheless, fractal and conventional morphometry may represent complementary analytical/

quantitative tools to elucidate the diversity of morphological patterns and functional parameters which characterize neural cells and brain structures.

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