3.08 The Evolution of Neuron Classes in the Neocortex of Mammals

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Glossary

Afrotheria A clade of placental mammals that is thought to have originated in Africa at a time when it was isolated from other continents. This clade contains Paenungulata (elephants, hyraxes, and sirenians), Afrosciricida (tenrecs and golden moles), Tubulidentata (aardvarks), and Macroscelidea (elephant shrews). Molecular data indicate that the Afrotheria diverged from other placental mammals 110–100 Mya.

Anthropoidea A primate clade that includes New World monkeys, Old World monkeys, apes, and humans. Tarsiers are the sister group to the Anthropoidea.

Boreoeutheria A clade of placental mammals that encompasses Euarchontoglires and Laurasiatheria. This taxonomic division within the Boreoeutheria occurred 95–85 Mya in the Cretaceous.

Cetartiodactyla A clade of placental mammals that includes cetaceans (whales, dolphins, and porpoises) and artiodactyls (even-toed ungulates). This phylogenetic grouping is based on molecular evidence indicating that cetaceans evolved from within the artiodactyls and have their closest relationship with the hippopotamus and then ruminants (cattle and deer).

Euarchontoglires A supraordinal clade of boreoeutherian placental mammals that includes Rodentia, Lagomorpha, Dermoptera, Scandentia, and Primates.

Hominidae A primate clade that includes great apes (orangutans, gorillas, chimpanzees, and bonobos) and humans. The hylobatids (gibbons and siamangs) are the sister taxon to the Hominidae.

homoplasy A structure that is the result of convergent evolution, where organisms that are not closely related independently acquire similar characteristics. Homoplasy is contrasted with homology, which means that structures have a common origin derived from a shared ancestor.

Laurasiatheria A supraordinal clade of boreoeutherian placental mammals that includes Cetartiodactyla, Perissodactyla, Carnivora, Pholidota, and Eulipotyphla.

marsupials A clade of mammals in which the female has a pouch where the young is nurtured through early infancy. There are approximately 280 species of living marsupials, with the majority being native to Australia and the remainder living in South America (however, there is a single North American native marsupial species, the Virginia opossum).

monotremes A clade of mammals that lay eggs, rather than giving birth to live young like marsupials and placental mammals. The extant representatives of this group, the platypus and the echidnas, are indigenous to Australia, New Guinea, and Tasmania. Fossil evidence, however, suggests that monotremes were once more widespread.
3.08.1 Introduction

A diverse array of neuron types populates the mammalian neocortex. Physiological activity within a cortical area, in turn, is largely determined by interactions among these different cell types and incoming afferents. Neurons in the cerebral cortex of mammals can be divided into two major classes on the basis of morphology and function, pyramidal excitatory cells, and inhibitory interneurons. Each class includes many subtypes that can be identified by their size, shape, dendritic and axonal morphology, and connectivity. These neuronal subtypes exhibit a variable distribution among cortical layers and regions, and some are differentially represented among species (see Hof et al., 1999, 2000; Hof and Sherwood, 2005). Neurons can be further classified based on their expression of various proteins, such as neurofilament proteins (NFPs), calcium-binding proteins, and neuropeptides.

The morphology of a given neuron, particularly of its dendritic arborizations, reflects the size of its receptive field and the specificity of its synaptic contacts. Thus, the structure of the dendritic arbor as well as the distribution of axonal terminal ramifications confer a high level of subcellular specificity in the localization of particular synaptic contacts on a given neuron. The three-dimensional distribution of the dendritic tree is also a key factor with respect to the type of information transferred to the neuron. A neuron with a dendritic tree restricted to a particular cortical layer may be receptive to a very limited pool of afferents, whereas widely expanding dendritic branches typical of large pyramidal neurons will receive highly diversified inputs in the cortical layers through which the dendrites course. Considering the variability in cortical morphology, size, and cellular organization in mammals, it is important to investigate how specific characteristics of cortical microcircuitry differ among species, and how these cellular phenotypes could be used to assess taxonomic affinities and functional differences among species.

3.08.2 Neuronal Typology and Chemical Specialization

3.08.2.1 Pyramidal Neurons

Pyramidal neurons are the principal excitatory neuronal class in the cerebral cortex. They are highly polarized neurons, with a major orientation axis orthogonal to the pial surface of the cerebral cortex. Their cell body is roughly triangular on cross section, although a large variety of morphologic types exist with elongate, horizontal or vertical fusiform, or inverted perikaryal shapes. They typically have a large number of dendrites that emanate from the apex and from the base of the cell body. The span of their dendrites may cover several millimeters and their somata are found in all cortical layers except layer I, with predominance in layers II, III, and V. Small pyramidal neurons in layers II and III of the neocortex have a restricted dendritic tree and form vast arrays of axonal collaterals with neighboring cortical domains, whereas medium to large pyramidal cells in deep layer III and layer V have a much more extensive dendritic tree and furnish long corticocortical connections. Layer V also contains very large pyramidal neurons disposed in clusters or as isolated, somewhat regularly spaced elements. These neurons are known to project to subcortical centers such as the basal ganglia, brainstem, and spinal cord. Finally, layer VI pyramidal cells exhibit a greater morphologic variability than those in other layers, and are involved in certain corticocortical as well as corticothalamic projections. The dendrites of pyramidal cells show large numbers of dendritic spines that receive most of the excitatory synaptic inputs. As many as 40,000 spines can be encountered on a large pyramidal neuron.

The major excitatory output of the neocortex is furnished by pyramidal cells. Their axon extends in most cases from the base of the perikaryon and courses toward the subcortical white matter and gives off several collateral branches that are directed to cortical domains generally located within the vicinity of the cell of origin. While many of these branches ascend in a radial, vertical pattern of arborization, a separate set of projections also travels horizontally over long distances. One function of the vertically oriented component of the recurrent collaterals may be to interconnect layers III
and V, the two major output layers of the neocortex. Horizontal intrinsic connections are positioned to recruit and coordinate the activity of modules of similar functional properties while inhibiting other domains. Together these recurrent projections function to set up local excitatory patterns and coordinate the output of neuronal ensembles. NFP immunoreactivity, chiefly recognized by the expression of dephosphorylated epitopes of NFP medium and heavy molecular weight subunits, characterizes a subpopulation of pyramidal neurons in the neocortex of mammals that exhibits clear regional and species differences in their distribution and densities (Hof et al., 1992, 1996; van der Gucht et al., 2001, 2005; Kirkcaldie et al., 2002; Boire et al., 2005; Figure 1). The expression of NFP has been studied most comprehensively in primates, where it has been shown that this protein is enriched in a subset of large pyramidal neurons that have an extensive dendritic arborization, are distributed in well-defined laminar positions, form highly specific long corticocortical projections, and show regional specificity in their distribution patterns (Campbell and Morrison, 1989; Campbell et al., 1991; Hof and Nimchinsky, 1992; Carmichael and Price, 1994; Hof and Morrison, 1995; Hof et al., 1995b; Nimchinsky et al., 1995, 1996, 1997; Preuss et al., 1997, 1999; Sherwood et al., 2003a; Vogt et al., 2001, 2005). Although the precise function of NFP is not completely understood, its restricted distribution among certain subsets of corticocortical circuits in primates suggests that it confers unique neurochemical and morphologic properties subserving a range of highly specialized functions in neocortical connectivity, as well as selective vulnerability to neurodegenerative diseases unique to humans (Hof et al., 1995a, 1995b; Nimchinsky et al., 1996; Bussière et al., 2003; Hof and Morrison, 2004). NFP may be present, to some degree, in functionally homologous subsets of cortical output neurons in many species.

Figure 1  Examples of cytoarchitecture and cellular typology using an antibody against NFP. a, Organization of the primary visual cortex in a long-tailed macaque monkey (Macaca fascicularis). NFP-immunoreactive neurons are predominant in layers III, IVB, and V. b and c, Distribution of NFP-immunoreactive neurons in the primary visual cortex of two canivores, the dog (b, Canis familiaris), and the California sea lion (c, Zalophus californianus). Note the prominent labeling of very large cells in layer V and smaller cells in layer III, whereas layer IV in the middle is devoid of labeled neurons. The large size and extensive dendrites of NFP-immunoreactive neurons is obvious. d, NFP-immunoreactive cells in the visual area of the camel (Camelus dromedarius). The labeled neurons are relatively small and predominate in the deep layers. e and f, NFP immunolabeling in the visual cortex of the pigmy sperm whale (e, Kogia breviceps), and of the beluga whale (f, Delphinapterus leucas). Note the prominent clusters of large immunoreactive neurons in layer IIIc/V only. Scale bar (on f): 300 μm. Reproduced from Hof, P. R. and Sherwood, C. C. 2005. Morphomolecular neuronal phenotypes in the neocortex reflect phylogenetic relationships among certain mammalian orders. Anat. Rec. A 287, 1153–1163, with permission from John Wiley & Sons, Inc.
3.08.2.2 Spiny Stellate Cells

Spiny stellate cells are the other class of cortical excitatory neurons and are found in highest numbers in neocortical layer IV. The spiny stellate cell is a small multipolar neuron with local dendritic and axonal arborizations. These neurons resemble pyramidal cells in that they are the only other cortical neurons with large numbers of dendritic spines, but differ from them in lacking most of an apical dendrite and having a restricted dendritic arbor that generally does not extend beyond the layer in which the cell body resides. The axons of spiny stellate neurons are primarily intrinsic and form links between layer IV, that receives a major input from the thalamus, and layers III, V, and VI. In some respects, the axonal arbor of spiny stellate cells mirrors the vertical plexuses of recurrent collaterals, albeit in a more restricted manner. Given its axonal distribution, spiny stellate neurons appear to function as a high-fidelity translator of thalamic inputs, maintaining strict topographic organization and setting up initial links of information transfer within a cortical area.

3.08.2.3 Inhibitory Interneurons: Basket, Chandelier, and Double Bouquet Cells

There is a large variety of interneuron types in the cerebral cortex. These neurons contain the neurotransmitter \( \gamma \)-aminobutyric acid (GABA), and exert strong local inhibitory influences on postsynaptic neurons. The dendritic and axonal arborizations of interneurons offer important clues as to their role in the regulation of pyramidal cell function. In addition, many morphologic classes of GABAergic interneurons can be further defined by a particular set of neurochemical characteristics. Three major subtypes of cortical interneurons are classically described, principally based on rodent and primate studies, namely basket, chandelier, and double bouquet cells. It must be noted, however, that interneurons exhibit a rich variety of size and morphologies, such as clutch cells, neurogliaform cells, Martinotti-type and Cajal–Retzius cells, bipolar cells and other stellate cells, and multipolar neurons, which all have diverse representations depending on the brain region as well as on the species studied.

Basket cells are characterized by axonal endings that form a basket of terminals surrounding a pyramidal cell soma and provide most of the inhibitory GABAergic synapses to the soma and proximal dendrites of pyramidal cells. One basket cell may contact numerous pyramidal cells, and in turn several basket cells can contribute to the pericellular basket of one pyramidal cell. The basket cells have a relatively large soma and multipolar morphology with dendrites extending in all directions for several hundred micrometers such that the vertically oriented dendrites cross several layers. Their axon arises vertically, quickly bifurcates and travels long distances, forming multiple pericellular arrays as it spreads horizontally. The basket cells predominate in layers III and V in the neocortex and are numerous amid hippocampal pyramidal neurons.

Chandelier cells generally have a variably bitufted or multipolar dendritic tree. The defining characteristic of this cell class is the very striking appearance of its axonal endings. In Golgi or immunohistochemical preparations, the axon terminals appear as vertically oriented cartridges, each consisting of a series of axonal swellings linked together by thin connecting pieces making them look like old-style chandeliers. These neurons synapse exclusively on the axon initial segment of pyramidal cells. Most of the chandelier cells are located in layer III and their primary target appears to be pyramidal cells in layer III, and to a lesser extent layer V. One pyramidal cell may receive inputs from multiple chandelier cells, and one chandelier cell may innervate more than one pyramidal cell. This cell exerts powerful inhibition on postsynaptic pyramidal cell firing.

Double bouquet cells are mostly prevalent in layers II and III, and are also present in layer V of the neocortex. These interneurons are characterized by a vertical bitufted dendritic tree and a tight bundle of vertically oriented varicose axon collaterals that traverse layers II through V. Many double bouquet cells synapse on spines of pyramidal cells and most of their remaining synapses are on fine dendritic shafts, in striking contrast to the basket and chandelier cells. Another subclass of double bouquet cell has similar synaptic targets but primarily in layer V, thus influencing the activity of different populations of pyramidal cells.

GABAergic interneurons can also be classified in nonoverlapping subtypes based on their content in the calcium-binding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR), as well as several neuropeptides (Hendry et al., 1989; Andressen et al., 1993; Condé et al., 1994; DeFelipe, 1997; Gonchar and Burkhalter, 1997; Glezer et al., 1998; Morrison et al., 1998; Hof et al., 1999; Markram et al., 2004). CB- and CR-expressing interneurons share many morphologic similarities, and are mainly bitufted, bipolar, and double bouquet neurons, as well as a few pyramidal neurons, with minimal overlap among these subpopulations in the rodent and primate neocortex (Rogers, 1992; DeFelipe, 1997; Morrison et al., 1998). PV-immunoreactive neurons are mainly observed in layers II
to V, and are principally basket and chandelier cells (Blümcke et al., 1990; Van Brederode et al., 1990; Hof and Nimchinsky, 1992; Condé et al., 1994; Nimchinsky et al., 1997). PV has also been reported to occur in certain pyramidal neurons in primate somatosensory and motor cortex (Preuss and Kaas, 1996; Sherwood et al., 2004).

### 3.08.3 Relationships of Neurochemical Phenotype and Phylogenetic Affinities

#### 3.08.3.1 General Phylogenetic Patterns

Phylogenetic patterns in the distribution of NFP and the three calcium-binding proteins appear to be associated with interspecific variation in other aspects of the cytoarchitecture of mammalian neocortex. Monotremes (i.e., echidnas and the platypus) are the sister taxon to all therian mammals. In these species, neurons containing CB, CR, and PV are found throughout the cortex, although CR-immunoreactive neurons are most dense in the piriform cortex where they predominate as a polymorphic phenotype. In monotremes (Hof et al., 1999), CB and PV comprise morphologic types that resemble those found in placental mammals, including large PV-immunoreactive multipolar neurons that are similar to basket cells. In echidnas, however, PV is present in a unique cell type characterized by a large pyramidal-like or multipolar morphology that is common in layers V and VI. Such PV-labeled neurons have not been observed in any other mammalian species. It is also worth noting that the Australian echidna (Tachyglossus aculeatus) presents NFP-expressing cells in layer V of its neocortex (Hassiotis et al., 2004). Although there are no studies of NFP staining in the most closely related taxon, the platypus, NFP is enriched in layer V neurons in the marsupial tammar wallaby (Macropus eugenii) throughout its cortex, as well as occasionally in layer III neurons of select sensory and association regions (Ashwell et al., 2005). In placental mammals, NFP-containing pyramidal neurons are found frequently in layers III, V, and VI. Thus, NFP-rich cells in layer V may be interpreted as a conservative trait among mammals, with variable patterns of NFP expression in other layers having arisen in different lineages in subsequent evolution (Figure 1).

All species of marsupials examined to date display comparable staining patterns in the neocortex for PV, CB, and CR (Hof et al., 1999). The most prevalent calcium-binding protein in marsupials appears to be CR, which is present in numerous small bipolar neurons located in layers II and III, as well as small pyramidal-like neurons in layers V and VI in the lateral cortex. Compared to CR-immunoreactive neurons, CB is more sparsely present in bipolar and bitufted neurons in the supragranular layers throughout the cortex, and in some larger multipolar neurons in the deep layers. A major difference between marsupials and other mammalian taxa is the remarkable paucity of PV-immunoreactive neurons and fibers. PV is observed only in a few small interneurons, whereas it is much more prevalent in other small-bodied mammals as well as in primates and carnivores. Surprisingly, PV-containing neurons in layer II have a morphology resembling double bouquet cells that are usually labeled by CB in rodents and primates. This may represent a neuronal specialization in certain marsupials that is not found in placental mammals.

A current limitation to interpreting regional patterns of distributions of neuron classes is the paucity of data from the phylogenetic groups that diverged close to the base of the adaptive radiation of placental mammals, the Xenarthra (i.e., sloths, anteaters, and armadillos) and Afrotheria (i.e., tenrecs, golden moles, elephant shrews, aardvarks, manatees, hyraxes, and elephants). Substantially more is known regarding variation in cortical architecture of the other placental mammals, the Boreoeutheria. Among boreoeutherian mammals, species showing a high degree of morphologic differentiation of neocortical areas, a variable development of layer IV, and substantial variation in neuronal size and packing densities across the cortical plate are also generally characterized by a balanced representation of the three calcium-binding proteins and morphological diversity of NFP-immunoreactive pyramidal neurons across cortical regions (Hof et al., 2000). In contrast, species characterized by greater cytoarchitectural monotony throughout the cortical mantle, a poorly defined or lack of layer IV in most regions, and the presence of very large pyramidal cells in all neocortical areas, display a predominance of CB- and CR-containing populations in comparison to PV-immunoreactive neurons, and rather uniform NFP-containing pyramidal cell morphology. The first type occurs in primates, rodents, carnivores, and to some extent megachiropterans, as well as in tree shrews and lagomorphs. Most of the taxa that are characterized by this cortical organization are members of the supraordinal group Euarchontoglires, with the exception of carnivores and bats. In contrast, the second type of cortical organizational pattern is present in cetaceans, artiodactyls, and perissodactyls, which are all
members of the Laurasiatheria. Thus, the distribution of these particular aspects of the cortical phenotype follows a major taxonomic division that occurred about 80–90 Mya at the base of the radiation of boreoeutherian mammals (Murphy et al., 2001a, 2001b). While these general patterns appear to differentiate cortical organization in major boreoeutherian clades, the lack of data from xenarthrans and afrotherians makes it difficult to clearly establish which character states are conservative and which are derived for placental mammals. This is a particular challenge because cortical cell types in monotremes and marsupials often differ significantly from placental mammals and from each other. Our examination of calcium-binding protein immunoreactivity in the xenarthran giant anteater (Myrmecophaga tridactyla), however, shows similarities with marsupials and cetartiodactyls in that PV-immunoreactive neurons are very sparse, whereas CB- and CR-immunoreactive neurons are expressed in a morphologically diverse population of cells that includes a high frequency of pyramidal neurons (Hof and Sherwood, 2005; Figures 2 and 3).

3.08.3.2 The Distribution of Some Neuron Classes Closely Matches Phylogenetic Affinities

Reports of the cyto- and chemoarchitecture of odontocete cetaceans, particularly of visual and auditory regions, and analysis of neocortical neurons in a few large artiodactyls have revealed commonalities in cortical organization between these sister taxa (Morgane et al., 1988, 1990; Glezer et al., 1992, 1993, 1998; Hof et al., 1992; 1999). In both groups, PV is present only in sparsely distributed large stellate neurons located in layer IIIc/V. A few small pyramidal neurons in layer III also exhibit PV immunoreactivity in dolphins. In cetaceans and artiodactyls, CB- and CR-immunoreactive neurons are more numerous than PV-immunoreactive cells, occurring in large fusiform, bipolar, or multipolar neurons in layers I, II, and superficial layer III. CB-containing neurons are much less numerous and less intensely stained than CR-immunoreactive neurons. The CR-containing neurons located in layer I have a morphology quite comparable to that of the bipolar/buftufted CB- or CR-expressing neurons typically seen in layer II of other mammals such as rats, carnivores, and primates (Ballesteros Yáñez et al., 2005), whereas the CR-containing neurons in layers II and III are much larger and more variable in shape than in other species, with a predominance of multipolar and fusiform types. These neurons have long dendrites that extend into layers I and III. Very large CR-immunoreactive neurons are also encountered in layers V and VI, especially in the neocortex of large artiodactyls, such as the giraffe (Giraffa camelopardalis), llama (Lama glama), and camel (Camelus dromedarius), whereas they are less numerous in the pig (Sus scrofa), and in smaller ruminants. A few pyramidal-like neurons in layer III are also faintly CR-containing in dolphins, and the large pyramidal neurons in layer IIIc/V contain low levels of CB. The distribution and morphology of NFP-immunoreactive neurons are also comparable in cetaceans and artiodactyls, but differ considerably from those in primates, carnivores, and rodents. In cetartiodactyls, NFP is expressed in very large pyramidal neurons located in the deep portion of layer III and in upper layer V (Hof et al., 1992). These neurons are present as clusters of three to six neurons, regularly spaced throughout the cortical mantle and intensely labeled with prominent apical dendrites extending well into layer I, with no major regional variability in their densities.

Another cell class that shows a restricted phylogenetic distribution is the spindle-shaped neuron, which is found exclusively in the cerebral cortex of hominids (i.e., great apes and humans). Spindle-shaped cells are characterized by a vertical, fusiform morphology, very large size, and high levels of NFP immunoreactivity (Nimchinsky et al., 1995, 1999). They are prevalent in a restricted sector of the anterior cingulate cortex (areas 25, 24a, and 24b; Vogt et al., 1995) and are also numerous in the anterodorsal agranular insular cortex (see The Evolution of Neuron Types and Cortical Histology in Apes and Humans, Role of Spindle Cells in the Social Cognition of Apes and Humans). These neurons are found exclusively in hominids and have not been reported in any other mammalian species investigated thus far (including other primate species; Nimchinsky et al., 1999; Hof et al., 2000). The volume of spindle-shaped cells is strongly correlated with encephalization, which is not the case for the other neuron types (Nimchinsky et al., 1999). These spindle-shaped cells are a particular type of projection neuron, as they send an axon in the subcortical white matter, although their exact domain(s) of projection cannot be ascertained in the species in which they are present. They may, however, provide well-defined projections, similar to Meynert cells or Betz cells (Sherwood et al., 2003b). Furthermore, although CR-immunoreactive pyramidal neurons have been reported in the entorhinal cortex of several mammalian species, CR-containing small pyramidal neurons in layer Va of anterior cingulate cortex (area 24) appear to be present exclusively in hominids, possibly indicating a recent evolutionary character in
this primate clade (Hof et al., 2001). These observations of multiple novel cellular specializations suggest the occurrence of functional modifications along the hominid lineage during the last 15–20 My in cortical regions that play major roles in the regulation of autonomic function, cognition, self-awareness, emotionality, and vocalization (Figures 2 and 3).

Figure 2 Immunoreactivity patterns of calcium-binding proteins in the neocortex of various mammals. a and b, CB (a) and PV (b) immunoreactivity in the primary somatosensory cortex of the echidna (Tachyglossus aculeatus). There is a large population of small neurons that express CB, whereas PV is contained in a heterogeneous collection of neurons, some very large and multipolar. This differs radically from other members of the Australian fauna (Hof et al., 1999), such as the koala (Phascolarctos cinereus, c), which displays a rather sparse population of CB-containing cells. d and e, The giant anteater (Myrmecophaga tridactyla) is also characterized by a sparse population of relatively large CB-immunoreactive multipolar neurons (d) and PV-expressing neurons (e). These patterns are fully distinct from that in rodents (f, CR immunoreactivity in the somatosensory cortex of the chinchilla, Chinchilla laniger), carnivores (g, CR immunoreactivity in a dog motor cortex displaying bipolar neurons as well as pyramidal-like neurons), and a cetacean visual cortex (h, the bottlenose dolphin, Tursiops truncatus). Layers I and II are particularly enriched in the cetacean (layer II is located about one-third down from the top of the photomicrograph). Panels (i–l) show details of the chemoarchitecture of the frontal cortex of a Siberian tiger (Panthera tigris). CR-immunoreactive neurons exhibit a typical distribution predominating in the superficial layers (i, k), and large multipolar CB-immunoreactive (j) and PV-immunoreactive (l) neurons are encountered in deep layer III. Large multipolar CR-containing neurons are found in layer III of a dog (Canis familiaris) primary motor area (m). Note the large size of this neuron and compare it to the CR-immunoreactive giant neuron located in layer VI of a giraffe (n, Giraffa camelopardalis), and to a typical bipolar neuron in layer III of the mouse visual cortex (o, Mus musculus). Scale bar (on n): 300 μm (a, b); 400 μm (f–i); 50 μm (c–e, j–o). Reproduced from Hof, P. R. and Sherwood, C. C. 2005. Morphomolecular neuronal phenotypes in the neocortex reflect phylogenetic relationships among certain mammalian orders. Anat. Rec. A 287, 1153–1163, with permission from John Wiley & Sons, Inc.
3.08.3.3 Other Phylogenetic Distributions of Cortical Neuron Classes Indicate Convergent Evolution

Although many aspects of cortical architecture reflect phylogenetic affinities, it is likely that many cortical phenotypes present examples of homoplasy, where certain characters have evolved independently in nonrelated groups of mammals. In this regard, it is noteworthy that there are several traits shared by Carnivora and Euarchontoglires, such as the distribution and typology of calcium-binding protein-containing neurons (Glezer et al., 1993, 1998; Hof et al., 1999; Ballesteros Yáñez et al., 2005). For example, in the dog neocortex, PV is present in a large population of morphologically diverse interneurons with a typology generally comparable to that observed in anthropoid primates. Some of these large multipolar neurons may be basket cells, due to the presence of PV-immunoreactive basket terminals around unstained pyramidal perikarya. Furthermore, CR is present in a very dense population of bipolar and double bouquet cells in layer II and the upper portion of layer III, as observed in primates and rodents. These aspects of chemoarchitecture in carnivores that are similar to primates, rodents, and other Euarchontoglires are not derived from a common ancestral state and hence have arisen due to convergent evolution. Other features of carnivore cortical organization resemble traits observed in their close relatives in the Laurasiatheria, the perissodactyls and cetartiodactyls, and so were probably inherited.

Figure 3 Schematic representation of the major calcium-binding protein-immunoreactive neuronal types in the mammalian neocortex. A few representative species of monotremes, marsupials, and placentals are shown. Many species have comparable neuronal types, especially with respect to small CR-immunoreactive bipolar neurons and CB-containing bitufted and double-bouquet cells. Primates, rodents, and carnivores, with the exception of CR, tend to show comparable patterns. The shading of the neurons indicates the relative intensity of the staining. Faintly labeled neurons are shown as empty symbols, moderately labeled neurons as gray symbols, and intensely labeled neurons as black symbols. Dotted triangles indicate the presence of PV-immunoreactive basket terminals on pyramidal neurons. Triangles represent pyramidal and pyramid-like neurons; black dots represent small multipolar or round neurons; diamonds represent large multipolar neurons and basket cells. Other symbols identify CB- and CR-immunoreactive bipolar, bitufted, and double bouquet cells, as well as neurons with atypical morphology. Note the presence in the monkey and the rat of CB-immunoreactive neurogliaform (represented as small star-shaped elements), and Martinotti cells (shown as large, ovoid horizontally oriented elements), in layers V and VI, that have not been reported in other species. On each panel, the dashed lines identify layers I and IV. Note the thick layer I and the absence of layer IV in cetaceans and ungulates. Layer IV is also not clearly defined in the hedgehog. Adapted from Hof, P. R., Glezer, I. I., Condé, F., et al. 1999. Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: Phylogenetic and developmental patterns. J. Chem. Neuroanat. 16, 77–116, Elsevier.
The role of calcium-binding protein in cortical integration is likely to be similar to a large degree among rodents, carnivores, and primates, suggesting that similar mechanisms exist across boreoeutherian mammals at least. However, differences are present at the level of particular neuronal subclasses, as recently revealed in a study of CB-expressing interneurons in primates compared to rodents, lagomorphs, carnivores, and artiodactyls (Ballesteros Yáñez et al., 2005). These authors reported that, in the nonprimate species, axon bundles of CB-immunoreactive double bouquet cells are not observed except for some in the visual cortex of carnivores, indicating that although somata that resemble typical CB-expressing cell types from primates can be found in these species, their axonal projections are likely to differ from primates. Whether such differences in axonal organization can be extended to PV- and CR-immunoreactive neurons remains to be demonstrated.

The degree to which functional interpretations of biochemical neuron types can be applied to all mammalian orders is difficult to determine owing to large differences in morphological phenotypes and distributions for any given cell type across species. The relative rarity of PV-immunoreactive neurons in cetaceans and artiodactyls could be interpreted as an ancestral retention for the Laurasiatheria because it also occurs in other laurasiatherians such as echolocating bats and hedgehogs, which may show many plesiomorphic features (Glezer et al., 1988). The neocortex of cetaceans and large artiodactyls appears to contain an inordinate number of cortical modules revealed by clusters of large NFP-containing pyramidal cells in layer IIc/V (Glezer et al., 1988; Morgane et al., 1988; Hof et al., 1992). Much cortical integration in cetaceans may take place in the cellular, thick layer I that contains 70% of the neocortical synapses in these species (Glezer and Morgane, 1990). Consistent with this observation, most CB- and CR-containing interneurons are located in layers I and II in cetaceans, and the few PV-immunoreactive cells lie in nearby layers IIc/V and VI pyramidal cells. The PV-immunoreactive neurons may represent basket cells, and axons of CB- and CR-immunoreactive interneurons may be located in a position to interact with inputs to the neocortex and connect the apical dendrites of the deep layers of pyramidal neurons (Glezer et al., 1988; Morgane et al., 1988). It is possible that in cetartiodactyls calcium-binding protein-immunoreactive neurons play a comparable role in neocortical microcircuits as in primates and rodents. The similarities in neurochemical specialization of the cetartiodactyl...
neocortex parallel the paleontological and molecular evidence, indicating that these species share a relatively recent common ancestor, and that much like primates, the evolution of the species with the largest brains (the delphinids) is a recent event (Marino et al., 2005).

Although there are major gaps in our knowledge of the evolutionary history of neocortical organization in mammals and of the chemical organization of the cerebral cortex in most species, collectively these observations indicate that brain organization and neurochemical cellular specialization reflect evolutionary relationships among many mammalian species.

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References
The Evolution of Neuron Classes in the Neocortex of Mammals


Further Reading


Calcium-binding protein-containing neuronal populations in 
mammalian visual cortex: A comparative study in whales, insectivores, bats, rodents, and primates. 

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distribution of the calcium-binding proteins parvalbumin, 
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