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An Architectonic Study of the Neocortex of the Short-Tailed Opossum (*Monodelphis domestica*)

Peiyan Wong and Jon H. Kaas

Department of Psychology, Vanderbilt University, Nashville, Tenn., USA

Abstract

Short-tailed opossums (*Monodelphis domestica*) belong to the branch of marsupial mammals that diverged from eutherian mammals approximately 180 million years ago. They are small in size, lack a marsupial pouch, and may have retained more morphological characteristics of early marsupial neocortex than most other marsupials. In the present study, we used several different histochemical and immunochemical procedures to reveal the architectonic characteristics of cortical areas in short-tailed opossums. Subdivisions of cortex were identified in brain sections cut in the coronal, sagittal, horizontal or tangential planes and processed for a calcium-binding protein, parvalbumin (PV), neurofilament protein epitopes recognized by SMI-32, the vesicle glutamate transporter 2 (VGluT2), myelin, cytochrome oxidase (CO), and Nissl substance. These different procedures revealed similar boundaries among areas, suggesting that functionally relevant borders were detected. The results allowed a fuller description and more precise demarcation of previously identified sensory areas, and the delineation of additional subdivisions of cortex. Area 17 (V1) was especially prominent, with a densely populated layer 4, high myelination levels, and dark staining of PV and VGluT2 immunopositive terminations. These architectonic features were present, albeit less pronounced, in somatosensory and auditory cortex. The major findings support the conclusion that short-tailed opossums have fewer cortical areas and their neocortex is less distinctly laminated than most other mammals.

Keywords

Marsupial; Cortical areas; Visual cortex; Frontal cortex; Somatosensory cortex; Auditory cortex; Retrosplenial cortex; Cingulate cortex

Introduction

We examined the cortical architecture of the short-tailed opossum (*Monodelphis domestica*) with the purpose of defining and providing a detailed description of neocortex subdivisions that are likely to have functional significance. Marsupial mammals diverged from eutherian mammals approximately 180 million years ago [Kumar and Hedges, 1998; Murphy et al., 2001; Woodburne et al., 2003]. Since then, marsupials have had considerable success and diversified, radiating into over 70 genera and 240 species [Grzimek, 1990]. Opossums are part of the marsupial radiation and over the last hundred million years have diverged into 15 genera, with more than 75 species [Gardner, 1993]. They are small, smooth-brained mammals that are found only in the New World. Opossums are important to include in comparative studies of neocortical evolution. Most of them have a small body size and some

lack a marsupial pouch, suggesting that they might have retained more primitive features than most other marsupials [Kirsch, 1977; Marshall, 1979; Reig et al., 1987]. Comparative studies suggest that opossums have retained more of the morphological characteristics of early marsupial neocortices than other marsupials [see Johnson, 1990; Rowe, 1990]. Features of neocortical organization that opossums share with small, smooth-brained eutherian mammals are likely to be those inherited from a common ancestor.

The short-tailed opossum is native to South America and is a ground-dwelling mammal that feeds mostly on plants and insects [Streilein, 1982]. It has large eyes, an elongated snout with well-developed vibrissae, and uses its forepaws to manipulate food items [Huffman et al., 1999]. Earlier studies have shown that the organization of neocortex in short-tailed opossums is generally less complex than in eutherian mammals. As in other marsupials, short-tailed opossums do not have a corpus callosum, and instead have a rather large anterior commissure [Owen, 1837], which plays the role of transferring information between the two hemispheres [Ebner, 1967; Martin, 1967; Foster and Ebner, 1977; Foster et al., 1981; Moreland et al., 1985]. As with most other mammals, studies in short-tailed opossums have shown that their neocortex contains a primary visual area (V1), or area 17, an auditory area (A), and a primary somatosensory area (area 3b), and they have no known motor area. The primary visual area, V1, of short-tailed opossums contains a complete topographic representation of the visual hemifield [Sousa et al., 1978; Kahn et al., 2000], and tracer injection studies have demonstrated dense connections between V1 and cortex lying along the rostrolateral border of V1, suggesting the presence of a secondary visual area [Kahn et al., 2000]. The organization of the auditory cortex in short-tailed opossums is relatively unknown, although the presence of a putative primary auditory cortex that is coextensive with the densely myelinated region in the temporal cortex can be assumed [Karlen and Krubitzer, 2006]. A complete somatosensory representation of the contralateral body surface is present in the primary somatosensory cortex, area 3b, of opossums [Pubols et al., 1976; Huffman et al., 1999; Frost et al., 2000; Catania et al., 2000b], with a large proportion devoted to the representation of the face and mouth [Frost et al., 2000; Catania et al., 2000b]. Microelectrode recording experiments have also revealed a second somatosensory area with a reversal of the somatotopic organization of S1 [Frost et al., 2000; Catania et al., 2000b]. The architectonic borders of this second somatosensory area are not clear, and the caudal portion of this area contains neurons that respond to both auditory and tactile stimulation [Catania et al., 2000b].

This present study is part of a larger comparative study aimed at determining the neocortical organization based on architectonic features revealed by histological and immunohistological techniques. A detailed and comprehensive characterization of the various cortical areas in short-tailed opossums will allow comparisons to made with better studied small-brained eutherian mammals, including laboratory rats [e.g., Krieg, 1946; Schober, 1986; Wise and Donoghue, 1986; Zilles and Wree, 1995; Swanson, 2003; Uylings et al., 2003], laboratory mice [e.g., Rose, 1912; Lorente de N, 1938; Wallace, 1983; Paxinos and Franklin, 2003], and to a lesser extent, squirrels [Kaas et al., 1972; Sereno et al., 1991; Wong and Kaas, 2008] and tree shrews [Zilles, 1978; Wong and Kaas, 2009]. Common features in cortical organization across infraclasses of mammals might be those present in early mammals [Kaas et al., 1970; Kaas, 1987].

We have used a variety of staining preparations in this study in an attempt to identify and give a detailed characterization of the various cortical areas. Apart from the standard Nissl, myelin and cytochrome oxidase (CO) histological stains, immunohistochemical stains such as vesicle glutamate transporter 2 (VGluT2), parvalbumin (PV), calbindin (CB), and SMI-32 were used. The VGluT2 immunostain reveals areal differences in thalamocortical terminations [Fujiyama et al., 2001; Kaneko and Fujiyama, 2002; Nahmani and Erisir,

2005]. Both PV and CB are calcium-binding proteins that reveal different subsets of GABA immunoreactive interneurons [Van Brederode et al., 1990]. In addition to revealing subsets of GABAergic non-pyramidal cells such as basket and double bouquet interneurons [Celio, 1986; DeFelipe and Jones, 1991; Conde et al., 1996; Hof et al., 1999], the PV immunostain labels afferent cortical terminations from sensory thalamic nuclei [Van Brederode et al., 1990; DeFelipe and Jones, 1991; de Venecia et al., 1995; Hackett et al., 1998; Latawiec et al., 2000; Cruikshank et al., 2001; Wong and Kaas, 2008]. The PV and CB immunostain not only reveals a subset of GABAergic non-pyramidal cells, it also reveals a subpopulation of pyramidal neurons [Preuss and Kaas, 1996; Hof et al., 1999, 2000]. SMI-32 is a non-phosphorylated epitope on neurofilaments that are present in a subset of pyramidal cells [Lee et al., 1987; Campbell and Morrison, 1989]. With this battery of staining methods used, we are able to provide an extensive description of the architectonic characteristics of the neocortex of short-tailed opossums.

Materials and Methods

Architectonic subdivisions of the neocortex were studied in seven short-tailed opossum (*Monodelphis domestica*) brains provided by Professor Leah Krubitzer at the University of California, Davis. All procedures were approved by the Vanderbilt Institutional Animal Care and Use Committee and followed NIH guidelines.

Tissue Preparation

The perfusions of the animals were done by Sarah Karlen at the University of California, Davis. All animals were given a lethal dose of sodium pentobarbital (100 mg/kg). The animals were perfused transcardially, in sequence, with 0.9% saline, 2% paraformaldehyde in 0.1 M PB and subsequently with 2% paraformaldehyde with 10% sucrose. Brains were stored after fixation in 0.1 M phosphate-buffered saline (PB) for transport to Vanderbilt University. Five of the seven brains were bisected and post-fixed for about 3 h in 4% paraformaldehyde and 10% sucrose in 0.1 M PB. The hemispheres were immersed in 30% sucrose solution for cryoprotection until they sank to the bottom of the vial. They were then sectioned at 40 μ m thickness in the coronal (n = 2), parasagittal (n = 2) or horizontal (n = 2) planes on a freezing microtome. Those brains sectioned horizontally or parasagittally were saved in three series, and brains that were sectioned coronally were divided into four series. For two of the brains, the hemispheres were separated from the brain stem and artificially flattened. These brains were then cut tangentially, parallel to the pia at 40 μ m thickness and saved in two series.

Histochemistry

One section from each coronal, sagittal, or horizontal series was processed for Nissl substance (with thionin). In some cases, a second series was processed for myelin using the Gallyas [1979] silver procedure, or for cytochrome oxidase (CO) [Wong-Riley, 1979]. In flattened brain sections, one of the two sections was processed for either the Gallyas [1979] silver procedure or for cytochrome oxidase (CO) [Wong-Riley, 1979].

Immunohistochemistry

In some cases, one or two series of brain sections was immunostained for SMI-32 (mouse monoclonal anti-SMI-32, catalog number SMI-32R from Covance Inc. Princeton, NJ; 1: 2000), parvalbumin (PV) (mouse monoclonal anti-PV, catalog number P3088 from Sigma-Aldrich, St. Louis, Mo; 1: 2000), calbindin (CB) (mouse monoclonal anti-CB, catalog number 235 from Swant, Bellinzona, Switzerland; 1: 5000), or vesicle glutamate transporter 2 (VGluT2) (mouse monoclonal anti-VGluT2, catalog number MAB5504 from Chemicon now part of Millipore, Billerica, Mass., USA; 1: 2000).

Light Microscopy and Anatomical Reconstruction

Cortical borders were revealed by laminar and cell density changes in the sections that have been processed for Nissl substance, myelin, CO, PV, CB, VGluT2, and SMI-32. The locations of borders were established by viewing sections at high power using a microscope. Nissl and PV preparations were the most useful for defining primary sensory areas. Other histochemical procedures were used for corroborating otherwise ambiguous borders. Processed sections were viewed under a Nikon E800 microscope (Nikon Inc., Melville, N.Y., USA) and digital photomicrographs of sections were acquired using a Nikon DXM1200 camera (Nikon Inc., Melville, N.Y., USA) mounted on the microscope with the ACT-1 program (Nikon Inc., Melville, N.Y., USA). Digitized images were adjusted for brightness and contrast using Adobe Photoshop (Adobe Systems Inc., San Jose, Calif., USA), but they were not otherwise altered.

The borders of architectonic subdivisions of cortex were defined by criteria specific to each set of brain sections, as described in Wong and Kaas [2008]. In brief, brain sections were outlined and architectonic borders were identified for each section using a Bausch and Lomb Microprojector (Bausch and Lomb, Rochester, N.Y., USA). Blood vessels and other landmarks were marked to aid in the alignment of results from adjacent sections. In general, the different histological procedures revealed almost identical boundaries between areas. This suggests that functionally relevant borders were being detected.

Results

Our current findings provide more detailed characterizations of previously proposed areas, as well as other subdivisions of the neocortex in short-tailed opossums. Some of these areas have been shown in brain sections cut parallel to the surface of manually flattened cortex [Kahn et al., 2000; Catania et al., 2000b], and similar sections are shown here (fig. 1). Descriptions of the cortical areas, region by region, follow.

Occipital Cortex

Area 17—Perhaps the most distinctive subdivision of neocortex is the large area 17, which occupies the caudomedial region of cortex (fig. 1). In opossums, layer 4 of area 17 is thick and densely populated with cells (fig. 2A). Layer 4 of adjoining cortical areas is less pronounced (fig. 3A). In contrast to layer 4, layer 3 is rather sparsely populated with cells (fig. 2A), giving rise to the banded appearance of area 17. Area 17 is more densely myelinated than the surrounding areas, such as the proposed second visual area (V2) and the prostriata area (PS) (fig. 1B; 3B; 7B). The dense myelinated fibers extend from the inner cortical layers to layer 3, with no distinct bands of Baillarger (fig. 2B). In sections cut parallel to the cortical surface, area 17 stands out as a darkly myelinated region (fig. 1B, E). As the medial portions of area 17 in the sections shown in figure 1 are deeper in cortex, they appear darker. CO expression in area 17 is stronger compared to the surrounding areas (fig. 2C; 3C) and is mainly concentrated in layer 4 (fig. 2C). In sections from flattened cortex, the borders of area 17 are clearly delineated by the reduction in CO expression in the adjoining areas (fig. 1A, C). Layer 4 of area 17 is darkly stained by the VGluT2 antibody due to the presence of dense thalamocortical terminations (fig. 2D). This dense VGluT2 staining drops off sharply at the borders of area 17 with the presumptive V2 and with PS (fig. 3D; 7D). In PV preparations, area 17 is more darkly stained for the PV antibody than the surrounding cortical areas (fig. 3E; 7E; 9D). At higher magnifications, the PV-immunopositive cells are scattered, but more numerous in layer 4 (fig. 2E). In size and shape, they appear to be interneurons. Layer 4 is also, darkly stained for PV-immunopositive terminations (fig. 2E). In sections tangent to the surface, area 17 is a darkly PV-stained region surrounded by PV-poor regions (fig. 1D). Area 17 shows a dense band of CB staining that terminates at the

area 17/presumed V2 and area 17/PS borders (fig. 3F; 7F). Densely stained SMI-32 immunopositive dendrites arborize in layer 5, and more so in layer 3 of area 17. This staining is largely absent in the cortical areas adjacent to area 17 (fig. 3G; 7G). SMI-32 immunostained cell bodies were not observed in area 17 (fig. 2F).

Remaining Occipital Areas—The remaining areas of the occipital cortex, considered here as part of the occipital-temporal-parietal cortex (OTP), consist of a secondary visual area that is adjacent to the rostrolateral border of area 17, and at least one other multimodal area where the neurons present are responsive to both auditory and visual stimulation [Huffman et al., 1999; Kahn et al., 2000]. Although the border between V2 and area 17 is distinct, the rostral border of V2 with the multimodal region is not.

To summarize, area 17 is distinguished from the rest of the occipital areas by a thick layer 4 that is densely populated with granule cells, dense myelination, and dark staining in CO, VGluT2, PV CB, and SMI-32 preparations. The architectonic border between area 17 and V2 is distinct, and the borders between V2 and other possible extrastriate areas in the OTP region are not.

Occipital-Temporal-Parietal Cortex (OTP)

The occipital-temporal-parietal cortex (OTP) is bordered rostrally by area 3b, rostroventrally by area A, caudally by area CT and caudomedially by V1. This region has a rather homogenous architectonic appearance, although it is likely that it contains more than one cortical area, including V2. As functional studies will be required for delimiting these borders, they are left unmarked in this study, and the architecture of this area is described as a whole. In Nissl preparations, layer 4 of OTP is less densely packed than in the three bordering sensory areas, area 17 (fig. 3A), area A (fig. 7A) and area 3b (fig. 9B), and layer 3 is more densely packed with cells than area CT (fig. 3A). OTP is also less myelinated than areas 17 (fig. 3B; 4A; 7B), A (fig. 5A; 7B), 3b (fig. 9C) and, to a lesser extent CT (fig. 3B; 4A). The low levels of myelination in OPT is observed in sections cut parallel to the cortical surface (fig. 1B, E). In CO preparations, OTP stain less darkly than areas 17 (fig. 1A, C; 3C), A (fig. 1A, C; 7C), 3b (fig. 1A, C) and CT (fig. 1A, C; 3C). OPT also stains poorly for VGluT2 (fig. 3D; 7D) and PV (fig. 3E; 7E; 9D) immunopositive terminations, and has a scattering of PV-immunostained cell bodies throughout the cortical layers (fig. 4C; 5C). At higher magnifications, the areal borders of OTP with the adjoining cortical areas is distinct due to the reduction of VGluT2 immunopositive terminations compared to area 17 (fig. 4B; 5B), CT (fig. 4B) and A (fig. 5B). CB preparations, OPT shows less staining than the surrounding areas (fig. 3F; 7F). In SMI-32 preparations, the borders between OTP and the adjoining areas, 17 (fig. 3G; 4D), A (fig. 5D; 7G), and 3b (not shown), with the exception of CT (fig. 3G; 4D; 6D), are distinct as OTP shows no staining for the SMI-32 antibody (fig. 3G; 4D; 5D).

Temporal Cortex (Auditory Cortex, A, and Caudal Temporal Area, CT)

Temporal cortex includes a core primary auditory field or fields (A), a caudal temporal area (CT), and a homogenous region between the two cortical areas.

Auditory Area (A)—In Nissl preparations, the auditory cortex (A) has a layer 4 that is densely populated with granule cells (fig. 6A), as expected for a sensory area. Layers 5 and 6 are less densely packed with cells (fig. 7A). Area A is densely myelinated compared to the adjacent cortical areas (fig. 7B), and at higher magnifications, the myelinated fibers extend up to layer 3, with no distinct bands of Baillarger (fig. 6B). In flattened sections, area A is a densely myelinated oval that is located rostrolateral to the occipital cortex, above the rhinal fissure (fig. 1B, E). Area A stains darker for CO compared to the adjoining cortical areas

(fig. 7C), and at higher magnification, a dark band of CO staining is present in layer 4, with some darkly stained cell bodies in layer 5 (fig. 6C).

In VGluT2 preparations, area A has a band of VGluT2 terminations in layer 4 (fig. 6D) that is thicker and more darkly stained than the adjoining cortical areas (fig. 7D). Area A also expresses more PV immunostaining compared to the adjoining areas, but less PV immunostaining than area 17 (fig. 7E). A distribution of darkly stained PV-immunopositive cell bodies is concentrated in layers 4 and 5 of area A, with layer 4 having the densest population of PV-immunopositive terminations (fig. 6E). The inner cortical layers of area A stain darkly for CB, and the dorsal border of area A with adjoining temporal cortex is marked by a sharp reduction in the level of CB staining (fig. 7F). In SMI-32 immunostained sections, area A shows dense staining compared to the adjacent cortical areas (fig. 7G), and at higher magnification, dense populations of short dendrites in layers 3 and 5 are stained for the SMI-32 antibody, with a sparse population of small, darkly SMI-32 immunostained cell bodies in layer 5 (fig. 6F).

Caudal Temporal Area (CT)—In Nissl preparations, CT has an appearance similar to the adjoining perirhinal cortex as it does not have a well-developed lamination pattern. CT has a densely packed layer 2, but is less densely packed with cells throughout the rest of the cortical layers compared to the dorsally adjoining cortex (fig. 3A). In myelin preparations, CT is more myelinated than the dorsally adjoining cortex (fig. 3B). In sections from flattened cortex, CT stands out as a darkly myelinated region (fig. 1B, E) that is immediately above the entorhinal cortex (EC). CT expresses more CO (fig. 3C), and has a thicker and darker band of VGluT2 staining in layer 4 (fig. 3D) than the surrounding areas. In PV-immunostained sections, CT has larger, more darkly stained PV-immunopositive cell bodies, with a dense concentration of PV-immunopositive terminations in layer 4/5 (fig. 3E). CT is more intensely stained for the CB antibody compared to the surrounding areas (fig. 3F), and does not show any staining for the SMI-32 antibody (fig. 3G).

In summary, area A is delineated from the surrounding cortical areas by a layer 4 that is densely packed with granule cells, dense myelination, and higher expression levels of CO protein, and VGluT2, PV, CB and SMI-32 antibodies. Area CT has a lower population of cells throughout the cortical layers, has a less distinct lamination pattern, is highly myelinated, and stains darkly for CO, VGluT2, PV, and CB, but not for SMI-32. Temporal cortex between areas A and CT is poorly laminated. Layer 4 is not highly differentiated, and CO, myelin, PV and VGluT2 levels are low, as in OTP.

Parietal Cortex

Primary Somatosensory Area (3b or S1)—In Nissl preparations, area 3b is more densely packed with cells than the surrounding cortical areas, with a darkly stained band in layer 4 (fig. 9B; 10A). At higher magnification, area 3b has a densely packed granular layer 4 and a moderately packed layer 5 of larger cell bodies (fig. 8A). Area 3b is more densely myelinated than the surrounding cortex (fig. 9C; 10B), and stands out as a darkly stained patch surrounded by myelin-poor regions in flattened cortex (fig. 1B, E). The myelinated fibers extend dorsally to layer 2, with no obvious bands of Baillarger (fig. 8B). Area 3b exhibits darker staining for CO in layer 4 compared to the surrounding areas (fig. 8C; 10C), and this is evident in the appropriate sections from flattened cortex as well (fig. 1A). Layer 4 of area 3b stains darkly for VGluT2-immunopositive terminations (fig. 8D), marking the border with the surrounding cortical areas, which have poor VGluT2 staining (fig. 10D). Area 3b stains more darkly for the PV antibody compared to the surrounding cortical areas (fig. 8E; 9D; 10E), as also observed in sections tangent to the surface (fig. 1D). At higher magnifications, area 3b contains a higher population of PV-immunopositive neurons in

layers 4 to 6, and a concentration of PV-immunopositive terminals in layer 4 (fig. 8E). In CB preparations, area 3b stains darker for the CB antibody compared to the surrounding cortical areas (fig. 10F). Area 3b stains darker for the SMI-32 antibody compared to the surrounding cortical areas (fig. 10G), with a dense population of SMI-32 immunostained dendrites in layer 3, and darkly stained dendrites, with a scattering of SMI-32 immunostained cell bodies in layer 5 (fig. 8F).

Secondary Somatosensory Area (S2)—The electrophysiologically defined S2 is located between area 3b and area A. The architectonic borders of S2 are not distinct and left unmarked. Architectonically, S2 stains lighter for CO (fig. 1A, C) and is more poorly myelinated (fig. 1B, E) compared to area 3b and area A.

Frontal-Parietal Area (FP)—The frontal-parietal area is a strip of poorly myelinated cortex between area 3b and the frontal area (fig. 1B, E; 9C). In Nissl preparations, area FP has poorly differentiated cortical layers. Area FP lacks the thick granular layer 4 that is present in area 3b, and is more sparsely populated with neurons in layers 2 and 3 than area F. Compared to the adjoining areas, area FP expresses less CO (fig. 1A). Area FP also stains poorly for PV-immunopositive terminations and has a lower population of PV-immunopositive cell bodies compared to areas 3b and F (fig. 9D).

In summary, area 3b can be delineated from the surrounding cortical areas by the densely populated layer 4 with granule cells, dense myelination, dark staining in CO, VGluT2, PV, CB and SMI-32 preparations. Area FP has poorly defined architectonic features compared to the adjoining areas F and 3b, and is poorly laminated, weakly myelinated, and expresses little CO and PV.

Frontal Cortex

The frontal cortex consists of at least a frontal area (F) and the surrounding areas. Area F is less densely populated with cells compared to area 3b, giving it an overall paler appearance in the Nissl stain (fig. 9B; 10A). The upper cortical layers 2 and 3 of area F are more densely populated with cells, whereas layer 4 is less developed and more sparsely populated with cells than area 3b (fig. 9B; 10A). Area F has similar myelination levels compared to area 3b, but can be delimited from area 3b by a myelin-poor region (FP) that lies between these two areas (fig. 1B, E; 9C). In CO preparations, area F stains darkly for CO (fig. 10C), and appears as a dark CO-patch in flattened sections (fig. 1A, C). Area F is less darkly stained in VGluT2 sections compared to area 3b (fig. 10D). In PV preparations, area F is a darkly stained patch in tangent sections (fig. 1D), and is populated with PV-immunostained cell bodies (fig. 9D; 10E). Area F can be delimited from area 3b in the PV stain as area F lacks the dense PV-immunopositive terminations that are present in layer 4 of area 3b (fig. 9D; 10E). In CB preparations, area F lacks the dense staining that is present in area 3b (fig. 10F). Layers 3 and 5 of area F are densely populated by SMI-32 immunopositive dendrites, with no visible SMI-32 immunopositive cell bodies (fig. 10G).

Retrosplenial Cortex

The retrosplenial cortex contains at least three cortical areas, the prostriata (PS), the retrosplenial dorsal (RSd), and retrosplenial ventral (RSv) areas, and occupies the caudal half of the medial wall.

Area Prostriata (PS)—Area PS lies along the medial border of area 17 and is differentiated from area 17 by less well-defined lamination, the lack of a thick, distinct layer 4 (fig. 3A; 7A), and reduced myelination (fig. 3B; 7B). In PV preparations, area PS has a sparse population of PV-immunostained cell bodies (fig. 3E; 7E). Area PS stains poorly for

CO (fig. 3C; 7C), VGluT2 (fig. 3D; 7D), CB (fig. 3F; 7F), and SMI-32 compared to area 17 (fig. 3G; 7G).

Retrosplenial Dorsal Area (RSd)—In Nissl preparations, layer 2 of RSd is densely populated with cells, giving it a darker appearance than the adjoining area PS, and lacks a distinct layer 4 (fig. 3A; 7A). RSd is sparsely myelinated compared to the surrounding areas (fig. 3B; 7B), and expresses less CO staining than PS (fig. 3C; 7C). In VGluT2 preparations, RSd, like area PS, stains poorly for the VGluT2 antibody (fig. 3D; 7D). RSd is more highly populated with PV-immunostained neurons than area PS (fig. 3E; 7E), whereas in other preparations such as the CB (fig. 3F; 7F) and SMI-32 (fig. 3G; 7G) immunostained sections, area RSd stains in a similar manner to PS.

Retrosplenial Ventral Area (RSv)—RSv does not have well-defined laminar layers but can be delimited from RSd due to the higher cell packing density in RSv that results in an overall darker appearance in the Nissl stain (fig. 3A; 7A). RSv is also more densely myelinated than RSd (fig. 3B; 7B). In CO preparations, no visible difference is observed between the staining patterns of RSd and RSv (fig. 3C; 7C). RSv is more densely populated with VGluT2- (fig. 3D; 7D) and PV-immunopositive terminations, and has a dense population of PV-immunopositive neurons (fig. 3E; 7E). In CB preparations, RSv, like RSd, stains poorly for the CB antibody (fig. 3F; 7F). Layers 3 and 5 of RSd show some staining of SMI-32-immunopositive dendrites, with no SMI-32-immunopositive cell bodies (fig. 3G; 7G).

Cingulate Cortex

The cingulate cortex occupies the rostral half of the medial wall, and consists of three areas, the dorsal (CGd), ventral (CGv) and rostral (CGr) cingulate areas.

Cingulate Dorsal Area (CGd)—CGd is not well laminated, and is less densely packed with cells, resulting in an overall paler appearance than the surrounding areas (fig. 10A). CGd is delimited from the lateral adjoining area by the sparsely populated layer 5 (fig. 10A). In myelin preparations, CGd is more densely myelinated (fig. 10B), and expresses more CO protein in layer 4 than the surrounding cortical areas (fig. 10C). Layer 4 of CGd stains darkly in the VGluT2 stain, unlike the adjoining cortical areas (fig. 10D). In PV and CB preparations, CGd expresses very little of either antibody (fig. 10E, F). CGd contains more SMI-32-immunostained dendrites in layer 3 compared to the adjacent cortical areas (fig. 10G).

Cingulate Ventral Area (CGv)—The laminar layers in CGv are not well differentiated. In the Nissl stain, CGv has a darker appearance than CGd, as CGv is more densely populated with cells, especially in layer 2 and 5/6 (fig. 10A). CGv is less densely myelinated (fig. 10B), and expresses less CO protein than CGd (fig. 10C). In VGluT2 preparations, CGv shows no visible staining for the VGluT2 antibody (fig. 10D). Layer 5/6 of CGv is densely populated with PV-immunopositive neurons and terminations (fig. 10E). CGv shows no visible staining in either CB (fig. 10F) or SMI-32 (fig. 10G) preparations.

Cingulate Rostral Area (CGr)—In Nissl preparations, CGr is marked by darker staining in layer 2 compared to area F due to a dense population of cells. CGr is less densely myelinated (fig. 11B) and expresses fewer CO proteins than area F (fig. 11C). CGr does not show staining in VGluT2 (fig. 11D), PV (fig. 11E), CB (fig. 11F), or SMI-32 (fig. 11G) preparations.

Discussion

In the present study, we used a variety of histological preparations to characterize architectonic subdivisions of neocortex in the short-tailed opossums. Opossums are of special interest because they are marsupials and thus represent one of the three major branches of the mammalian radiation. In addition, they have retained many of the morphological characteristics of early marsupials and other early mammals, including a relatively small brain with proportionately little neocortex. Thus, their neocortex might resemble that of early mammals more so than most other extant mammals. Our results indicate that the neocortex in these opossums is rather poorly differentiated into layers and areas with different laminar characteristics, and that most of the neocortex is occupied by primary visual, somatosensory, and auditory areas. These primary areas have architectonic features found in primary areas of other mammals, although they are muted and less distinct. Previous physiological and anatomical studies have identified the sensory fields in opossums [Lende, 1963; Pubols et al., 1976; Frost et al., 2000; Kahn et al., 2000; Catania et al., 2000b]. We were able to distinguish several other fields, including cingulate and retrosplenial areas that appear to be homologs of fields identified in eutherian (placental) mammals. Although there is physiological evidence for a second visual area, V2, and a second somatosensory area, S2, in short-tailed opossums [Kahn et al., 2000; Catania et al., 2000b], these representations were not architectonically distinct from adjoining fields, other than the primary fields. Overall, the cortical organization of short-tailed opossums resemble that of other small-brained mammals in having fewer and poorly differentiated areas [Krubitzer et al., 1997; Catania et al., 1999, 2000b], consistent with the view that early mammals had little neocortex, and this cortex had only a few, poorly differentiated areas [Kaas, 2007]. Cortical fields of the present study are discussed in relation to previous findings in marsupials and other mammals below.

Occipital Cortex

The occipital cortex in short-tailed opossums (*Monodelphis domestica*) consists of an architectonically distinct area 17, or V1, possibly a second visual area, V2, and a caudal temporal area, CT. Area 17, or V1 has a retino-topic map of the visual field, with most of area 17 devoted to the central 30 to 50 degrees representation of the contralateral visual hemifield [Kahn et al., 2000]. The retino-topic organization of V1 in short-tailed opossums is similar to that found in other marsupials, such as the South American opossum [Sousa et al., 1978; Volchan et al., 1988], Virginia opossum [Kaas, 1980], quoll [Rosa et al., 1999], and wallaby [Vidyasagar et al., 1992], and other mammals [Kaas, 1980]. Area 17 of short-tailed opossums is easily identified as an area with a layer 4 that is densely populated with granule cells and is densely myelinated [Kahn et al., 2000]. The architectonic characteristics of area 17 and its distinct rostral border in Nissl and myelin preparations have been observed in a range of marsupials [Beck et al., 1996; Rosa et al., 1999], and many small, lissencephalic eutherian mammals including rats [Krieg, 1946; Zilles et al., 1980, 1984; Reid and Juraska, 1991; Swanson, 2003], mice [Rose, 1931; Caviness, 1975; Paxinos and Franklin, 2003; Van der Gucht et al., 2007], rabbits [Rose and Malis, 1965], tenrecs [Krubitzer et al., 1997], hedgehogs [Kaas et al., 1970; Catania et al., 2000a] and shrews [Catania et al., 1999]. The extent of area 17 is distinct in flattened preparations. In addition to dense myelination, area 17 also darkly stains for CO, as it is metabolically more active than the surrounding cortical areas. In the Virginia opossum, which is in the same infraclass as the short-tailed opossum, area 17 or V1 receives inputs from the lateral geniculate nucleus [Benevento and Ebner, 1971] and the lateral portion of the lateral posterior nucleus [Coleman and Clerici, 1981] that corresponds to part of the pulvinar complex of primates. Area 17 of short-tailed opossums might receive similar thalamic projections, as layer 4 stains darkly for VGluT2 and PV immunopositive thalamocortical terminations.

The lateral border of area 17 is formed by area 18 or V2, as in other mammals [Rosa and Krubitzer, 1999]; however, the lateral border of V2 is unclear and has been left unmarked in this study. Neurons in V2 have large receptive fields and a reversal in retinotopy is observed at the V1/V2 boundary [Kahn et al., 2000]. Neuroanatomical studies of cortical connections in short-tailed opossums [Kahn et al., 2000] produced similar results to those from studies of brush-tailed possums [Crewther et al., 1984], South American opossums [Martinich et al., 2000], Virginia opossum [Benevento and Ebner, 1971] and mouse opossums [Bravo et al., 1990]. Namely, connections are observed between V1 and the presumptive location of V2, which is immediately rostrolateral to V1. Architectonically, the cortex immediately rostrolateral to area 17 in the short-tailed opossums is poorly myelinated and expresses less CO, suggesting that it is less metabolically active than area 17. In addition, layer 4 of that region is poorly populated with VGluT2 and PV immunopositive terminals, indicating that there is only sparse input from the thalamus.

Occipital-Temporal-Parietal Cortex (OTP)

The occipital-temporal-parietal cortex includes the peristriate cortex of Huffman et al. [1999], and the multimodal auditory and visual region of Kahn et al. [2000]. This region of neocortex lacks the pronounced architectonic features of core sensory areas 17, A and 3b, but rather has the appearance of a higher-order or association area or areas. Due to the homogenous architectonic appearance of OTP, it is difficult to subdivide this area of cortex. Caudally, the region of OTP that borders area 17 shows visual responses and is likely V2 [Kahn et al., 2000]. The ventral portion of the occipital-temporal-parietal cortex (OTP), between areas A and CT, is not well differentiated and it does not have the characteristics of sensory cortex. By position, the cortex might have auditory and visual functions [Wong et al., 2008].

Temporal Cortex

The auditory cortex, which includes the putative primary auditory area, A1, occupies much of the temporal cortex in short-tailed opossums. Electrophysiological studies in short-tailed opossums have shown that the neurons in the rostral pole of the auditory cortex are responsive to both auditory and tactile stimulation, suggesting the presence of a multimodal area between area A and the somatosensory cortex [Catania et al., 2000b]. This is similar to the auditory cortex of Virginia opossums, where the secondary somatosensory area has neurons responsive to auditory stimuli [Pubols, 1977; Beck et al., 1996]. In other marsupials, such as the brush-tailed possum [Gates and Aitkin, 1982] and the Northern quoll [Aitkin et al., 1986], primary auditory area (A1) is tonotopically organized and receives projections from the medial geniculate nucleus [Goldby, 1943; Neylon and Haight, 1983; Aitkin and Gates, 1983; Kudo et al., 1989] as in placental mammals [Ryugo and Killackey, 1974; Merzenich et al., 1976; Luethke et al., 1988; Wong et al., 2008]. Architectonically, the auditory cortex of short-tailed opossums has characteristics that are similar to those in other marsupials, including the Virginia opossum, brush-tailed possum and Northern quoll [Beck et al., 1996; Huffman et al., 1999; Elston and Manger, 1999; Catania et al., 2000b], such as dense myelination, dark CO staining, and a thick, densely packed layer 4 with granule cells. Here, we have further characterized the architectonic properties of the auditory cortex in short-tailed opossums. The dense CO staining of area A suggests that it is metabolically active. In addition, area A stains darkly for VGluT2 and PV immunopositive terminations, indicating the presence of dense thalamocortical projections. In brain sections cut tangentially to the pia, the topographic location of area A in short-tailed opossums is similar to A1 of placental mammals [e.g. Ryugo and Killackey, 1974; Merzenich et al., 1976; Luethke et al., 1988; Wong et al., 2008].

Area CT has been previously identified as a dark myelinated wedge-shaped region in flattened cortex containing neurons responsive to visual stimulation [Kahn et al., 2000] and receiving projections from area 17 [Kahn et al., 2000]. Area CT is metabolically active as it expresses relatively high levels of CO, and it is densely myelinated. Layer 4 of area CT apparently receives dense inputs from the thalamus, as it stains densely for VGluT2 and PV immunopositive terminations. In comparison with other marsupials, area CT of short-tailed opossums is in a similar location and has a similar appearance to area CT in the Virginia opossum [Beck et al., 1996], and the medial temporal region of the brush-tailed possum [Crewther et al., 1984].

Parietal Cortex

The parietal cortex of the marsupials consists of at least two areas, the primary somatosensory cortex (3b or S1) and a secondary somatosensory cortex (S2) [for review see Johnson, 1990; Rowe, 1990]. Electrophysiological studies in short-tailed opossums have shown that area 3b contains a complete, inverted representation of the contralateral body surface [Huffman et al., 1999; Catania et al., 2000b; Frost et al., 2000], with a poorly developed motor component embedded within [Lende, 1963; Pubols et al., 1976; Catania et al., 2000b; Frost et al., 2000]. The representation of the contralateral body surface is organized such that the tail representation is most medial, followed by the hindlimb, trunk, forelimb, forepaw, face and oral representations in a mediolateral progression [Catania et al., 2000b; Frost et al., 2000]. This topographic pattern is similar to the primary somatosensory areas of placental mammals [see Kaas, 1983 for review]. Area 3b of short-tailed opossums has similar architectonic characteristics [Huffman et al., 1999; Catania et al., 2000b; Frost et al., 2000] to other marsupials, such as the wallaby [Ashwell et al., 1996], Virginia opossum [Foster et al., 1981; Beck et al., 1996] and the brush-tailed possum [Weller, 1972], and placental mammals [e.g., Sur et al., 1978; Chapin and Lin, 1984; Krubitzer et al., 1986; Dawson and Killackey, 1987; Slutsky et al., 2000; Remple et al., 2003; Wong and Kaas, 2008]. These characteristics include a well developed, densely packed granular layer 4 and dense myelination. Area 3b of short-tailed opossums is further characterized by dark CO staining, suggesting that it has high metabolic demands. In addition, there is denser staining of VGluT2 and PV immunopositive terminations compared to the surrounding cortical areas, suggesting the presence of a higher proportion of thalamocortical terminations in area 3b, possibly originating from the ventroposterior nucleus, as has been shown in the Virginia opossum [Diamond and Utley, 1963; Pubols, 1968]. Unlike other marsupials, such as the brush-tailed possum, Virginia opossum, striped possum or Tammar wallaby, the short-tailed opossum has no architectonically definable barrel field or barrel-like cortex in area 3b [Woolsey et al., 1975; Waite et al., 1991, 1998; Weller, 1993; Beck et al., 1996; Elston and Manger, 1999].

Cortex immediately ventral and caudal to area 3b, in the expected location of S2, is responsive to somatosensory and auditory stimulation [Huffman et al., 1999]. S2 has a mirror reversal of the organization found in area 3b [Frost et al., 2000; Catania et al., 2000b]. The somatotopic organization of S2 in short-tailed opossums is similar to that found in the Virginia opossum [Pubols, 1977; Beck et al., 1996] and placental mammals [e.g., Kaas, 1983; Krubitzer et al., 1986; Catania and Kaas, 1995; Krubitzer, 1995]. S2 of short-tailed opossums at least partially overlaps the auditory cortex [Catania et al., 2000b]. Architectonically, S2 of most marsupials can be identified in the Nissl stain by broad layers 3 and 5, with a poorly defined granular layer 4 [Adey and Kerr, 1954], and is generally less densely myelinated than the adjoining S1 and auditory areas [e.g., Beck et al., 1996; Huffman et al., 1999; Catania et al., 2000b]. As the borders of S2 are unclear, they are left unmarked in this study.

Rostral to area 3b is a strip of poorly myelinated and poorly laminated cortex, the frontal-parietal area (FP). The functional significance of this area is unknown. From its shape, topographic location and architectonic properties, area FP is reminiscent of area 3a of cats [Dykes et al., 1980, 1986; Felleman et al., 1983] and primates [Jones and Porter, 1980; Huffman and Krubitzer, 2001; Krubitzer et al., 2004]. This poorly myelinated region, area FP, separates area F from area 3b, and architectonically resembles the somatosensory rostral area of the Virginia opossum [Beck et al., 1996]. The connections of area 3b in Virginia opossums with cortex along the rostral border, as well as the caudal border of area 3b [Beck et al., 1996] implicate rostral and caudal bordering strips of cortex in somatosensory functions.

Frontal Cortex

The frontal cortex of short-tailed opossums contains at least one architectonically distinct field, the frontal field (F), which was defined as the frontal myelinated field (FM) by Karlen and Krubitzer [2006]. Architectonically, area F of short-tailed opossums is similar to the frontal region of Virginia opossums, in that these two areas stain darkly for CO and are highly myelinated [Beck et al., 1996]. In addition, area F of short-tailed opossums is somewhat similar to the frontal region of the tammar wallaby, where there is dark staining in SMI-32 [Ashwell et al., 2005] and the upper cortical layers are thicker than the adjoining cortical areas [Mayner, 1989]. Area F of short-tailed opossums is more darkly stained for CO, however, compared to the surrounding cortical areas, whereas the frontal region of the tammar wallaby stains lighter for CO than the adjacent cortical areas [Ashwell et al., 2005]. Area F receives less dense inputs from the thalamus than area 3b, as area F stains lighter for VGluT2 and PV immunopositive terminations in layer 4 than area 3b.

We found no architectonic evidence for a motor area, M1, rostral to S1 in short-tailed opossums, and separate motor cortex appears to be lacking in marsupials, with an overlap of motor and somatosensory functions in the somatosensory cortex [see Beck et al., 1996; Haight and Neylon, 1979].

Retrosplenial Cortex

Area prostriata (PS), a visual limbic area that contains the peripheral vision of the contralateral visual hemifield, was first identified in primates by Sanides [1970]. This area has been identified as the splenial visual area in cats [see Rosa, 1999, for review], medial area 18 (18b) in rats and mice [Krieg, 1946; Caviness, 1975; Zilles and Wree, 1995; Wang and Burkhalter, 2007], and as prostriata in grey squirrels [Wong and Kaas, 2008] and tree shrews [Wong and Kaas, 2009]. In the short-tailed opossums, the area identified as area PS is in the approximate expected location of area PS in placental mammals [see Rosa, 1999 for review], which is adjoining area 17 along the medial wall. Architectonically, area PS of short-tailed opossums resembles that of placental mammals [for example, see Wong and Kaas, 2008, 2009], with poor laminar characteristics, an indistinct layer 4, and poor myelination. In addition, area PS stains poorly for VGluT2 and PV immunopositive terminations, suggesting that it receives less input from the thalamus compared to the adjoining area 17, and for CO, which further suggests that it is not as metabolically active as the adjacent area 17. Area PS in the short-tailed opossum might also correspond to the rostromedial visual area of the brown antechinus, a small Australian marsupial carnivore, as both areas stain poorly for CB, PV and SMI-32 [Ashwell et al., 2008].

Other areas of the retrosplenial cortex include the retrosplenial dorsal (RSd) and ventral (RSv) areas. Both areas have poorly defined laminar patterns and show characteristics of periallocortex, making them easily distinguishable from the adjoining cortical areas. Architectonically, RSd lacks a distinct layer 4 and is poorly myelinated. It is also less

metabolically active than the surrounding areas as it expresses less CO staining and has reduced inputs from the thalamus, as evidenced by the low proportion of VGluT2 and PV immunopositive thalamocortical terminations. RSd may correspond to the retrosplenial agranular area of the tammar wallaby [Ashwell et al., 2005], retrosplenial dorsal cortex of the brown antechinus [Ashwell et al., 2008], and the retrosplenial agranular area of placental mammals such as grey squirrels [e.g., Wong and Kaas, 2008], rats [e.g., Palomero-Gallagher and Zilles, 2004], and tree shrews [e.g., Zilles, 1978; Wong and Kaas, 2009]. As with the RSd, RSv lacks a distinct layer 4, but RSv can be differentiated from RSd by the higher cell packing density and dense myelination. In addition, RSv has a greater population of VGluT2 and PV immunopositive terminations, which suggests that RSv receives more inputs from thalamic nuclei than RSd. RSv might correspond to the retrosplenial granular area of the tammar wallaby [Ashwell et al., 2005], retrosplenial granular cortex, both parts a and b, of the brown antechinus [Ashwell et al., 2008], and the retrosplenial granular area of placental mammals such as grey squirrels [e.g., Wong and Kaas, 2008], rats [Palomero-Gallagher and Zilles, 2004], and tree shrews [e.g., Zilles, 1978; Wong and Kaas, 2009].

Cingulate Cortex

Although the short-tailed opossums lack a corpus callosum, the cingulate cortex occupies most of the anterior portion of the medial wall, as with most placental mammals [e.g., Zilles, 1990; Vogt et al., 1992; Wong and Kaas, 2008]. The cingulate cortex is divided into the dorsal cingulate (CGd), ventral cingulate (CGv) and rostral cingulate (CGr) areas. These three areas do not have well-defined laminar characteristics and lack a distinct granular layer 4, as in the tammar wallaby [Mayner, 1989; Ashwell et al., 2005] and other placental mammals such as rats [Zilles, 1990; Vogt et al., 2004], grey squirrels [Wong and Kaas, 2008], and tree shrews [Wong and Kaas, 2009]. CGd is distinguished from CGv as it has a lower packing density and is more highly myelinated than CGv. CGd is populated with VGluT2 immunopositive terminations, whereas CGv is populated with PV immunopositive terminations. This suggests that both areas receive different subsets of inputs from thalamic nuclei. CGd is also metabolically more active than CGv, as CGd expresses more CO. CGr does not have well defined architectonic characteristics, except for a distinctive layer 2 that is densely packed with cells. The lack of staining in PV and VGluT2 preparations suggests that CGr receives almost no input from the thalamus.

Conclusions

Although the neocortex of short-tailed opossums is not highly differentiated, primary visual, auditory, and somatosensory areas are distinct, and they share architectonic features with these fields in other mammals. In addition, frontal (F), caudotemporal (CT), retrosplenial, and cingulate areas of cortex were identified. Overall, short-tailed opossums have at least four visual areas (area 17 or V1, area 18 or V2, prostriata, and CT), and perhaps five somatosensory areas (area 3b or S1, S2, rostral and caudal somatosensory strips, and possibly a parietal ventral somatosensory area) [see Beck et al., 1996]. There is at least one primary auditory area (A1), and possibly an auditory belt of one or more secondary auditory areas. To this cortical map, we add the frontal area, dorsal and ventral retrosplenial areas, and three cingulate areas. Although not described here, the perirhinal region might contain two or three fields, including an 'insular' region devoted to taste. There is no evidence for a separate motor area, and somatosensory areas have motor functions [Catania et al., 2000b]. Although the full number of cortical areas is not yet established, we can estimate the number with some confidence as reaching or exceeding eighteen, but fewer than twenty-five. This estimate is consistent with other results from opossums and possum species, and it seems reasonable to conclude that early marsupials were opossum-like in body and lifestyle, and

they had small brains with proportionately little neocortex compared to most extant mammals [see Johnson, 1990; Rowe, 1990].

Cortical areas in these early marsupials were not highly differentiated from each other structurally, although primary sensory areas demonstrated moderate levels of specialization. Yet, they likely had close to twenty distinct cortical areas, each serving different sets of functions. At least a number of these areas are shared with extant monotremes [Krubitzer et al., 1995], indicating that mammals at the time of the marsupial-monotreme divergence 230 million years ago [Murphy et al., 2001] already had a cortex of some complexity. Perhaps surprisingly, the neocortex of small-brained placental mammals, such as tenrecs [Krubitzer et al., 1997], hedgehogs [Catania et al., 2000a], and shrews [Catania et al., 1999], is only poorly or moderately differentiated architectonically into distinct areas, and these areas appear to be largely those present in opossums, with the addition of at least one motor field. If early placental (eutherian) mammals were similar, this would suggest that neocortex was fairly stable in relative size, structural differentiation, and functional organization from soon after the emergence of early mammals, perhaps about 240 to 250 million years ago, through the subsequent major radiations of marsupials and eutherian mammals, occurring between 110 and 60 million years ago. This suggests that the profound expansions of neocortex, with structural specializations and the proliferation of areas now present in several orders of mammals and so evident in humans, has occurred only within the last third of the evolutionary history of mammals.

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References

- Adey WR, Kerr DI. The cerebral representation of deep somatic sensibility in the marsupial phalanger and the rabbit; an evoked potential and histological study. *J Comp Neurol.* 1954; 100:597–624. [PubMed: 13192212]
- Aitkin LM, Gates GR. Connections of the auditory cortex of the brush-tailed possum, *Trichosurus vulpecula*. *Brain Behav Evol.* 1983; 22:75–88. [PubMed: 6303495]
- Aitkin LM, Irvine DR, Nelson JE, Merzenich MM, Clarey JC. Frequency representation in the auditory midbrain and forebrain of a marsupial, the northern native cat (*Dasyurus hallucatus*). *Brain Behav Evol.* 1986; 29:17–28. [PubMed: 3594197]
- Ashwell KW, Marotte LR, Li L, Waite PM. Anterior commissure of the wallaby (*Macropus eugenii*): adult morphology and development. *J Comp Neurol.* 1996; 366:478–494. [PubMed: 8907360]
- Ashwell KW, McAllan BM, Mai JK, Paxinos G. Cortical cyto- and chemoarchitecture in three small Australian marsupial carnivores: *Sminthopsis macroura*, *Antechinus stuartii* and *Phascogale calura*. *Brain Behav Evol.* 2008; 72:215–232. [PubMed: 18946209]
- Ashwell KW, Zhang LL, Marotte LR. Cyto- and chemoarchitecture of the cortex of the tammar wallaby (*Macropus eugenii*): areal organization. *Brain Behav Evol.* 2005; 66:114–136. [PubMed: 15942162]
- Beck PD, Pospichal MW, Kaas JH. Topography, architecture, and connections of somatosensory cortex in opossums: evidence for five somatosensory areas. *J Comp Neurol.* 1996; 366:109–133. [PubMed: 8866849]
- Benevento LA, Ebner FF. The areas and layers of corticocortical terminations in the visual cortex of the Virginia opossum. *J Comp Neurol.* 1971; 141:157–189. [PubMed: 5541350]
- Bravo H, Olavarría J, Martinich S. Patterns of interhemispheric and striate-peristriate connections in visual cortex of the South American marsupial *Marmosa elegans* (mouse opossum). *Anat Embryol (Berl).* 1990; 182:583–589. [PubMed: 2075916]

- Campbell MJ, Morrison JH. Monoclonal antibody to neurofilament protein (SMI-32) labels a subpopulation of pyramidal neurons in the human and monkey neocortex. *J Comp Neurol.* 1989; 282:191–205. [PubMed: 2496154]
- Catania KC, Collins CE, Kaas JH. Organization of sensory cortex in the East African hedgehog (*Atelerix albiventris*). *J Comp Neurol.* 2000a; 421:256–274. [PubMed: 10813786]
- Catania KC, Jain N, Franca JG, Volchan E, Kaas JH. The organization of somatosensory cortex in the short-tailed opossum (*Monodelphis domestica*). *Somatosens Mot Res.* 2000b; 17:39–51. [PubMed: 10833083]
- Catania KC, Kaas JH. Organization of the somatosensory cortex of the star-nosed mole. *J Comp Neurol.* 1995; 351:549–567. [PubMed: 7721983]
- Catania KC, Lyon DC, Mock OB, Kaas JH. Cortical organization in shrews: evidence from five species. *J Comp Neurol.* 1999; 410:55–72. [PubMed: 10397395]
- Caviness VSJ. Architectonic map of neocortex of the normal mouse. *J Comp Neurol.* 1975; 164:247–263. [PubMed: 1184785]
- Celio MR. Parvalbumin in most gamma-aminobutyric acid-containing neurons of the rat cerebral cortex. *Science.* 1986; 231:995–997. [PubMed: 3945815]
- Chapin JK, Lin CS. Mapping the body representation in the SI cortex of anesthetized and awake rats. *J Comp Neurol.* 1984; 229:199–213. [PubMed: 6438190]
- Coleman J, Clerici WJ. Organization of thalamic projections to visual cortex in opossum. *Brain Behav Evol.* 1981; 18:41–59. [PubMed: 7237104]
- Conde F, Lund JS, Lewis DA. The hierarchical development of monkey visual cortical regions as revealed by the maturation of parvalbumin-immunoreactive neurons. *Brain Res Dev.* 1996; 96:261–276.
- Crewther DP, Crewther SG, Sanderson KJ. Primary visual cortex in the brushtailed possum: receptive field properties and corticocortical connections. *Brain Behav Evol.* 1984; 24:184–197. [PubMed: 6093921]
- Cruikshank SJ, Killackey HP, Metharate R. Parvalbumin and calbindin are differentially distributed within primary and secondary subregions of the mouse auditory forebrain. *Neuroscience.* 2001; 105:553–569. [PubMed: 11516823]
- Dawson DR, Killackey HP. The organization and mutability of the forepaw and hindpaw representations in the somatosensory cortex of the neonatal rat. *J Comp Neurol.* 1987; 256:246–256. [PubMed: 3558880]
- DeFelipe J, Jones EG. Parvalbumin immunoreactivity reveals layer IV of monkey cerebral cortex as a mosaic of microzones of thalamic afferent terminations. *Brain Res.* 1991; 562:39–47. [PubMed: 1799871]
- Diamond IT, Utley JD. Thalamic retrograde degeneration study of sensory cortex in opossum. *J Comp Neurol.* 1963; 120:129–160. [PubMed: 14027634]
- Dykes RW, Herron P, Lin CS. Ventroposterior thalamic regions projecting to cytoarchitectonic areas 3a and 3b in the cat. *J Neurophysiol.* 1986; 56:1521–1541. [PubMed: 3027273]
- Dykes RW, Rasmussen DD, Hoeltzel PB. Ventroposterior thalamic regions projecting to cytoarchitectonic areas 3a and 3b in the cat. *J Neurophysiol.* 1980; 56:1527–1546. [PubMed: 7411175]
- Ebner FF. Afferent connections to neocortex in the opossum (*Didelphis virginiana*). *J Comp Neurol.* 1967; 129:241–268. [PubMed: 6034492]
- Elston GN, Manger PR. The organization and connections of somatosensory cortex in the brush-tailed possum (*Trichosurus vulpecula*): evidence for multiple, topographically organized and interconnected representations in an Australian marsupial. *Somatosens Mot Res.* 1999; 16:312–337. [PubMed: 10632029]
- Felleman DJ, Wall JT, Cusick CG, Kaas JH. The representation of the body surface in S-I of cats. *J Neurosci.* 1983; 3:1648–1669. [PubMed: 6875661]
- Foster RE, Donoghue JP, Ebner FF. Laminar organization of efferent cells in the parietal cortex of the Virginia opossum. *Exp Brain Res.* 1981; 43:330–336. [PubMed: 7262228]

- Frost SB, Milliken GW, Plautz EJ, Masterton RB, Nudo RJ. Somatosensory and motor representations in cerebral cortex of a primitive mammal (*Monodelphis domestica*): a window into the early evolution of sensorimotor cortex. *J Comp Neurol*. 2000; 421:29–51. [PubMed: 10813771]
- Fujiyama F, Furuta T, Kaneko T. Immunocytochemical localization of candidates for vesicular glutamate transporters in the rat cerebral cortex. *J Comp Neurol*. 2001; 435:379–387. [PubMed: 11406819]
- Gallyas F. Silver staining of myelin by means of physical development. *Neurol Res*. 1979; 1:203–209. [PubMed: 95356]
- Gardner, AL. Order Didelphimorphia. In: Wilson, DE.; Reeder, DM., editors. *Mammal Species of the World: A Taxonomic and Geographic Reference*. Washington, DC: Johns Hopkins University Press; 1993. p. 15-23.
- Gates GR, Aitkin LM. Auditory cortex in the marsupial possum, *Trichosurus vulpecula*. *Hear Res*. 1982; 7:1–11. [PubMed: 7096213]
- Goldby F. An experimental study of the thalamus in the phalanger, *Trichosurus vulpecula*. *J Anat*. 1943; 77:195–224.4. [PubMed: 17104927]
- Grzimek, B. Grzimek's Encyclopedia of Mammals. New York: McGraw-Hill; 1990.
- Hackett TA, Stepniewska I, Kaas JH. Thalamocortical connections of the parabelt auditory cortex in macaque monkeys. *J Comp Neurol*. 1998; 400:271–286. [PubMed: 9766404]
- Haight JR, Neylon L. The organization of neocortical projections from the ventrolateral thalamic nucleus in the brush-tailed possum, *Trichosurus vulpecula*, and the problem of motor and somatic sensory convergence within the mammalian brain. *J Anat*. 1979; 129:673–694. [PubMed: 536306]
- Hof PR, Glezer I, Conde F, Flagg RA, Rubin MB, Nimchinsky EA, Vogt Weisenhorn DM. Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. *J Chem Neuroanat*. 1999; 16:77–116. [PubMed: 10223310]
- Hof PR, Glezer II, Nimchinsky EA, Erwin JM. Neurochemical and cellular specializations in the mammalian neocortex reflect phylogenetic relationships: evidence from primates, cetaceans, and artiodactyls. *Brain Behav Evol*. 2000; 55:300–310. [PubMed: 10971015]
- Huffman KJ, Krubitzer L. Area 3a: topographic organization and cortical connections in marmoset monkeys. *Cereb Cortex*. 2001; 11:849–867. [PubMed: 11532890]
- Huffman KJ, Nelson J, Clarey J, Krubitzer L. Organization of somatosensory cortex in three species of marsupials, *Dasyurus hallucatus*, *Dactylopsila trivirgata*, and *Monodelphis domestica*: neural correlates of morphological specializations. *J Comp Neurol*. 1999; 403:5–32. [PubMed: 10075440]
- Foster RE, Ebner F. Interhemispheric connections between the neocortical forepaw representations in the Virginia opossum. *Neurosci abstr*. 1977; 3:67.
- Johnson, JI. Comparative development of somatic sensory cortex. In: Jones, EG.; Peters, A., editors. *Cerebral Corte*. New York: Plenum Press; 1990. p. 335-449.
- Jones EG, Porter R. What is area 3a? *Brain Res*. 1980; 203:1–43. [PubMed: 6994855]
- Kaas, JH. A comparative survey of visual cortex organization in mammals. In: Ebbesson, SOE., editor. *Comparative Neurology of the Telencephalon*. New York: Plenum Press; 1980. p. 483-501.
- Kaas JH. What, if anything, is SI? Organization of first somatosensory area of cortex. *Physiol Rev*. 1983; 63:206–231. [PubMed: 6401864]
- Kaas JH. The organization of neocortex in mammals: implications for theories of brain function. *Annu Rev Psychol*. 1987; 38:129–151. [PubMed: 3548573]
- Kaas, JH. Reconstructing the organization of neocortex of the first mammals and subsequent modifications. In: Kaas, JH.; Krubitzer, LA., editors. *Evolution of Nervous Systems in Mammals*. London: Elsevier Press; 2007. p. 499-516.
- Kaas JH, Hall WC, Diamond IT. Cortical visual areas I and II in the hedgehog: relation between evoked potential maps and architectonic subdivisions. *J Neurophysiol*. 1970; 33:595–615. [PubMed: 5453053]
- Kaas JH, Hall WC, Diamond IT. Visual cortex of the grey squirrel (*Sciurus carolinensis*): architectonic subdivisions and connections from the visual thalamus. *J Comp Neurol*. 1972; 145:273–305. [PubMed: 5030907]

- Kahn DM, Huffman KJ, Krubitzer L. Organization and connections of V1 in *Monodelphis domestica*. *J Comp Neurol*. 2000; 428:337–354. [PubMed: 11064371]
- Kaneko T, Fujiyama F. Complementary distribution of vesicular glutamate transporters in the central nervous system. *Neurosci Res*. 2002; 42:243–250. [PubMed: 11985876]
- Karlen SJ, Krubitzer L. Phenotypic diversity is the cornerstone of evolution: variation in cortical field size within short-tailed opossums. *J Comp Neurol*. 2006; 499:990–999. [PubMed: 17072834]
- Karlen SJ, Krubitzer L. The functional and anatomical organization of marsupial neocortex: evidence for parallel evolution across mammals. *Prog Neurobiol*. 2007; 82:122–141. [PubMed: 17507143]
- Kirsch, JAW. The classification of marsupials. In: Hunsaker, D., editor. *The Biology of Marsupials*. New York: Academic Press; 1977. p. 1-50.
- Krieg WJS. Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. *J Comp Neurol*. 1946; 84:221–275. [PubMed: 20982805]
- Krubitzer L, Künzle H, Kaas J. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J Comp Neurol*. 1997; 379:399–414. [PubMed: 9067832]
- Krubitzer L, Manger P, Pettigrew J, Calford M. Organization of somatosensory cortex in monotremes: in search of the prototypical plan. *J Comp Neurol*. 1995; 351:261–306. [PubMed: 7699113]
- Krubitzer LA. The organization of neocortex in mammals: are species differences really so different? *Trends Neurosci*. 1995; 18:408–417. [PubMed: 7482807]
- Krubitzer LA, Huffman KJ, Disbrow E, Recanzone G. Organization of area 3a in macaque monkeys: contributions to the cortical phenotype. *J Comp Neurol*. 2004; 471:97–111. [PubMed: 14983479]
- Krubitzer LA, Sesma MA, Kaas JH. Micro-electrode maps, myeloarchitecture, and cortical connections of three somatotopically organized representations of the body surface in the parietal cortex of squirrels. *J Comp Neurol*. 1986; 250:403–430. [PubMed: 3760247]
- Kudo M, Aitkin LM, Nelson JE. Auditory forebrain organization of an Australian marsupial, the northern native cat (*Dasyurus hallucatus*). *J Comp Neurol*. 1989; 279:28–42. [PubMed: 2464009]
- Kumar S, Hedges SB. A molecular time-scale for vertebrate evolution. *Nature*. 1998; 392:917–920. [PubMed: 9582070]
- Latawiec D, Martin KA, Meskenaite V. Termination of the geniculocortical projection in the striate cortex of macaque monkey: a quantitative immunoelectron microscopic study. *J Comp Neurol*. 2000; 419:306–319. [PubMed: 10723007]
- Lee VM, Carden MJ, Schlaepfer WW, Trojanowski JQ. Monoclonal antibodies distinguish several differentially phosphorylated states of the two largest rat neurofilament subunits (NF-H and NF-M) and demonstrate their existence in the normal nervous system of adult rats. *J Neurosci*. 1987; 7:3474–3488. [PubMed: 3119789]
- Lende RA. Sensory representation in the cerebral cortex of the opossum (*Didelphis virginiana*). *J Comp Neurol*. 1963; 121:395–340. [PubMed: 14100022]
- Lorente de Nó, R. *Architectonics and Structure of the Cerebral Cortex*. Oxford, UK: Oxford University Press; 1938.
- Luehke LE, Krubitzer LA, Kaas JH. Cortical connections of electrophysiologically and architectonically defined subdivisions of auditory cortex in squirrels. *J Comp Neurol*. 1988; 268:181–203. [PubMed: 3360984]
- Marshall LG. Evolution of metatherian and eutherian (mammalian) characters: a review based on cladistic methodology. *Zool J Linn Soc*. 1979; 66:369–410.
- Martin GF. Interneocortical connections in the opossum, *Didelphis virginiana*. *Anat Rec*. 1967; 157:607–615. [PubMed: 6035000]
- Martinich S, Pontes MN, Rocha-Miranda CE. Patterns of corticocortical, corticotectal, and commissural connections in the opossum visual cortex. *J Comp Neurol*. 2000; 416:224–244. [PubMed: 10581468]
- Mayner L. A cyto-architectonic study of the cortex of the tammar wallaby, *Macropus eugenii*. *Brain Behav Evol*. 1989; 33:303–316. [PubMed: 2758318]
- Merzenich MM, Kaas JH, Roth GL. Auditory cortex in the grey squirrel: tonotopic organization and architectonic fields. *J Comp Neurol*. 1976; 166:387–401. [PubMed: 1270613]

- Moreland GE, Masterton RB, Glendenning KK. Origin of interhemispheric fibers in acallosal opossum (with a comparison to callosal origins in rat). *J Comp Neurol*. 1985; 241:82–98. [PubMed: 4056114]
- Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ryder OA, O'Brien SJ. Molecular phylogenetics and the origins of placental mammals. *Nature*. 2001; 409:614–618. [PubMed: 11214319]
- Nahmani M, Erisir A. VGlut2 immunohistochemistry identifies thalamocortical terminals in layer 4 of adult and developing visual cortex. *J Comp Neurol*. 2005; 484:458–473. [PubMed: 15770654]
- Neylon L, Haight JR. Neocortical projections of the suprageniculate and posterior thalamic nuclei in the marsupial brush-tailed possum, *Trichosurus vulpecula* (Phalangeridae), with a comparative commentary on the organization of the posterior thalamus in marsupial and placental mammals. *J Comp Neurol*. 1983; 217:357–375. [PubMed: 6886058]
- Owen R. On the structure of the brain in marsupial animals. *Phil Trans R Soc London*. 1837:87–96.
- Palomero-Gallagher, N.; Zilles, K. Isocortex. In: Paxinos, G., editor. *The Rat Nervous System*. London: Elsevier Press; 2004. p. 729-757.
- Paxinos, G.; Franklin, KBJ. *The Mouse Brain in Stereotaxic Coordinates*. New York: Academic Press; 2003.
- Preuss TM, Kaas JH. Parvalbumin-like immunoreactivity of layer V pyramidal cells in the motor and somatosensory cortex of adult primates. *Brain Res*. 1996; 712:353–357. [PubMed: 8814914]
- Pubols BH. Retrograde degeneration study of somatic sensory thalamocortical connections in brain of Virginia opossum. *Brain Res*. 1968; 7:232–251. [PubMed: 5638868]
- Pubols BH. The second somatic sensory area (SmII) of opossum neocortex. *J Comp Neurol*. 1977; 174:71–78. [PubMed: 864034]
- Pubols BH, Pubols LM, DiPette DJ, Sheely JC. Opossum somatic sensory cortex: a microelectrode mapping study. *J Comp Neurol*. 1976; 165:229–245. [PubMed: 1245614]
- Reid SN, Juraska JM. The cytoarchitectonic boundaries of the monocular and binocular areas of the rat primary visual cortex. *Brain Res*. 1991; 563:293–296. [PubMed: 1786543]
- Reig, OA.; Kirsch, JAW.; Marshall, LG. Systematic relationships of the living and the neocenozoic American 'opossum-like' marsupials (Suborder Didelphimorphia), with comments on the classification of these and of the Cretaceous and Paleogene New World and European metatherians. In: Archer, M., editor. *Possums and Opossums: Studies in Evolution*. Sydney: Surrey Beatty & Sons and the Royal Zoological Society of New South Wales; 1987. p. 1-89.
- Remple MS, Henry EC, Catania KC. Organization of somatosensory cortex in the laboratory rat (*Rattus norvegicus*): Evidence for two lateral areas joined at the representation of the teeth. *J Comp Neurol*. 2003; 467:105–118. [PubMed: 14574683]
- Rosa MG. Topographic organisation of extrastriate areas in the flying fox: implications for the evolution of mammalian visual cortex. *J Comp Neurol*. 1999; 411:503–523. [PubMed: 10413783]
- Rosa MG, Krubitzer LA. The evolution of visual cortex: where is V2? *Trends Neurosci*. 1999; 22:242–248. [PubMed: 10354599]
- Rosa MG, Krubitzer LA, Molnár Z, Nelson JE. Organization of visual cortex in the northern quoll, *Dasyurus hallucatus*: evidence for a homologue of the second visual area in marsupials. *Eur J Neurosci*. 1999; 11:907–915. [PubMed: 10103084]
- Rose JE, Malis LI. Geniculo-striate connections in the rabbit. II. Cytoarchitectonic structure of the striate region and of the dorsal lateral geniculate body; organization of the geniculo-striate projections. *J Comp Neurol*. 1965; 125:121–137. [PubMed: 5866591]
- Rose M. Histologische Lokalisation der Grosshirnrinde der kleinen Säugetiere (Rodentia, Insectivora, Chiroptera). *J Psychol Neurol*. 1912; 19:389–479.
- Rose M. Cytoarchitektonischer Atlas der Grosshirnrinde der Maus. *J Psychol Neurol*. 1931; 43:353–430.
- Rowe, M. Organization of the cerebral cortex in monotremes and marsupials. In: Jones, EG.; Peters, A., editors. *Cerebral Cortex*. New York: Plenum Press; 1990. p. 263-334.
- Ryugo DK, Killackey HP. Differential telencephalic projections of the medial and ventral divisions of the medial geniculate body of the rat. *Brain Res*. 1974; 82:173–177. [PubMed: 4611594]

- Sanides, F. Functional architecture of motor and sensory cortices in primates in the light of a new concept of neocortex evolution. In: Montagna, W., editor. *The Primate Brain*. New York: Appleton-Century-Crofts; 1970. p. 137-208.
- Schober W. The rat cortex in stereotaxic coordinates. *J Hirnforsch*. 1986; 27:121–143. [PubMed: 3722803]
- Sereno MI, Rodman HR, Karten HJ. Organization of visual cortex in the California ground squirrel. *Soc Neurosci Abstr*. 1991; 17:844.
- Slutsky DA, Manger PR, Krubitzer LA. Multiple somatosensory areas in the anterior parietal cortex of the California ground squirrel (*Spermophilus beecheyii*). *J Comp Neurol*. 2000; 416:521–539. [PubMed: 10660882]
- Sousa AB, Gattass R, Oswaldo-Cruz E. The projection of the opossum's visual field on the cerebral cortex. *J Comp Neurol*. 1978; 177:569–587. [PubMed: 624791]
- Streilein, KE. Behavior, ecology, and distribution of South American marsupials. In: Mares, MA.; Genoways, HH., editors. *Mammalian Biology in South America*. Linesville, PA: University of Pittsburgh Press; 1982. p. 251-271.
- Sur M, Nelson RJ, Kaas JH. The representation of the body surface in somatosensory area I of the grey squirrel. *J Comp Neurol*. 1978; 179:425–449. [PubMed: 417097]
- Swanson, L. *Brain Maps: Structure of the Rat Brain*. New York: Academic Press; 2003.
- Uylings HB, Groenewegen HJ, Kolb B. Do rats have a prefrontal cortex? *Behav Brain Res*. 2003; 146:3–17. [PubMed: 14643455]
- Van Brederode JF, Mulligan KA, Hendrickson AE. Calcium-binding proteins as markers for subpopulations of GABAergic neurons in monkey striate cortex. *J Comp Neurol*. 1990; 298:1–22. [PubMed: 2170466]
- Van der Gucht E, Hof PR, Van Brussel L, Burnat K, Arckens L. Neurofilament protein and neuronal activity markers define regional architectonic parcellation in the mouse visual cortex. *Cereb Cortex*. 2007; 17:2805–2819. [PubMed: 17337746]
- de Venecia RK, Smelser CB, Lossman SD, Mc-Mullen NT. Complementary expression of parvalbumin and calbindin D-28k delineates subdivisions of the rabbit medial geniculate body. *J Comp Neurol*. 1995; 359:595–612. [PubMed: 7499550]
- Vidyasagar TR, Wye-Dvorak J, Henry GH, Mark RF. Cytoarchitecture and visual field representation in area 17 of the tammar wallaby (*Macropus eugenii*). *J Comp Neurol*. 1992; 325:291–300. [PubMed: 1281175]
- Vogt BA, Finch DM, Olson CR. Functional heterogeneity in cingulate cortex: the anterior executive and posterior evaluative regions. *Cereb Cortex*. 1992; 2:435–443. [PubMed: 1477524]
- Vogt, BA.; Vogt, L.; Farber, NB. Cingulate cortex and disease models. In: Paxinos, G., editor. *The Rat Nervous System*. London: Elsevier; 2004. p. 704-727.
- Volchan E, Bernardes RF, Rocha-Miranda CE, Gleiser L, Gawryszewski LG. The ipsilateral field representation in the striate cortex of the opossum. *Exp Brain Res*. 1988; 73:297–304. [PubMed: 3215306]
- Waite PM, Marotte LR, Leamey CA, Mark RF. Development of whisker-related patterns in marsupials: factors controlling timing. *Trends Neurosci*. 1998; 21:265–269. [PubMed: 9641540]
- Waite PM, Marotte LR, Mark RF. Development of whisker representation in the cortex of the tammar wallaby *Macropus eugenii*. *Brain Res Dev Brain Res*. 1991; 58:35–41.
- Wallace MN. Organization of the mouse cerebral cortex: a histochemical study using glycogen phosphorylase. *Brain Res*. 1983; 267:201–216. [PubMed: 6871673]
- Wang Q, Burkhalter A. Area map of mouse visual cortex. *J Comp Neurol*. 2007; 502:339–357. [PubMed: 17366604]
- Weller WL. Barrels in somatic sensory neocortex of the marsupial *Trichosurus vulpecula* (brush-tailed possum). *Brain Res*. 1972; 43:11–24. [PubMed: 4115094]
- Weller WL. SmI cortical barrels in an Australian marsupial, *Trichosurus vulpecula* (brush-tailed possum): structural organization, patterned distribution, and somatotopic relationships. *J Comp Neurol*. 1993; 337:471–492. [PubMed: 8282853]
- Wise, SP.; Donoghue, JP. *Motor Cortex of Rodents*. New York: Plenum Press; 1986.

- Wong P, Gharbawie OA, Luethke LE, Kaas JH. Thalamic connections of architectonic subdivisions of temporal cortex in grey squirrels (*Sciurus carolinensis*). J Comp Neurol. 2008; 510:440–461. [PubMed: 18666125]
- Wong P, Kaas JH. Architectonic subdivisions of neocortex in the gray squirrel (*Sciurus carolinensis*). Anat Rec. 2008; 291:1301–1333.
- Wong P, Kaas JH. Architectonic subdivisions of neocortex in the tree shrew (*Tupaia belangeri*). Anat Rec. 2009 In press.
- Wong-Riley M. Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res. 1979; 171:11–28. [PubMed: 223730]
- Woodburne MO, Rich TH, Springer MS. The evolution of tribospheny and the antiquity of mammalian clades. Mol Phylogenet Evol. 2003; 28:360–385. [PubMed: 12878472]
- Woolsey TA, Welker C, Schwartz RH. Comparative anatomical studies of the SmL face cortex with special reference to the occurrence of ‘barrels’ in layer IV. J Comp Neurol. 1975; 164:79–94. [PubMed: 809494]
- Zilles K. A quantitative approach to cyto-architectonics: 1. The areal pattern of the cortex of *Tupaia Belangeri*. Anat Embryol (Berl). 1978; 153:195–212. [PubMed: 677471]
- Zilles, K. Organization of the neocortex. In: Tees, B.; Kolb, RC., editors. The Cerebral Cortex of the Rat. MIT Press; 1990. p. 19-20.
- Zilles, K.; Wree, A. Cortex: Areal and laminar structure. In: Paxinos, G., editor. The Rat Nervous System. Sydney: Academic Press; 1995. p. 649-685.
- Zilles K, Wree A, Schleicher A, Divac I. The monocular and binocular subfields of the rat’s primary visual cortex: a quantitative morphological approach. J Comp Neurol. 1984; 226:391–402. [PubMed: 6611357]
- Zilles K, Zilles B, Schleicher A. A quantitative approach to cytoarchitectonics. VI. The areal pattern of the cortex of the albino rat. Anat Embryol (Berl). 1980; 159:335–360. [PubMed: 6970009]

Abbreviations used in this paper

17	Primary visual area
3b	Primary somatosensory area
A	Auditory area
CB	Calbindin
CBL	Cerebellum
CG	Cingulate area
CGd	Cingulate dorsal area
CGr	Cingulate rostral area
CGv	Cingulate ventral area
CO	Cytochrome oxidase
CT	Caudal temporal area
EC	Ectorhinal cortex
F	Frontal cortex
FP	Frontal-parietal cortex
IC	Inferior colliculus
OB	Olfactory bulb
OTP	Occipital-temporal-parietal cortex

PB	Phosphate buffer
PBS	Phosphate buffer with saline
Pir	Piriform cortex
PS	Prostriata
PV	Parvalbumin
RS	Retrosplenial area
RSd	Retrosplenial dorsal area
RSv	Retrosplenial ventral area
S2	Secondary somatosensory area
SC	Superior colliculus
Thal	Thalamus
V1	Primary visual area
V2	Secondary visual area
VGlut2	Vesicle Glutamate Transporter 2

Fig. 1.

Architectonic characteristics of the cortical areas in tangential sections of flattened cortex. Panels **A** and **B** are adjacent sections stained for CO and myelin, respectively. Panels **C** and **D** are adjacent sections from another example stained for CO and PV, respectively. Panel **E** is from a third example stained for myelin. Primary sensory areas, such as area 17, auditory (A) area, and somatosensory (3b) area have distinct architectonic characteristics, as they are darkly stained for CO, myelin and PV. The extents of these three areas, and a fourth architectonically definable area, the caudal temporal area (CT), are outlined on the schematic brain map in panel **F**. The borders depicted by dotted lines are approximate and obtained from previous functional studies [S2 from Catania et al., 2000b; V2 from Kahn et al., 2000]. The cortical region between areas 17, A and 3b, the occipital-temporal-parietal cortex (OTP), does not have well defined architectonic characteristics and there was no clear architectonic evidence for further subdivisions. See list of abbreviations for other areas. Rostral is to the left and dorsal is to the top of the page. Scale bar in panel **F** = 1 mm.,

Fig. 2.

The laminar characteristics of area 17 at higher magnification. Each panel presents part of a main section processed for a different architectonic feature (see Methods). Six main cortical layers, with no apparent sublamination, are observed. As expected of sensory cortices, layer 4 is thick and densely populated with granule cells. Area 17 is also densely myelinated and expresses high levels of CO. Short horizontal lines on the sections illustrated here, and in the following figures, indicate cortical layers 1 to 6. Scale bar in panel **F** = 0.25 mm.

Fig. 3. Architectonic characteristics of the caudal region of neocortex. Coronal sections were processed for **A** Nissl substance, **B** myelin, **C** CO, **D** VGLUT2, **E** PV, **F** CB, **G** SMI-32. Brain sections illustrated in panels **A**, **B**, **D** and **E** are from one sample, and panels **C**, **F**, and **G** are from another sample. The vertical lines on the dorsolateral and medial view of the brain in **H** show the level from which the sections were taken in panels **A**–**G**. The thicker portion of the line marks the regions illustrated in panels **A**–**G**. Arrowheads on the sections illustrated here and in the following figures mark the architectonic borders. For the sections illustrated, medial is to the right and dorsal is towards the top of the page. Scale bar for sections (**G**) = 1 mm. Scale bar for dorsolateral (**H1**) and medial (**H2**) view of the cerebral hemisphere (**H**) = 2.5 mm.

Fig. 4. The laminar characteristics of the caudal portion of occipital-temporal-parietal (OTP) cortex. The borders of OTP with area 17 and CT are clearly delimited by the low staining levels of VGLUT2-immunopositive terminations (**B**), PV-immunopositive terminations (**C**), and SMI-32 (**D**). Scale bar in panel **D** = 0.5 mm.

Color version available online

Fig. 5.
The laminar characteristics of the rostral portion of occipital-temporal-parietal (OTP) cortex. The borders of OTP with area 17 and auditory area, A, are clearly delimited by the low staining levels of myelination (A), VGlut2-immunopositive terminations (B), PV-immunopositive terminations (C), and SMI-32 (D). Scale bar in panel D = 0.5mm.

Fig. 6.

The laminar characteristics of auditory area at higher magnification. Layer 4 is densely populated with granule cells. Area A is also densely myelinated and expresses high levels of CO. Scale bar in panel F = 0.25 mm.

Fig. 7.

Architectonic characteristics of occipital, temporal and retrosplenial cortex. Coronal sections were processed for **A** Nissl substance, **B** myelin, **C** CO, **D** VGlut2, **E** PV, **F** CB, **G** SMI-32. Brain sections illustrated in panels **A**, **B**, **C** and **F** are from one case, and in panels **D**, **E** and **G** are from another case. The vertical line on the dorsolateral view of the brain in **H** shows the level from which the sections were taken for panels **A**–**G**. The thicker portion of the line marks the regions illustrated in panels **A**–**G**. For the sections illustrated, medial is to the right and dorsal is towards the top of the page. Scale bar for sections (**G**) = 1 mm. Scale bar for the brain (**H**) = 2.5 mm.

Fig. 8.

The laminar characteristics of primary somatosensory area (3b) at higher magnification. As with other primary sensory areas, area 3b has a layer 4 that is densely populated with granule cells and is also densely myelinated and expresses high levels of CO. Scale bar in panel **F** = 0.25 mm.

Fig. 9.

Architectonic characteristics of occipital, parietal and frontal cortex. Sagittal sections were processed for **B** Nissl substance, **C** myelin, **D** PV. The horizontal line on the dorsolateral view of the brain in **A** shows the level from which the sections were taken for panels **B–D**. The thicker portion of the line marks the regions illustrated in panels **B–D**. For the sections illustrated, rostral is to the right and dorsal is towards the top of the page. Scale bar for sections (**D**) = 1 mm. Scale bar for the brain (**A**) = 2.5 mm.

Fig. 10.

Architectonic characteristics of parietal and cingulate cortex. Coronal sections were processed for **A** Nissl substance, **B** myelin, **C** CO, **D** VGLUT2, **E** PV, **F** CB, **G** SMI-32. Brain sections illustrated in panels **A**, **C**, **E** and **F** are from one case and those in panels **B**, **D** and **G** are from another. The vertical lines on the dorsolateral (**H1**) and medial (**H2**) views of the brain shows the level from which the sections were taken for panels **A–G**. The thicker portion of the line marks the regions illustrated in panels **A–G**. For the sections illustrated, medial is to the left and dorsal is towards the top of the page. Scale bar for sections (**G**) = 1 mm. Scale bar for the brains (**H**) = 2.5 mm.

Fig. 11.

Architectonic characteristics of frontal and cingulate cortex. Coronal sections were processed for **A** Nissl substance, **B** myelin, **C** CO, **D** VGLUT2, **E** PV, **F** CB, **G** SMI-32. Brain sections illustrated in panels **A**, **C** and **F** are from one case and those in panels **B**, **D**, **E** and **G** are from another. The vertical line on the medial view of the brain in **H** shows the level from which the sections were taken for panels **A–G**. The thicker portion of the line marks the regions illustrated in panels **A–G**. For the sections illustrated, medial is to the right and dorsal is towards the top of the page. Scale bar for sections (**G**) = 1 mm. Scale bar for the brain (**H**) = 2.5 mm.