THE JOURNAL OF COMPARATIVE NEUROLOGY 428:337–354 (2000)

Organization and Connections of V1 in *Monodelphis domestica*

DIANNA M. KAHN, KELLY J. HUFFMAN, AND LEAH KRUBITZER*
Center for Neuroscience and Department of Psychology, University of California at Davis, Davis, California 95616

ABSTRACT

We examined the internal organization and connections of the primary visual area, V1, in the South American marsupial *Monodelphis domestica*. Multiunit electrophysiological recording techniques were used to record from neurons at multiple sites. Receptive field location, size, progressions, and reversals were systematically examined to determine the visuotopic organization of V1 and its boundaries with adjacent visual areas. As in other mammals, a virtually complete representation of the visual hemifield was observed in V1, which was coextensive with a region of dense myelination. The vertical meridian was represented at the rostrolateral boundary of the field, the upper visual quadrant was represented caudolaterally, whereas the lower visual quadrant was represented rostromedially. Injections of fluorescent tracers into V1 revealed dense connections with cortex immediately adjacent to the rostrolateral boundary, in peristriate cortex (PS or V2). Connections were also consistently observed with a caudotemporal area (CT), entorhinal cortex (EC), and multimodal cortex (auditory/visual, A/V). These results demonstrate that *M. domestica* possess a highly differentiated neocortex with clear functional and architectonic cortical field boundaries, as well as discrete patterns of cortical connections. Some connections of V1 are similar to those observed in eutherian mammals, such as connections with V2 and extrastriate areas (e.g., CT), which suggests that there are general features of visual system organization that all mammals posses due to retention from a common ancestor. On the other hand, connections of V1 with EC and multimodal cortex may be a primitive feature of visual cortex that was lost in some lineages, may be a derived feature of marsupial neocortex, or may be a feature particular to mammals with small brains. J. Comp. Neurol. 428:337–354, 2000.

Indexing terms: marsupials; primary visual area; evolution; fluorescent tracers; multiunit electrophysiology

The visual system is unquestionably the most widely studied sensory system in mammals, perhaps this is because we consider ourselves to be highly visual animals, and, therefore, the visual system to be a focal point around which higher processes such as cognition, learning, and memory are built. Work on the nonhuman primate visual system has validated this belief by demonstrating that the visual neocortex is very complexly organized and is composed of multiple areas, or fields, involved in processing various features of the stimulus.

However, the complexity that we seek to understand is also one of the largest obstacles in understanding basic elements of processing. Our laboratory's approach to understanding how complex systems were generated in evolution is to identify the organization of the forerunner from which more complex systems evolved. Comparative analyses of different species are essential for describing a general plan of organization of the mammalian brain, as well as for identifying variations to this plan. By observing similarities and differences across species, one can then posit how cortical areas were modified, how new areas were added, and how these modifications were integrated with general features of organization to generate a complexly organized and interconnected visual system.

Although studies of other mammals have led to a better understanding of the basic features of organization of the mammalian visual system, the number of species exam-

Grant sponsor: NIH; Grant number: RO1 NS35103-04A1; Grant sponsor: Whitehall Foundation; Grant number: M97-20.

*Correspondence to: Leah Krubitzer, Center for Neuroscience, 1544 Newton Court, Davis, CA 95616. E-mail: lakrubitzer@ucdavis.edu

Received 13 June 2000; Revised 27 July 2000; Accepted 29 August 2000

ined to date is limited, and our understanding of the common ancestral form is at best rudimentary (e.g., Van Essen, 1979; Kaas, 1980, 1988; Tyler et al., 1998). For instance, studies on mammals such as cats (e.g., Tusa et al., 1978; Gilbert and Wiesel, 1979; Montero, 1981), some species of rodents (e.g., Adams and Forrester, 1968; Montero et al., 1973a,b; Dräger, 1975; Wagor et al., 1980; Malach, 1989), and primates (e.g., Allman and Kaas, 1971; Gattass et al., 1987; Krubitzer and Kaas, 1993; Rosa et al., 1997) have demonstrated that the visual system of these animals share common features, in that they possess a primary visual area (V1) with similar patterns of connections and retinotopic organization. However, some fundamental issues of visual system organization remain unresolved. For example, while the second visual area, V2, has been identified in both cats (Tusa et al., 1979) and primates (Allman and Kaas, 1971; Gattass et al., 1981), the differences between these two species in terms of modular organization (Bonhoeffer and Grinvald, 1993; Roe and Ts'o, 1995) makes it difficult to infer what the ancestral state may have been. This issue of V2 becomes more confusing when one considers the very different proposals of extrastriate visual cortex organization in rodents (see Rosa and Krubitzer, 1999).

Studies of mammals whose ancestors diverged early in evolution can provide insight into features of the cortex possessed by early ancestors. These features may characterize fundamental building blocks of the mammalian visual system. Marsupials are an extant lineage of metatherian mammals whose ancestors diverged from the eutherian line approximately 135 million years ago (Kirsch, 1977), and they offer a unique opportunity to study a visual system that may be representative of the ancestral mammalian brain.

Previous work on marsupials has shown that they share some features of visual system organization and patterns of connections with placental mammals. For example, electrophysiological studies of V1 in the quoll (Rosa et al., 1999), wallaby (Vidyasagar et al., 1992), and South American opossum (Sousa et al., 1978) have demonstrated a topographic representation of the visual field similar to that described for V1 of eutherian mammals. Cytoarchitectonic studies have shown similarities between marsupial and placental mammals in terms of thickness of cell layers and distribution of cells, although there seems to be as much variation among marsupials as there is among placentals (Tyler et al., 1998). Studies of connections (e.g., Benevento and Ebner, 1971; Crewther et al., 1984; Bravo et al.,1990; Martinich et al., 2000) demonstrate projections from V1 to a second visual area, V2, as well as other extrastriate areas. However, it remains to be established whether these extrastriate areas are homologous in different species.

We examined the topographic organization and connections of V1 in the short-tailed opossum for three reasons. The first is to expand our understanding of general features of visual processing in mammals. Comparative studies of a wide variety of species, including marsupials, are essential to ascertain a general plan of mammalian cortical organization. The second reason we are examining this species, rather than the more common North American opossum, is that the short-tailed opossum has a well-developed visual system, and the amount of cortex devoted to visual processing is relatively large (Huffman et al., 1999a). Also, the eye is larger and easier to stabilize for visual mapping studies. The final reason we have chosen this species is the growing prominence of these animals in studies of neural development. Work in our laboratory, which focuses on the evolution of cortical areas, has led us to consider the factors involved in cortical development (Huffman et al., 1999b). For these reasons, we have found it necessary to establish normal patterns of cortical organization and connections in the adult opossum.

Abbreviations

A	auditory area
A/V	multimodal auditory & visual region
CT	caudotemporal area
EC	entorhinal cortex
HM	horizontal meridian
OB	olfactory bulb
PS	peristriate cortex
PYR	pyriform cortex
RS	rhinal sulcus
S	somatosensory area
S1	primary somatosensory area
V1	primary visual area
V2	second visual area
VM	vertical meridian

MATERIALS AND METHODS

Multiunit electrophysiological recording methods and anatomical tracing techniques were used to investigate the visuotopic organization and connections of the primary visual area, V1, in 9 South American short-tailed opossums (*Monodelphis domestica*). Maps of the visual hemifield were obtained for V1 in five animals. In an additional four animals, the corticocortical connections were determined by injecting neuroanatomical tracers into V1 and plotting retrogradely labeled cells. Both data sets were related to cortical myeloarchitecture obtained from tissue sectioned in the tangential plane, or from cytoarchitecture of coronally sectioned brains.

Surgical preparation and anesthetics

Anesthesia was induced with a cocktail of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg) administered intramuscularly. A total of 0.2 ml of 1% lidocaine, a local anesthetic, was injected at the scalp and into the temporal muscle. Maintenance doses of pentobarbital sodium (20 mg/kg) were administered subcutaneously at roughly 30-minute intervals to maintain a surgical level of anesthesia. For the acute electrophysiological experiments, subcutaneous injections of lidocaine were administered into the skin surrounding the eye, and the pupil was dilated with atropine. Subcutaneous injections of lactated Ringer's solution were administered every 3 to 4 hours to maintain hydration. For all experiments, body temperature was maintained by placing the animal on a heating pad, and body temperature, heart rate, and respiration were monitored throughout the experiment. Once a surgical level of anesthesia was reached, the skin was cut at the midline of the scalp, and a craniotomy was performed to expose the entire primary visual area and much of the surrounding cortex.

For the electrophysiological recording experiments, the dura was retracted and the exposed cortex was covered with dimethylsiloxane to prevent desiccation and to maintain cortical temperature. A small screw was inserted into

the skull of the contralateral hemisphere, and the head was stabilized by using dental acrylic, which cemented the skull screw to a metal bar attached to a magnetic base. The exposed cortex was imaged by using a CCD camera (Optronics Engineering, Zeiss, Thornwood, NY), and this image was used as a reference map to relate the electrode penetrations to cortical vasculature. To stabilize the eye, an eye ring was sutured to the sclera of the eye contralateral to the exposed cortex, and then secured in place with surgical glue. The eye ring was cemented to a metal bar attached to the magnetic base, which stabilized the head. A thin clear layer of dimethylsiloxane was applied to the eye to prevent desiccation of the cornea. All experimental protocols were approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis.

Electrophysiological recordings

Five animals underwent electrophysiological recording. Each animal was placed within an opaque plastic globe, and the eye contralateral to the exposed cortex of the animal was positioned so that it was at the center of the sphere. The entire visual field of one eye was determined by reflecting a beam of light from its tapetum onto the globe. Although visual inspection of the eye with an ophthalmoscope allowed us to view the vasculature and the optic disc with clarity, we were unable to project retinal landmarks on to the globe. These procedures are similar to those described in previous studies in owl monkeys (e.g., Allman and Kaas, 1971). Multiunit electrophysiological recordings of the visual cortex were done by using tungsten microelectrodes (0.020 inches, 5 MΩ). The electrode was manually advanced to a depth of 200–400 μm from the pial surface, approximately in layer IV. Although neural responses to visual stimulation could be obtained in other layers, those in layer IV were most robust and allowed us to determine receptive field boundaries with greater accuracy. To obtain the receptive fields for neurons in a given penetration, the contralateral retina was stimulated with full field flashes of light, and moving or flashing circles and bars of light presented through the globe. Receptive fields were drawn directly onto the globe, and the retinotopic organization of a region of the cortex was determined from receptive fields for groups of neurons at several recording sites spaced approximately 300–500 μm apart. Stability of the eye was tested by returning the electrode to a known cortical position over the course of the experiment, and determining the visual field represented at that point. Upon completion of the recording, two probes were placed in the cortex to aid with reconstruction of the tissue.

Tracer injections and histology

Four adult short-tailed opossums were used to determine the cortical connections of V1. All procedures were conducted under sterile surgical conditions. In each case, the animal was anesthetized with ketamine hydrochloride, xylazine, and pentobarbital sodium as described above. A craniotomy was performed over the caudal portion of the left cerebral hemisphere. A Hamilton syringe was lowered through the dura, approximately 300 μm into the cortex, and 0.3–0.5 μl of either 7% fluoroemerald (FE), fluororuby (FR), or both (in separate injections) were injected into the cortex. An acrylic skull cap was then placed over the opening, and the temporal muscle and skin were sutured. The animal was allowed to recover for a survival time of 4–7 days to allow for transport of the tracer.

At the end of all experiments, the animals were killed with a lethal dose of pentobarbital sodium (250 mg/kg) and transcardially perfused with 0.9% saline (and 0.1% heparin for electrophysiological cases), followed by 4% paraformaldehyde in phosphate buffer (PB; pH 7.4), and then 4% paraformaldehyde with 10% sucrose in PB. The brain was removed, and the cortex was removed from the brainstem and diencephalon. The cortices were flattened between two glass slides and immersed in 30% sucrose overnight. The flattened tissue was sectioned on a freezing microtome at 35–40 μm in a plane parallel to the cortical surface. For the electrophysiological recording cases, all cortical sections were stained for myelin (Gallyas, 1979). For anatomical tracing cases, one series of sections was stained for myelin, and one series of sections was mounted for fluorescent microscopy. In one case (99-53), the whole brain was cut in the coronal plane at 40 μm, and alternate sections were stained for Nissl, myelin, or mounted for fluorescent microscopy.

Data analysis

For each electrophysiological recording case, camera lucida drawings of individual myelin-stained sections were made by using a stereomicroscope. Each drawing contained the outline of the section, blood vessels, tissue artifacts, probes, and architectonic borders determined from these sections. All drawn sections were aligned by using these landmarks and compiled into one reconstruction of the architectonic boundaries. By matching blood vessel patterns obtained from the digital image of the exposed cortex with blood vessels, probes, and other artifacts in the histologically processed tissue, the electrode penetrations were plotted onto reconstructed myelin drawings to produce one comprehensive reconstruction of both architectonic boundaries and electrophysiological recordings. By correlating receptive field progressions for neurons with electrode penetrations, maps of V1 were generated.

For cases in which anatomical tracers were injected, the injection sites and retrogradely labeled cell bodies were plotted by using an X/Y stage encoding system (MD Plot, Minnesota Datametrics, MN) that was mounted to a fluorescent microscope and connected to a PC. Each case was reconstructed by aligning myelin-stained sections with drawings of fluorescent sections, using probes and other tissue artifacts as landmarks. Drawings were then compiled into one comprehensive reconstruction as described above. In this way, the myeloarchitectonic features of the neocortex, including cortical field boundaries were related to areal patterns of connections.

RESULTS

In the following results, we first describe the visuotopic organization of V1, as determined by multiunit electrophysiological recording techniques, and we then relate functional maps of visual cortex to cortical architecture. The final section details the cortical connections of V1, as revealed by analysis of retrograde transport of the fluorochromes injected into V1.

Visuotopic organization of V1

Multiunit electrophysiological recording techniques were used to explore the dorsolateral surface of the occipital lobe, and maps of the visual hemifield were obtained in V1 (Fig. 1). Detailed maps were obtained in three cases, and partial maps were obtained from two cases. Recordings from neurons on the dorsolateral surface of V1, which comprised approximately 90% of the entire field as defined architectonically, revealed that most of V1 was devoted to representing the central 50 degrees of vision (Fig. 1). The region of cortex inaccessible to our electrode (on the curve of the midsagittal sulcus), comprised only a small portion of V1, yet presumably contained the representation of most of the peripheral hemifield.

The visuotopy of V1 was similar to that described in other marsupials (Sousa et al., 1978; Vidyasagar et al., 1992; Rosa, et al., 1999; see Rowe, 1990 for review). The vertical meridian formed the rostrolateral border of the field, the upper quadrant was represented caudally, and the lower quadrant was represented rostrally. Neurons in the most medial sites represented a more peripheral location in the visual hemifield (30–60 degrees azimuth), whereas neurons located laterally in V1 represented more central portions of the visual field (20–0 degrees azimuth), including the vertical meridian (Figs. 2, 3). Thus, as recording sites progressed from medial to lateral in V1, receptive fields for neurons at those sites moved from the peripheral visual field to the vertical meridian (e.g., Fig. 2, RFs 7-10, 15-17; Fig. 3, RFs 1-4, 12-16). Although we were unable to determine the exact location of the representation of the horizontal meridian, because we could not project retinal landmarks onto the globe, we could approximate its location based on the physiologically defined location of the blind spot (Fig. 1). Furthermore, a clear rostrocaudal progression of representation from lower to upper visual quadrant could be identified in V1 (Fig. 4). As recording sites moved from caudal to rostral in V1, receptive fields for neurons at those sites moved from the upper visual quadrant to the lower visual quadrant (e.g., Fig. 4, RFs 1-6, 7-11).

Differences in receptive field size for different portions of the visual hemifield were not systematically studied; however, one general pattern emerged. Receptive fields for neurons located lateral in V1, at the representation of the vertical meridian, tended to be smaller than receptive fields for neurons closer to the medial wall, which represented more peripheral locations of the visual hemifield. This can be seen in Figures 3, 4, and is demonstrated more dramatically in Figure 5. However, given that we were recording from multiple, rather than single units, interpretation of these results is difficult. Changes in receptive field sizes of multiple units do not necessarily reflect changes in receptive field sizes of individual neurons.

The data obtained for cortex outside of V1 were too sparse to come to any firm conclusions regarding the visuotopic organization of extrastriate areas. However, in several instances, reversals in receptive field progression were observed at the V1/V2 border (Figs. 2, 3). As recording sites moved medial to lateral in V1, corresponding receptive fields for neurons at those sites moved from the peripheral visual hemifield to the vertical meridian. As the lateral border of V1 was crossed, a reversal in receptive field progression was observed (e.g., Fig. 3, RFs 5, 11, 17, 22).

Myeloarchitectonic subdivisions of visual cortex

In all cases, the maps generated in V1 by using electrophysiological recording techniques corresponded closely with myelo- and cytoarchitectonic boundaries. Architectonic boundaries of V1 were consistently identified in cortex that was sectioned tangentially and stained for myelin (Fig. 6). V1 was a densely myelinated area located at the caudomedial pole of the occipital cortex. In myelin-stained sections cut in the coronal plane, V1 was characterized by dark myelination in layers III–VI (Fig. 7C).

Located along the lateral border of V1 was a very lightly myelinated peristriate area (PS, the presumptive V2). Although the V1/V2 boundary was readily determined in all planes of section, the rostral boundary of V2 was difficult to discern. Cortex immediately lateral to V2 was lightly to moderately myelinated and termed A/V (multimodal auditory/visual area) in a previous investigation (Huffman et al., 1999a), because neurons in this region responded to both auditory and visual stimulation.

A darkly myelinated wedge-shaped region in caudotemporal cortex, located lateral to V2 and immediately superior to the rhinal fissure, was distinct in each case (Figs. 6, 7D). This caudal temporal area, CT, has been described in a previous study of the Virginia opossum (Beck et al., 1996) and the short-tailed opossum (Huffman et al., 1999a), and contained neurons responsive to visual stimulation. CT has not yet been systematically mapped in any marsupial.

Auditory cortex (A) was located lateral to A/V, and included a darkly myelinated region, the putative primary auditory area, A1. The primary somatosensory area (S1) was also darkly myelinated, and was located lateral to V2 and immediately rostral to A1. Cortex immediately lateral to S1 was moderately to lightly myelinated and contained neurons responsive to somatosensory stimulation (see Huffman et al., 1999a).

Cytoarchitecture

In cortex that was sectioned coronally and stained for Nissl substance, V1 was characterized by six well-developed layers with a wide granular cell layer IV, and a relatively thicker layer I, compared with peristriate regions (Fig. 7A). The most salient feature of V1 compared with other visual areas was its clear stratification and the lack of large pyramidal cells. Layer I in all areas consisted of fibers and was nearly devoid of cell bodies. In V1, layer II appeared as a thin stripe of very densely packed medium-sized cells. Layer III contained small cells that were less densely packed than in layer II. Layer IV contained small, densely packed cells. Layer V consisted of large, moderately packed cells, whereas layer VI was sparsely populated with comparatively smaller cells. In myelin-stained sections, layers II through VI stained darkly for myelin. The V1/V2 border was readily distinguished as the point in which the stratification of layers was difficult to discern, where large pyramidal cells in layer V were abundant, and where the density of myelin diminished significantly (Fig. 7C).

Lamination in area CT appeared markedly different than in V1 or V2 in both Nissl and myelin-stained sections (Fig. 7B,D). In CT, only layers I, II, and VI could be

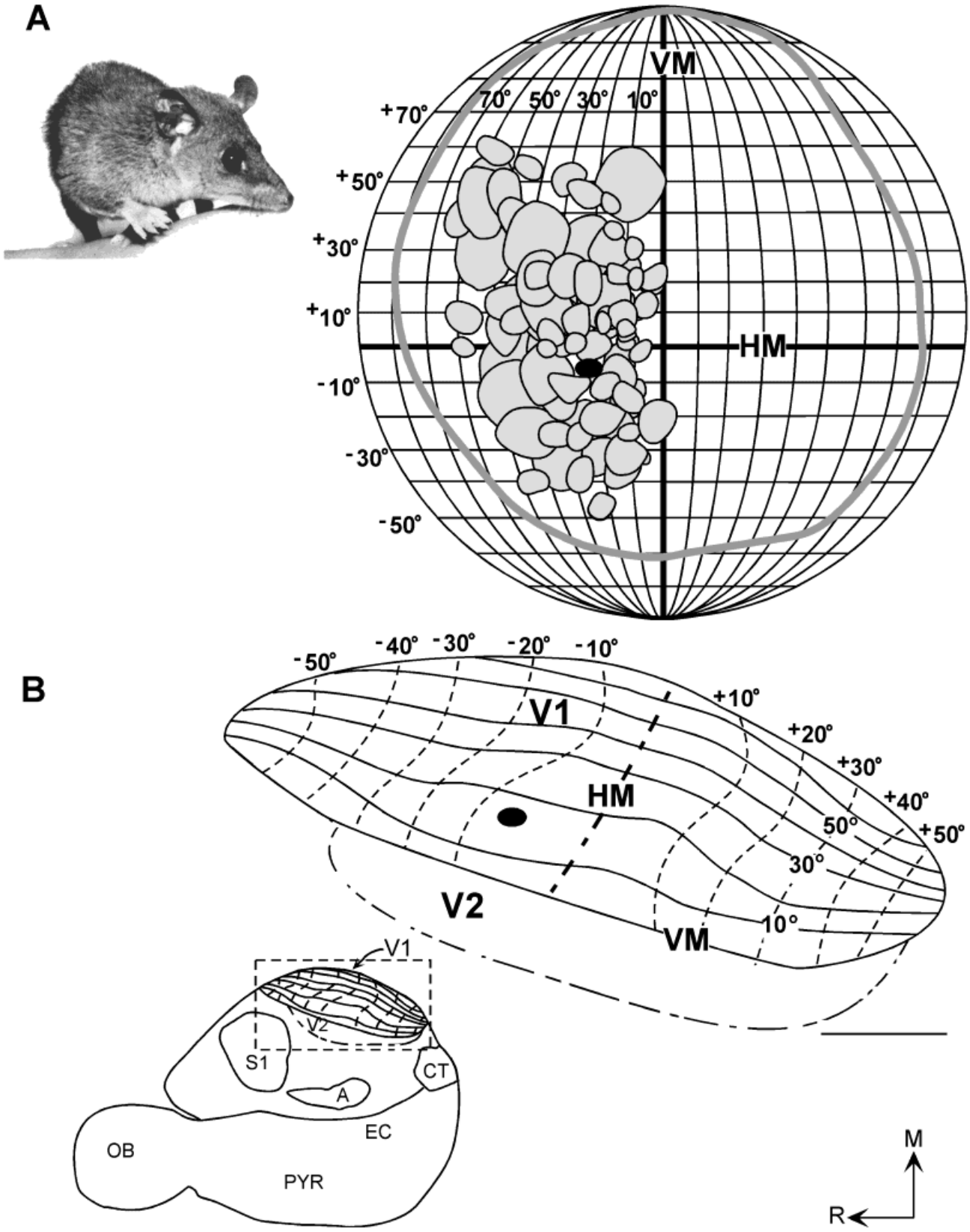

Fig. 1. **A:** A summary of visuotopic organization and extent of the visual field viewed through the right eye of the opossum. **B:** The dorsolateral view of a flattened section of the left hemisphere shows the overall organization of the neocortex. The top figure is a magnified view of the inset and depicts the visuotopic organization of V1. Solid lines represent isoazimuths; dashed lines represent isoelevations. The location of the horizontal meridian was estimated based on the position of the blind spot (black oval). We projected all receptive fields recorded for all cases onto an equatorial-azimuthal chart to determine the extent of the visual hemifield represented on the dorsolateral surface of the neocortex. Receptive fields are represented by circles, each square represents 10 degrees of azimuth and elevation. The peripheral and upper peripheral visual field were not mapped and were likely represented by neurons along the medial and caudomedial wall of the neocortex. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in B.

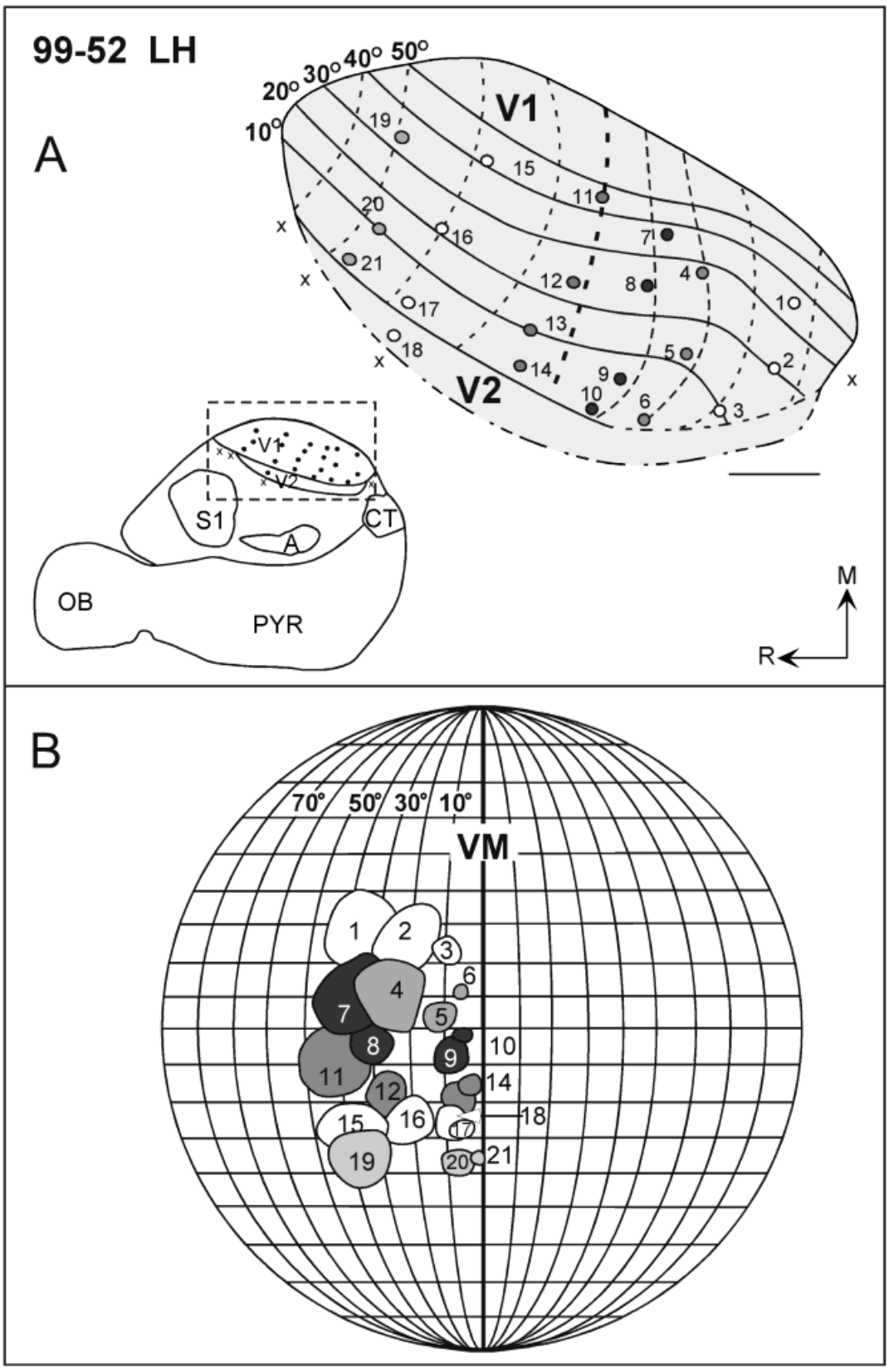

Fig. 2. Recording sites in V1 in the left hemisphere (**A**) and receptive fields for neurons at those sites drawn onto a globe (**B**) in case 99-52. As recording sites moved from medial to lateral in V1, receptive fields for neurons at those sites moved from the peripheral hemifield toward the vertical meridian. Note the smaller receptive field sizes along the vertical meridian. Recording sites are indicated by dots, and recording sites where no visual evoked activity was observed are denoted by small "x"s. For the purposes of illustration, rows of sequential progressions are indicated by sequences of numbers (e.g., 1-5). Solid lines mark the architectonic and electrophysiological boundary of V1, and dashed lines indicate the physiological border only. Other conventions are the same as in the previous figure. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in A.

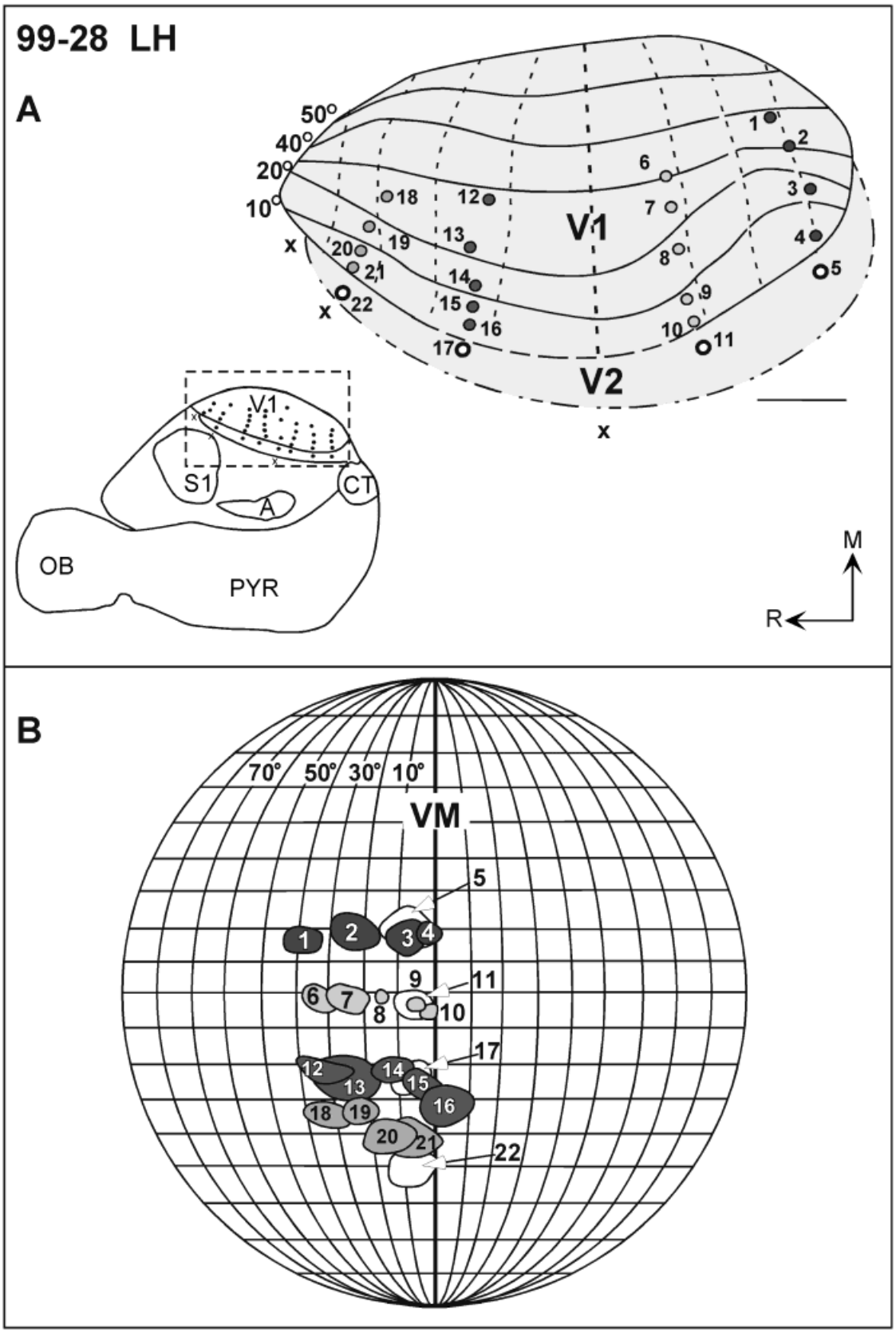

Fig. 3. Recording sites in V1 in the left hemisphere (**A**) and receptive fields for neurons at those sites drawn onto a globe (**B**) in case 99-28. As recording sites progressed from medial to lateral in V1, receptive fields for neurons at those sites progressed from the peripheral hemifield toward the vertical meridian. In this case, a few neurons were recorded outside of V1, along the rostrolateral border of the field. As recording sites progressed across the border, receptive fields for neurons at those sites reversed away from the vertical meridian and became larger. Receptive field reversals, indicated by arrows, were used to determine the physiological border of V1 and location of the vertical meridian. The locations of receptive field reversal sites correspond to recording sites in a region just lateral to the V1 border, in PS (V2). For illustration purposes, the enlarged map does not contain the entire array of electrode penetrations. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in A.

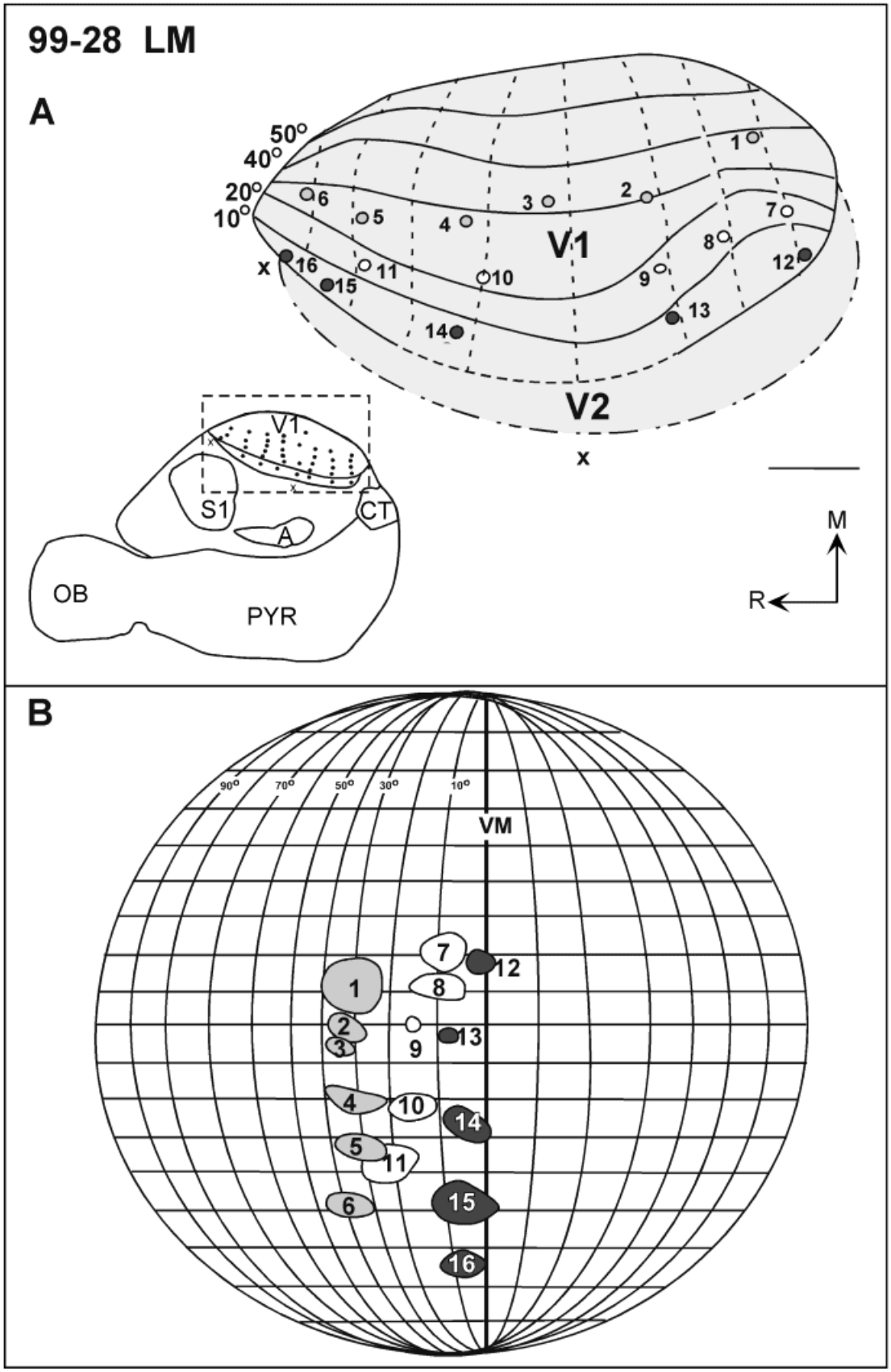

Fig. 4. Recording sites in V1 in the left hemisphere (**A**) and receptive fields for neurons at those sites drawn onto a globe (**B**) in case 99-28. This illustration depicts a progression of receptive fields from the upper to the lower visual hemifield representation. As recording sites moved from caudal to rostral in V1, receptive fields for neurons at those sites moved from the upper to the lower hemifield. For illustration purposes, the enlarged map does not contain the entire array of the electrode penetrations. Rows of sequential progressions are indicated by sequential numbers. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in A.

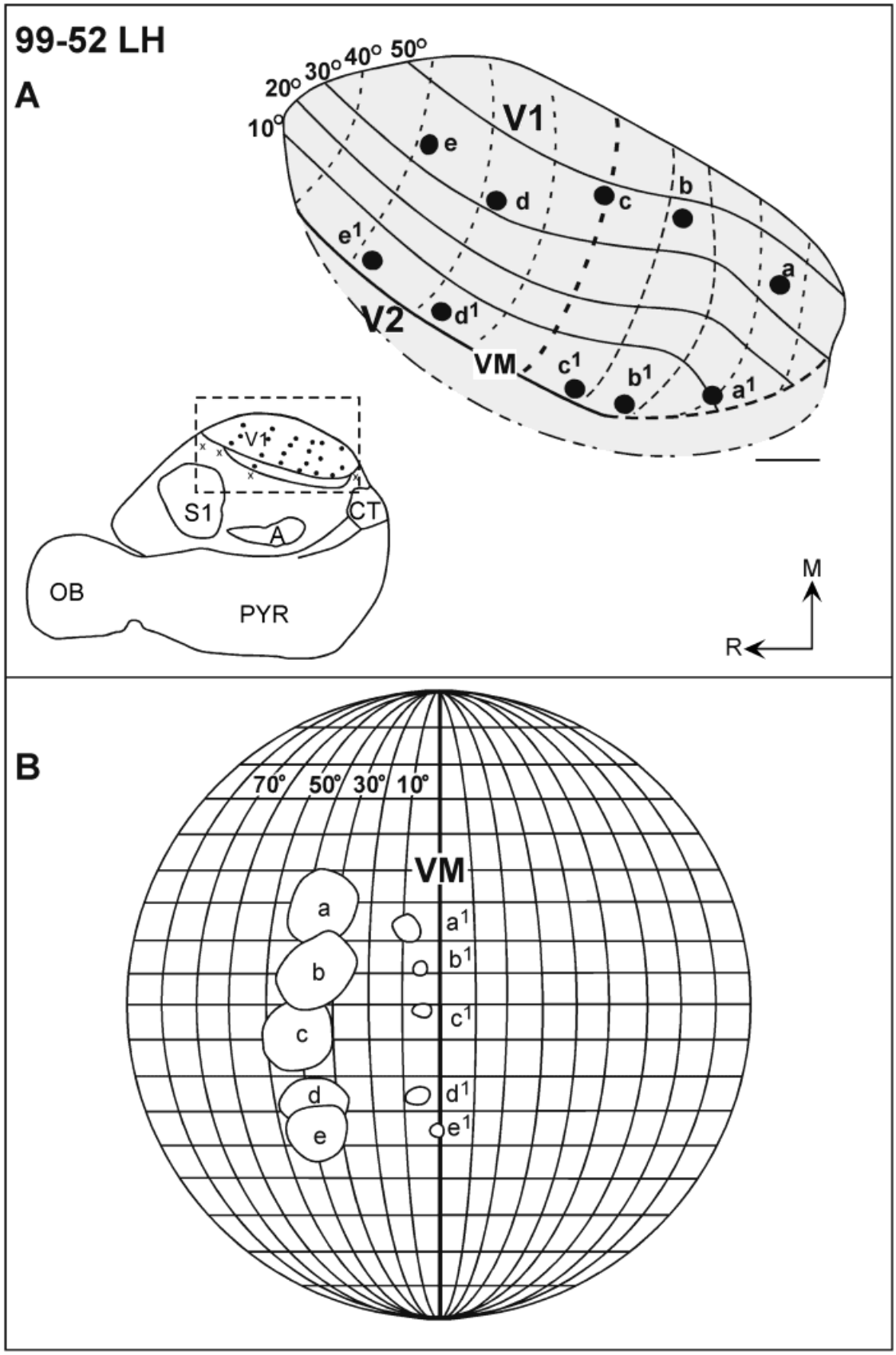

Fig. 5. Recording sites in V1 in the left hemisphere (**A**) and receptive fields for neurons at those sites drawn onto a globe (**B**) in case 99-52. This illustration demonstrates the tendency for neurons to have smaller receptive fields at the vertical meridian, compared with almost identical elevation location in the peripheral visual field. Recording sites a–e (indicated by black dots in A), are located medially in the cortex, RFs a^1 to e^1 were located near the lateral border of V1. RFs a–e and a^1 to e^1 corresponded to recording sites in A. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in A.

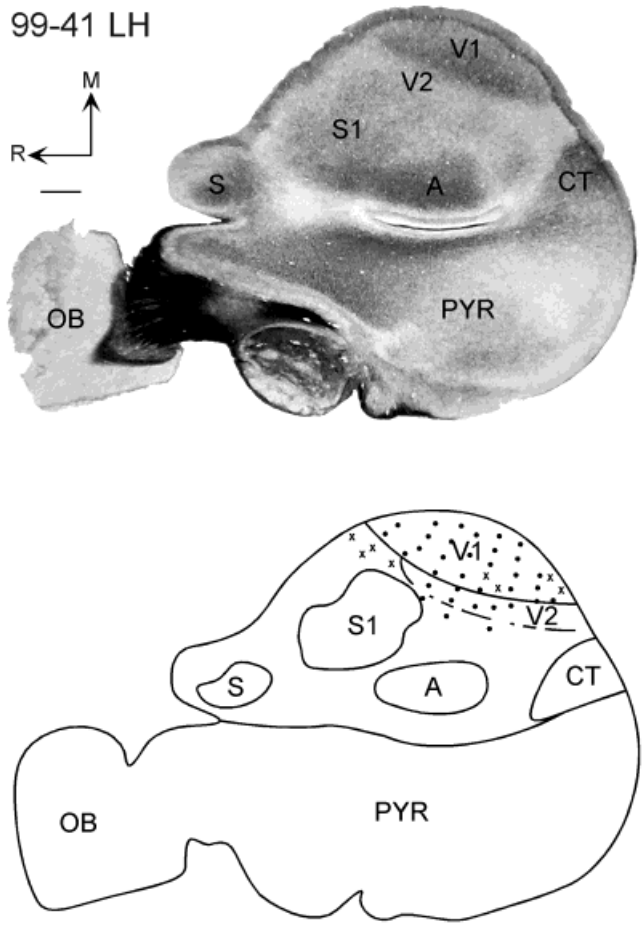

Fig. 6. Cortical myeloarchitecture and functional organization of the opossum neocortex, which was flattened and cut parallel to the cortical surface. Top is a digital image of a section stained for myelin. Sensory areas V1, CT, A, and S1 were darkly myelinated, with V1 located at the caudomedial pole, whereas CT was lateral to V1 and V2. A was located rostral to CT, and S1 on the rostral pole of the neocortex. PS (V2) appeared as a lightly myelinated region along the lateral border of V1 (after Huffman et al., 1999a). The bottom drawing is a reconstruction of the functional boundaries, which were defined by using multiunit electrophysiological recording techniques. Black dots represent electrode penetrations in case 99-41. By matching probes placed during electrophysiological recording experiments, the recording results can be superimposed on the cortical myeloarchitecture. In all cases, myeloarchitectonic boundaries of primary sensory areas could be clearly discerned, and the V1 and V2 borders matched almost perfectly with electrophysiologically identified boundaries. Thin lines indicate architectonic boundaries determined from the entire series of sections stained for myelin. The dashed line represents electrophysiological border only. X's indicate recording sites that did not respond to visual stimulation. Other conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1mm.

identified, and all other layers were virtually indistinguishable. In Nissl-stained sections, layer II was a thin stripe of darkly labeled, densely packed cells, and layer VI appeared as a cell sparse lightly stained layer. In myelin-stained sections, a thick stripe of darkly labeled fibers in the middle layers allowed CT to be distinguished from surrounding regions of cortex.

Cortical connections

A total of six injections of the neuroanatomical tracers flouroemerald (FE), fluororuby (FR), or both, were placed in V1, and retrogradely labeled cell bodies were plotted in the ipsilateral hemisphere (Figs. 8–12). In two cases, both FE and FR were injected (97-5 and 97-7, Figs. 9, 10), and in two cases (96-18 and 99-53, Figs 11, 12), only one tracer was injected into V1. In all, a total of five injections were made into three brains that were sectioned tangentially, and one tracer injected into one brain that was sectioned coronally. Examination of retrogradely labeled cell bodies revealed intrinsic connections within V1, as well as connections with other visual and multimodal areas of the cortex.

Intrinsic connections of V1. In all cases, the injection sites were confined to the neocortex, and in most cases spanned all cortical layers (e.g., Fig. 12B,C). All injections were placed in the caudal portion of V1, in the representation of the upper visual quadrant (Fig. 8). Three injections were placed at the caudal pole of V1 (Figs. 9, 10A). This location corresponds to the peripheral upper visual field representation (see Figs. 1, 9–11). Three injections were placed further rostral, in a location closer to the representation of the upper visual field near the vertical meridian (Figs. 10B, 11).

A dense halo of labeled cells was observed in all cases approximately 200 μm around the injection site, which we included as part of our injection site (Figs. 9–11). In all cases, intrinsic connections were observed immediately around the injection site, as well as further removed from the injection site. Labeled cells immediately around the injection site (approximately 500 μm to 1 mm) spread in all directions and formed patches (Fig. 8). In some cases (97-5FE, 97-7FR, and 97-7FE on Figs. 9B, 10A,B, respectively), labeled cells at more distant locations were observed. For instance, in case 97-5FE (Fig. 9B), a small injection near the caudal pole of V1 resulted in labeled cell bodies more than 4 mm rostral to the injection site, in the expected location of the lower visual quadrant. This pattern was also observed for two other injections (Fig. 10).

Extrastriate connections. Although labeled cells in V2 were sparse, there was some evidence of topography of connections of V2 projections to V1. In two cases in which small injections were made in the caudal region of V1, in the peripheral upper quadrant representation of the visual field, retrogradely labeled cells were located in the caudal region of V2 (Figs. 9A, 10A). Injections placed more rostrally (e.g., Figs. 9B, 10B) resulted in labeled cells slightly more rostral in V2. With the exception of 96-18 (Fig. 11), these labeling patterns provided some evidence for topographic organization of connections between V1 and V2.

The caudotemporal visual area (CT) contained labeled cells from all injections (Figs. 9–12). For three injections, CT was the most densely labeled extrastriate area (Figs. 9, 11). For these three injections, retrogradely labeled cells were concentrated predominantly in the rostromedial half of CT. For the other three injections, sparse label was found throughout CT (Figs. 10, 12). Labeled cells were also observed in entorhinal cortex (EC), along the rhinal sulcus, immediately lateral and rostral to area CT for five injections (Figs. 9, 10A, 11, 12). For two of these injections, label in EC was extremely sparse (Figs. 10A, 12). Examination of the tissue indicates that the injection site was

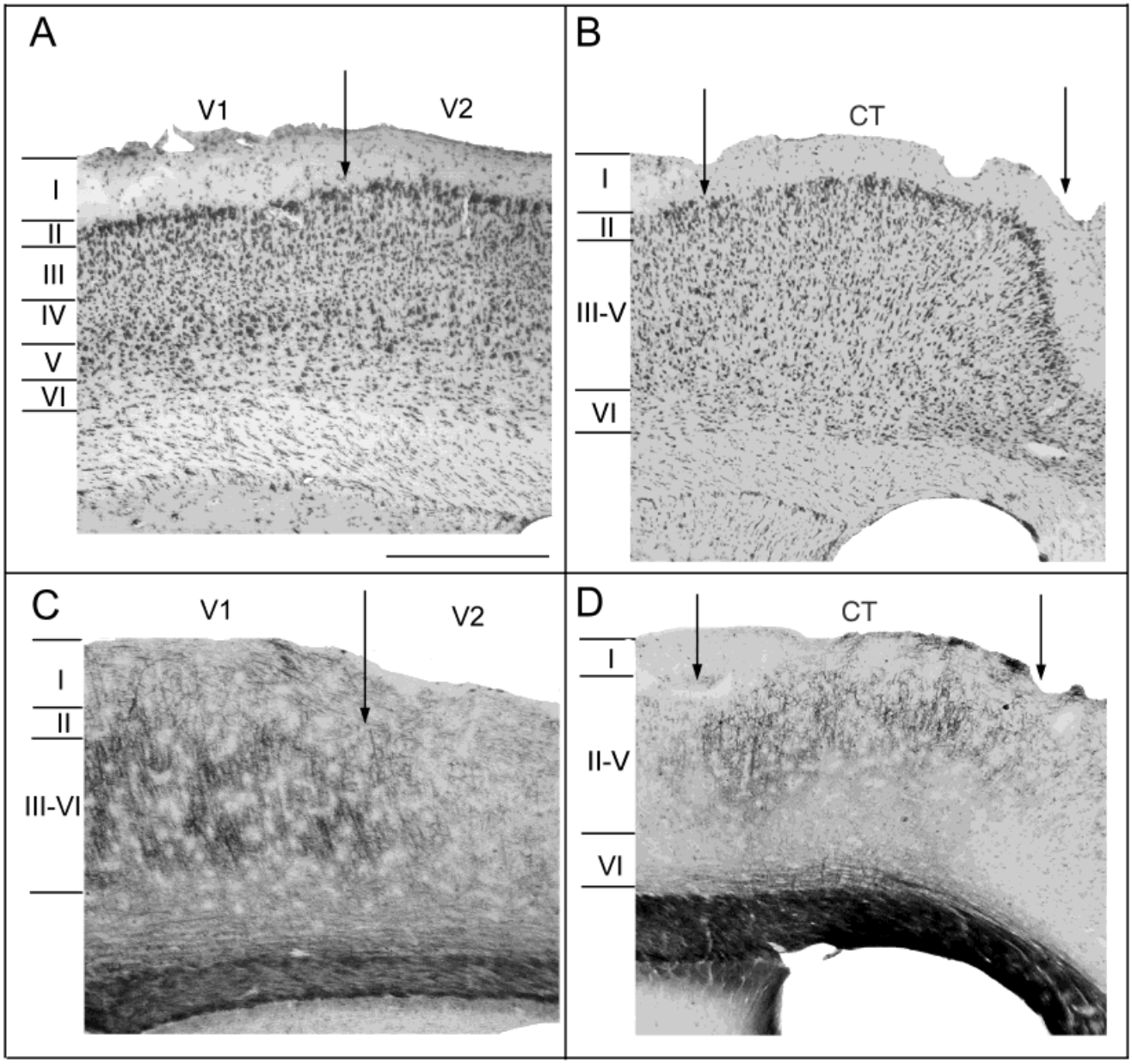

Fig. 7. Lightfield photomicrographs revealing cytoarchitecture of visual cortex that has been sectioned coronally and stained for Nissl (**A,B**) and myelin (**C,D**). The V1/V2 border (arrows in A and C) was characterized by a marked reduction in the width of layers I and IV and a decrease in density of cells in layer IV (A). In CT, the layers were less distinct, and there was a narrowing of all layers toward the rhinal sulcus (B). Myelin-stained sections in V1 were patchy through layers IV and V and less dense in layers II/III (C). In CT, the middle cortical layers stained densely compared with surrounding regions of the cortex (D). Other conventions are the same as in previous figures. For abbreviations, see list. Scale bar = 500 μm in A (applies to A–D).

confined to the neocortex, and did not extend to the underlying hippocampus. Therefore labeled cell bodies in EC were the direct result of our V1 injections.

For two injections (Figs. 9B, 10B), labeled cells were observed in the auditory area A and for three injections, in the multimodal area A/V (Figs. 9B, 10B). The label in A in both cases was very sparse and was composed of only a few cells, whereas the label in A/V was sparse but widely distributed.

Laminar connections. The injection in the coronally sectioned cortex was in the caudal portion of V1, close to the medial wall, in the upper quadrant of the peripheral visual field representation. The distribution of retrogradely labeled cells, shown in Figure 12A, was relatively sparse. Cells labeled with FR were observed in cortical layers II–VI near the injection site. Immediately adjacent to the injection site, labeled cells were mostly in the more superficial layers, II and III. Groups of labeled cells in V1

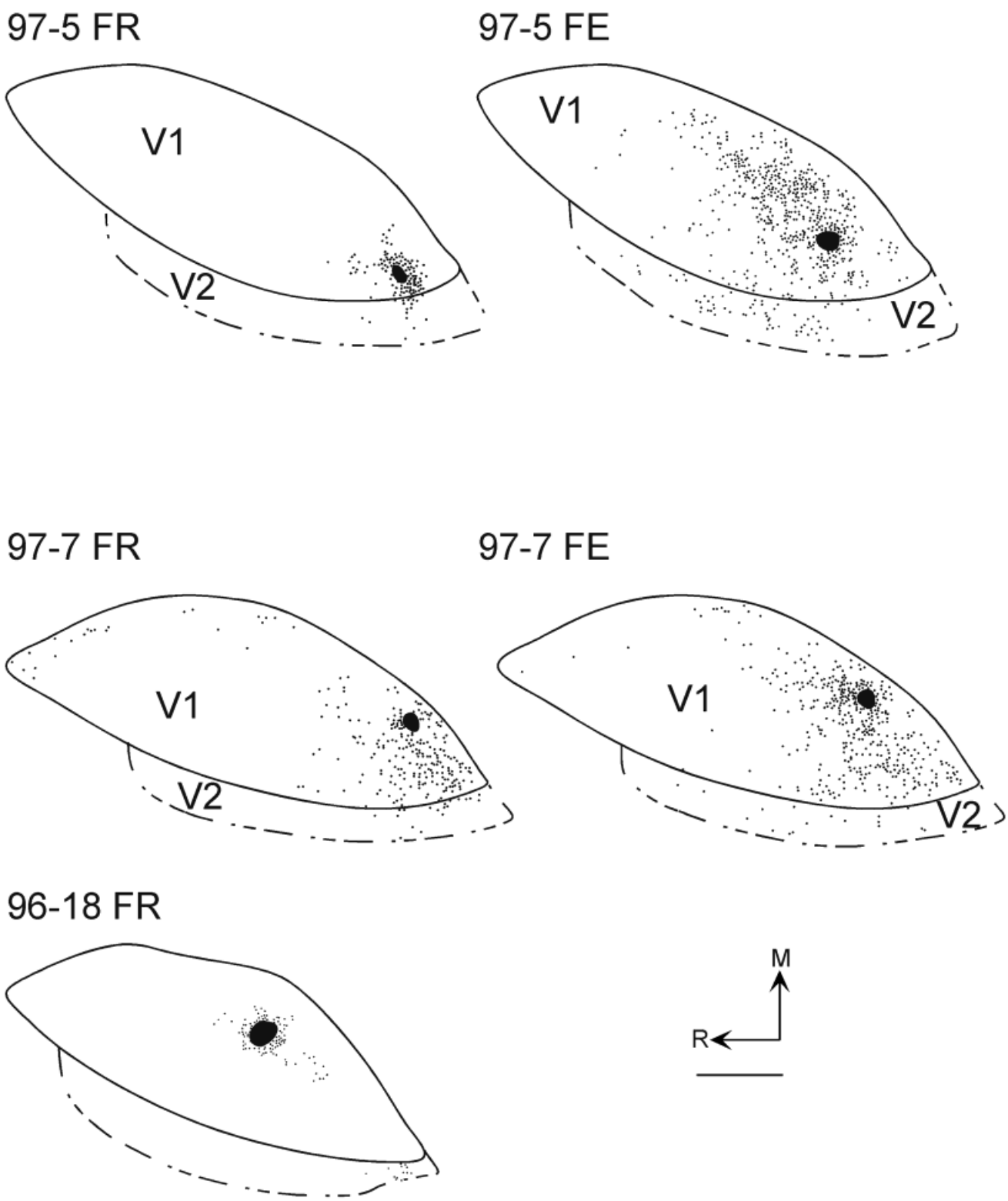

Fig. 8. Reconstruction of fluororuby (FR) and fluoroemerald (FE) labeled cells in V1 and V2. Drawings depict dorsal views of flattened left hemispheres in three animals (97-5, 97-7, and 96-18). Patches of retrogradely labeled cells (small dots) could clearly be seen near the injection sites (large dots) in 97-5 FE and 97-7 FE. Labeled cells were also observed in the far rostral pole of V1 in cases 97-7 FR and 97-7 FE. In these two cases, the injection sites were placed caudally in V1 close to the medial wall, in the cortical representation of the periphery of the upper quadrant of the visual field. The FE injections in cases 97-5 and 97-7 resulted in sparse labeling throughout the caudal to rostral extent of V2, whereas an injection site placed more medially in V1 resulted in labeled cells solely in the far caudal region of V2 in case 96-18 FR. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm.

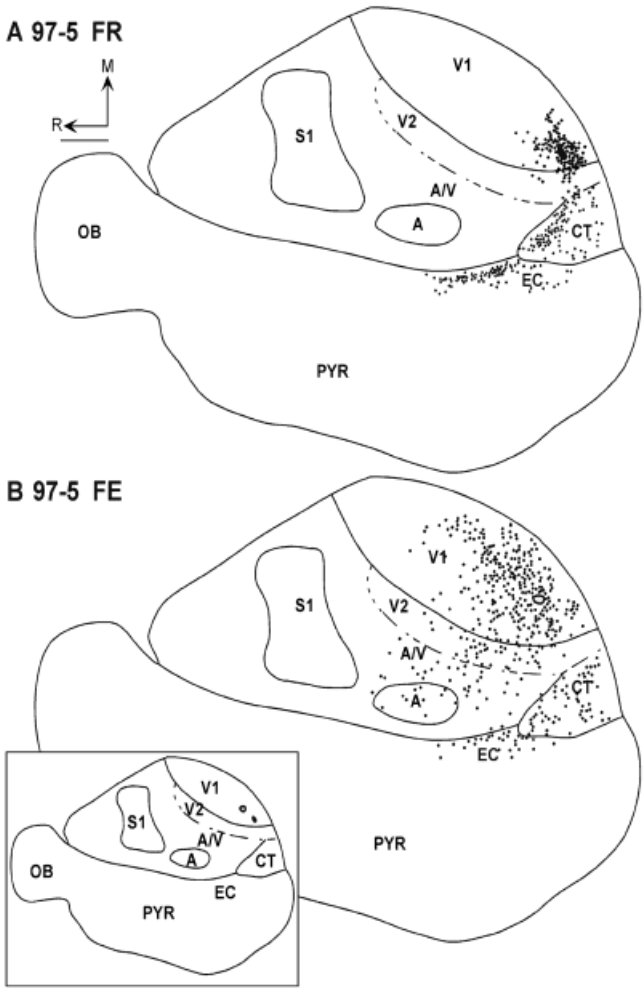

Fig. 9. A reconstruction of fluororuby (FR; **A**) and fluoroemerald (FE; **B**) injection sites in V1 and the location of retrogradely labeled cell bodies (dots) relative to myeloarchitectonic boundaries in case 97-5. Intrinsic connections within V1 were identified near the injection site, and distant from the injection site. CT, V2, A, A/V, and EC provided input to V1. Labeling was dense along the rostral border of CT, and in EC, along the caudal region of the rhinal sulcus. This cortex was flattened and cut parallel to the cortical surface so that the total patterns of connections could be readily appreciated. Inset on bottom left shows relative locations of FE (open dot) and FR (black dot) injections. Solid lines mark architectonic boundaries. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in A (applies to A,B).

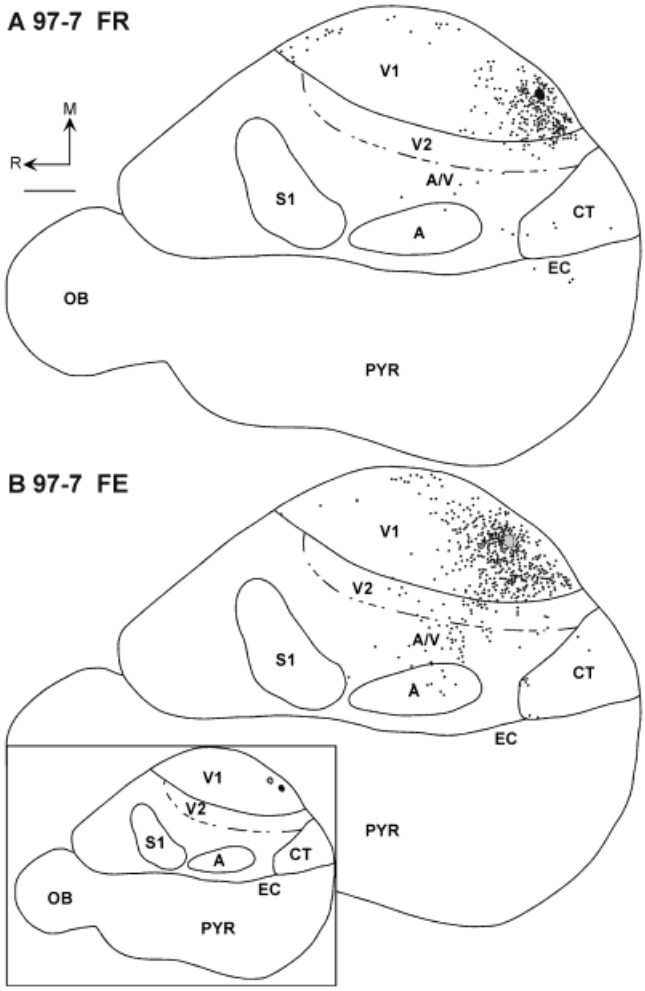

Fig. 10. A reconstruction of fluororuby (FR; **A**) and flouroemerald (FE; **B**) injection sites in V1, and the location of labeled cell bodies relative to myeloarchitectonic boundaries in case 97–7. The FR injection (black dot) was placed slightly more caudal than the FE injection (open dot), and FR labeled cells are located more caudally in V2. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm in A (applies to A,B).

were also observed further from the injection site, in layers II–VI. However, most labeled cells were predominantly located in layer IV (Fig. 12D,F). In V2, labeled cells appeared sparsely distributed throughout layers II–IV (Fig. 12E,G). A few large pyramidal cells were labeled in layers V and VI. In area CT, retrogradely labeled cells could be clearly seen in layers II–IV.

DISCUSSION

In the present study, we used electrophysiological recording techniques and architectonic analysis to examine the functional organization of the primary visual cortex in the short-tailed opossum. In addition, we placed anatomical tracers into V1 to reveal patterns of corticocortical connections and correlated these results with electrophysiological recording results and architecture of visual cortex.

In the current study, V1 was architectonically distinct in both Nissl- and myelin-stained tissue and had a clear visuotopic organization. Discrete patterns of connections were observed with two extrastriate areas, multimodal cortex and entorhinal cortex. These results are in contrast with earlier speculations that marsupials possess brains that are more generalized, with cortical areas that are less distinct than in placental mammals (e.g., Ebner, 1969; Walsh and Ebner, 1970; Jerison, 1973; see Rowe, 1990). Here, we compare our findings with those in other marsupials and discuss these findings in the context of brain evolution.

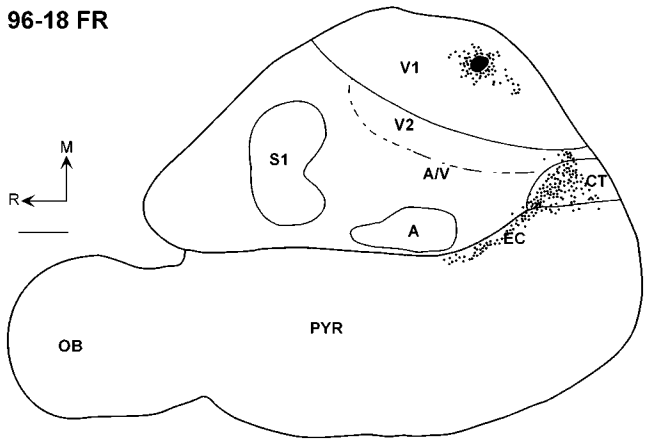

Fig. 11. A reconstruction of a fluororuby (FR) injection site in V1, and the location of labeled cell bodies relative to myeloarchitectonic boundaries in case 96-18. Backlabeled cells appear to be confined close the injection site in V1. Dense regions of FR labeled cells were also observed in CT and EC. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bar = 1 mm.

Visuotopic organization

V1. Most definitions of the primary visual cortex in marsupials are based on studies of cortical architecture or connections, and information on the functional organization of the marsupial visual cortex is limited. The topographic organization of V1 has been described in a few species of marsupial including the quoll (Rosa et al., 1999), wallaby (Vidyasagar et al., 1992), and opossum (Sousa et al., 1978; Volchan et al., 1988). In each of these species, the periphery of the visual field was represented in medial portions of V1, the upper quadrant was represented caudally, and the lower quadrant was represented rostrally. The vertical meridian was represented along the rostrolateral border of V1. This finding is similar to the current description of V1 in the short-tailed opossum. As in previous investigations, the receptive field sizes tended to be smaller for neurons at the vertical meridian than at the peripheral field representation (Christensen and Hill, 1970; Crewther et al., 1984). Likewise, most of V1 was devoted to representing the central 30–50 degrees of the contralateral visual hemifield. This cortical magnification of central vision is consistent with studies in the marsupial retina, which demonstrated a higher density of ganglion cells in the central retina (Hokoç and Oswaldo-Cruz, 1979; Tancred, 1981).

Similar retinotopic maps of V1 have been described in several eutherian mammals with well-developed visual systems, including squirrels (Kaas et al., 1972, 1989), cats (Tusa et al., 1978), ferrets (Law et al., 1988), tree shrews (Humphrey and Norton, 1980), megachiropteran bats (Rosa et al., 1993), and primates (e.g., Allman and Kaas, 1971; Gattass et al., 1987). Even in species that are not considered to be highly visual, such as mice, V1 contains a retinotopic map, although the amount of cortex it assumes is relatively small and the receptive field size of neurons therein is large (Wagor et al., 1980).

Extrastriate visual areas. In marsupials, complete electrophysiological maps of extrastriate cortex have been obtained only in the quoll (Rosa et al., 1999). Therefore, descriptions of extrastriate cortex have relied mainly on connectional and architectural data. Multiunit electrophysiological mapping of the quoll demonstrated that the peristriate belt immediately lateral to the V1 border contained a single, complete representation of the visual hemifield, similar to the topographic organization of V2 that has been described in placental mammals (see Kaas, 1980 for review). In the present study, complete electrophysiological maps could not be obtained; however, several recordings were made in V2. Neurons in V2 had relatively large receptive fields, and receptive field progression reversed at the V1/V2 boundary. Although previous investigations in short-tailed opossum revealed that cortex rostral and lateral to V2 contains neurons responsive to visual stimulation (Huffman et al., 1999a), this area has not been systematically examined in any marsupial.

Cortical architecture

When electrophysiological recording results were coregistered with cortical architecture in both tangentially and coronally sectioned tissue, it was found that the electrophysiologically defined V1 was coextensive with a myelin dark region and the classically defined striate cortex (Figs. 6, 7). V1 has been described as a darkly myelinated region with a clear rostral border in marsupials (e.g., Beck et al., 1996; Rosa et al., 1999), and a variety of placental mammals (e.g., Kaas et al., 1989; Krubitzer and Kaas, 1990; Rosa et al., 1993; Krubitzer et al., 1997). Likewise, the cytoarchitecture of striate cortex in the short-tailed opossum is similar to what has been observed in other marsupials and in placental mammals. For example, V1 in all marsupials and placental mammals examined consists of six layers, with a cell dense layer IV, large cells in layer V, and a cell sparse layer I (see Rowe, 1990 for review). Unlike V1, the rostral boundary of V2 of the opossum is more difficult to discern in our preparations. This is also true for a variety of eutherian mammals, particularly those with small brains, such as insectivores (Kaas et al., 1970; Krubitzer et al., 1997). In studies of the South American opossum (Martinich et al., 1990, 2000), the boundaries of V2 were determined by using combined neuroanatomical and histochemical techniques. The authors reported that V2 appeared to be modularly organized and was segregated into CO dense and light regions, and commissural rings. We have not observed modular types of patterns in CO-stained sections of V2 in the short-tailed opossum.

Another extrastriate area of opossum that was interconnected with V1 was the myelin-dense region located on the caudolateral pole of the neocortex, termed CT. Although a previous study did report visual responses from this region, the area was not systematically mapped (Huffman et al, 1999a). The location and appearance of CT was similar to area CT in the Virginia opossum (Beck et al., 1996) and to the medial temporal region described in the Australian brush-tailed possum (Crewther et al., 1984).

Cortical connections

The present study consistently revealed direct ipsilateral connections of V1 with three cortical areas: V2, CT, and EC. Investigations of connections in other marsupials have shown a similar pattern of connections. Anatomical tracing studies in the brush-tailed possum (Crewther et al., 1984) and mouse opossum (Bravo et al., 1990) revealed

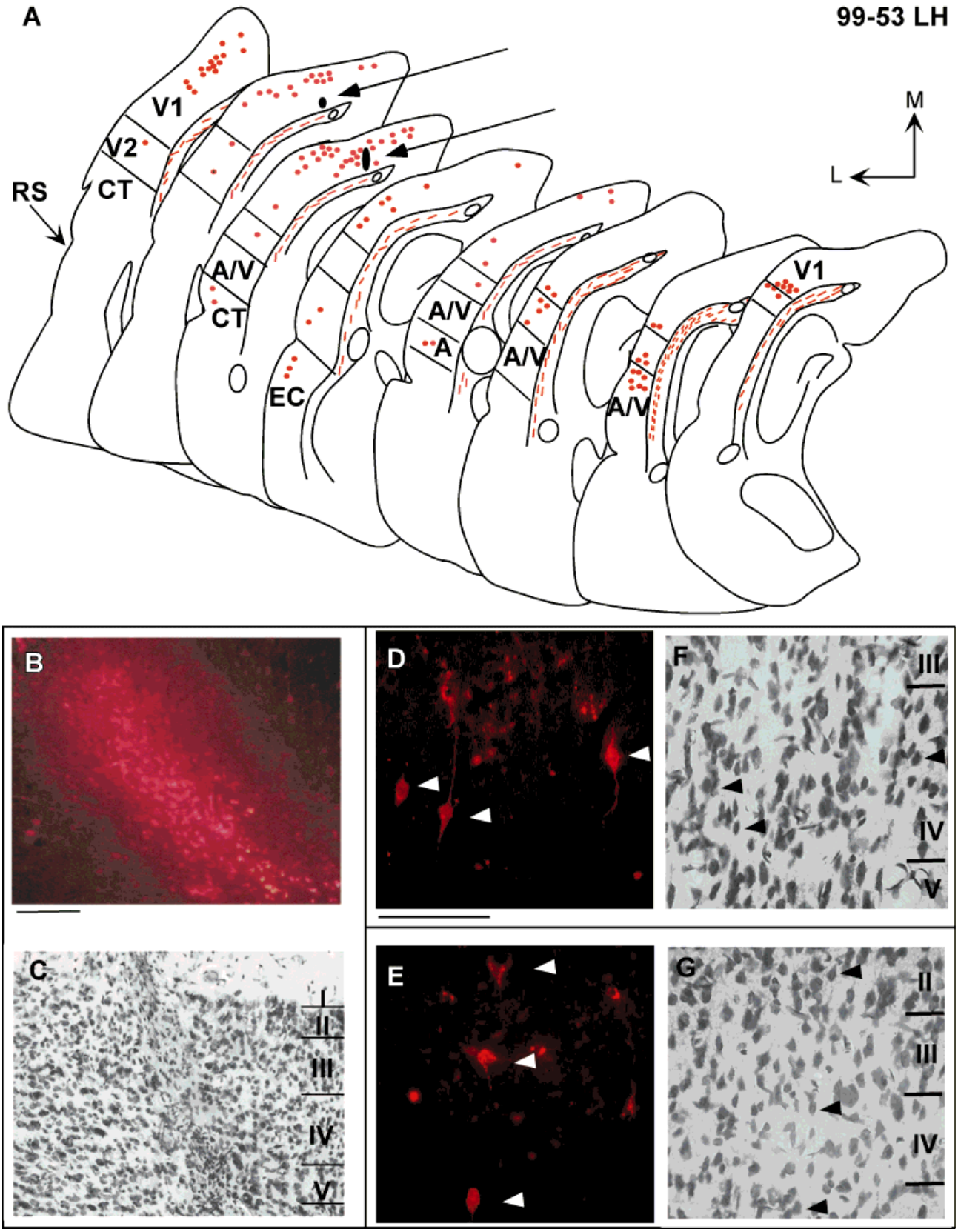

Fig. 12. Patterns of labeling in coronally sectioned tissue. **A:** Camera lucida drawings of retrogradely labeled fluororuby (FR) cells (red dots) resulting from an injection in V1 (arrows). Drawings of sections are in sequential order from caudal (left) to rostral (right). The injection spanned all cortical layers, as can be seen in digital images of the FR injection site (**B**) and in the adjacent Nissl-stained section (**C**). Labeled cells in V1 were observed in layers II–VI but were predominantly in layer IV (**D,F**). In V2, labeled cells were observed in layers II–V (**E,G**). D and E are digital images of FR-labeled cells in V1 (D) and V2 (E), next to the adjacent Nissl-stained section (F,G). Digital images were printed with Adobe Photoshop 4.0 software. Conventions are the same as in previous figures. R, rostral; M, medial. For abbreviations, see list. Scale bars = 100 μm (applies to B–G).

connections between V1 and the adjacent peristriate belt, the presumptive V2. Similarly, Benevento and Ebner (1971) reported degenerated fibers in a similar location after lesions to V1 in the Virginia opossum. In a recent study by Martinich et al. (2000), connections were observed between V1 and an area immediately rostrolateral to V1, termed the anterolateral peristriate cortex. Thus, accumulating evidence from both electrophysiological recording studies (Rosa et al., 1999; present investigation) and studies of connections indicate that marsupials possess a single visual area immediately rostrolateral to V1 that appears to be homologous to V2 described in other mammals.

V1 also received direct projections from CT. Investigations of cortical organization in different marsupials revealed connections with an architectonically similar cortical region. Lesion studies in the Virginia opossum (Benevento and Ebner, 1971) showed connections between V1 and a cytoarchitectonically distinct region located dorsal to the rhinal sulcus and posterior to the temporal cortex, which the authors termed the posterolateral peristriate cortex (PSpl). Other studies that used neuroanatomical tracing techniques in the South American opossum *Didelphis aurita,* showed V1 projections from the same area (Martinich, 2000). Similarly, Crewther et al. (1984) reported connections between V1 and a region of cortex just lateral to PS (V2) in the caudal pole of the cortex in the brush-tailed possum. Although the authors termed this region of cortex MT and LT (medial and lateral temporal regions), it appears to be in the same location as CT in the present investigation. An earlier investigation of the mouse opossum (Bravo et al., 1990) yielded similar results after horseradish peroxidase injections into V1. Although the authors did not assign a name to that cortical field, it corresponds to the location of CT, PSpl, and MT/LT described in other marsupials.

The connections with multimodal cortex and auditory cortex that were inconsistently observed in the present investigation have not been directly reported in other studies of marsupials. However, examination of the patterns of connections in all of the marsupials examined indicate that connections with cortex rostral and lateral to V2 (PS) can be observed and are in the approximate location of A/V and A in the present investigation (Benevento and Ebner, 1971; Crewther et al., 1984; Bravo et al., 1990; Martinich et al., 2000).

Extrastriate cortex in eutherian mammals has been subdivided differently for different mammals (see Kaas and Krubitzer, 1991 for review), and even in the same mammal investigators have varied in how they subdivide the neocortex (see Kaas, 1997 for review). Although connections of V1 have been reported with extrastriate areas other than V2, such as MT in primates (e.g., Weller and Kaas, 1983; Krubitzer and Kaas, 1993; Preuss et al., 1993), OTr and OTc in squirrels (Kaas et al., 1989), lateral parastriate cortex in hedgehogs (Gould and Ebner, 1978), or the Clare-Bishop area in cats (Sherk, 1990), data are too sparse to draw any reliable conclusions regarding homologies of these extrastriate areas.

Connections between the caudal region of the entorhinal cortex (EC) and V1 were also observed. This result was consistent across different animals, and in most cases, labeling in EC was extremely robust. This result is surprising because other studies of marsupial neocortex using similar techniques have not reported these connections. A recent study of connections of V1 in the South American opossum (Martinich et al., 2000) showed transported tracer in a band of cortex along the rhinal sulcus, but the location was rostral to that observed in the present investigations. Direct projections from EC to V1 have been demonstrated in the rat (Swanson and Köhler, 1986; Insausti et al., 1997), although these projections were not as robust as in the opossum. Given the paucity of data on connections between entorhinal cortex in V1 in marsupials and in other mammals, it is not clear whether such connections reflect a primitive feature of mammals that was lost in most eutherian lineages, or a feature of mammals with small neocortices and few cortical areas.

Implications for the evolution of visual cortex

The results of the present investigation, when compared with previous investigations of visual cortex in marsupials indicate that there are at least three common visual cortical areas: V1, V2, and CT. These areas were likely to be present in the common ancestor of all marsupials, and, thus, a universal feature of marsupial brains. In monotremes, mammals that in some respects are thought to reflect an even more primitive state than marsupials, there seem to be at least two visual areas (Vc & Vr; Krubitzer, 1998). As in other mammals, each of these two regions contains a complete representation of the visual hemifield. Although there is some evidence of retinotopy, the internal organization of these areas is not as precise as in marsupials and other mammals. Based on anatomical and functional organization of this area, it has been proposed that Vc in monotremes is a homologue of V1 (Krubitzer, 1998). Although monotremes contain a second field (Vr), it is not clear whether this field is homologous to V2 described in other mammals.

Thus, comparative studies in monotremes and marsupials indicate that their common ancestor possessed at least a primary visual area (V1) and possibly another visual area (Vr/V2). The imprecision of the organization, compared with animals with well-developed visual systems, and the large receptive fields of neurons in monotremes suggests that the ancestral visual system was rudimentary and that the earliest mammals may have performed poorly at making fine visual discriminations necessary for prey capture, predator avoidance, and more diverse foraging. Another possibility is that the apparent lack of a well-developed visual system in monotremes is a derived feature. The organization of the visual system observed in extant marsupials suggests that it was refined in evolution as species became more dependent on visual processing for survival. Such changes include an increase in the number of visual areas and an expansion of the amount of cortex devoted to visual processing, at least in those marsupials in which the visual system is well developed. In some eutherian lineages, such as insectivores (e.g., Kaas et al., 1970; Krubitzer et al., 1997) and some rodents (see Rosa and Krubitzer, 1999 for review), the status of the visual system is much like that proposed for the common ancestor of marsupials (i.e., containing only two or three visual areas). In other eutherians, such as cats, some rodents, and primates, there seems to be an independent expansion of the visual system like that observed in marsupials with well developed visual systems. This expansion includes an extraordinary increase in the amount of cortex devoted to visual process-

ing and an increase in the number of areas added to visual processing networks (see Krubitzer, 2000, for review).

Differences in cortical connections of V1 have also been observed in various mammals, but they may be a reflection of total brain size rather than a feature of primitive brains. For instance, in most mammals, connections of V1 are limited to V2 and one or two other extrastriate visual areas (Kaas, 1980; Kaas and Krubitzer, 1991). However, in mammals with smaller brains, such as rats (e.g., Swanson and Köhler, 1986; Insausti et al., 1997), and in this instance the opossum, connections are observed not only with extrastriate visual cortex but also with multimodal areas of the brain and with entorhinal cortex. Multimodal areas of cortex are often considered to be "higher order" areas in mammals with large brains, and represent ends of complex processing hierarchies. It has been proposed that animals with large brains have reduced long-range connections and increased local connections (Ringo, 1991; Ringo et al., 1994; Krubitzer et al., 1997; Manger et al., 1998). Thus, information is processed locally by closely related areas before being transmitted to more distant areas, such as multimodal areas in posterior parietal and insular cortex. This reduction in long-range connections allows for potentially increased processing capacity within any given sensory system, and hence, increased discriminatory and perceptual abilities, at the cost of global interactions between sensory systems and sensory and motor areas. Connections between V1 and multimodal areas in mammals with smaller brains, like the opossum, indicate that primary areas have access to higher order areas by means of monosynaptic connections. This reduced connectivity would result in decreased conduction time for processing potentially relevant stimuli but may be at the cost of discriminatory abilities of the animal. Thus, smaller brains with fewer cortical areas may have fundamental differences in processing strategies due, in part, to selection for optimal connection length. These differences in connectional layouts in large vs. small brains, impacts the types of discrete vs. global processing strategies that are generated in different-sized brains.

ACKNOWLEDGMENTS

We thank Elizabeth Disbrow, Barbara Chapman, and Marty Usrey for helpful comments on this manuscript, Daniel Slutsky for assistance with visual mapping, and Monika Sum and Monica Greene for assistance with histologic processing. L.K. received support from the NIH and the Whitehall Foundation.

LITERATURE CITED

Adams AD, Forrester JM. 1968. The projection of the rat's visual field on the cerebral cortex. Q J Exp Physiol Cogn Med Sci 53:327–336.

Allman JM, Kaas JH. 1971. Representation of the visual field in striate and adjoining cortex of the owl monkey (*Aotus trivirgatus*). Brain Res 35:89–106.

Beck PD, Pospichal MW, Kaas JH. 1996. Topography, architecture, and connections of somatosensory cortex in opossums: evidence for five somatosensory areas. J Comp Neurol 366:109–133.

Benevento LA, Ebner FF. 1971. The areas and layers of corticocortical terminations in the visual cortex of the Virginia opossum. J Comp Neurol 141:157–189.

Bonhoeffer T, Grinvald A. 1993. The layout of iso-orientation domains in area 18 of cat visual cortex: optical imaging reveals a pinwheel-like organization. J Neurosci 13:4157–4180.

Bravo H, Olavarría J, Martinich S. 1990. Patterns of interhemispheric and striate-peristriate connections in visual cortex of the South American marsupial *Marmosa elegans* (mouse opossum). Anat Embryol (Berl) 182:583–589.

Christensen JL, Hill RM. 1970. Response properties of single cells of a marsupial visual cortex. Am J Optom Arch Am Acad Optom 47:547–556.

Crewther DP, Crewther SG, Sanderson KJ. 1984. Primary visual cortex in the brushtailed possum: receptive field properties and corticocortical connections. Brain Behav Evol 24:184–197.

Dräger UC. 1975. Receptive fields of single cells and topography in mouse visual cortex. J Comp Neurol 160:269–290.

Ebner FF. 1969. A comparison of primitive forebrain organization in metatherian and eutherian mammals. Ann N Y Acad Sci 167:241–257.

Gallyas F. 1979. Silver staining of myelin by means of physical development. Neurol Res 1:203–209.

Gattass R, Gross CG, Sandell JH. 1981. Visual topography of V2 in the macaque. J Comp Neurol 201:519–539.

Gattass R, Sousa APB, Rosa MGP. 1987. Visual topography of V1 in the *Cebus* monkey. J Comp Neurol 259:529–548.

Gilbert CD, Wiesel TN. 1979. Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. Nature 280:120–125.

Gould HJ III, Ebner FF. 1978. Connections of the visual cortex in the hedgehog (*Paraechinus hypomelas*). J Comp Neurol 177:473–502.

Hokoç JN, Oswaldo-Cruz E. 1979. A regional specialization in the opossum's retina: quantitative analysis of the ganglion cell layer. J Comp Neurol 183:385–395.

Huffman KJ, Nelson J, Clarey J, Krubitzer L. 1999a. Organization of somatosensory cortex in three species of marsupials, *Dasyurus hallucatus*, *Dactylopsila trivirgata*, and *Monodelphis domestica*: neural correlates of morphological specializations. J Comp Neurol 403:5–32.

Huffman KJ, Molnár Z, Van Dellen A, Kahn DM, Blakemore C, Krubitzer L. 1999b. Formation of cortical fields on a reduced cortical sheet. J Neurosci 19:9939–9952.

Humphrey AL, Norton TT. 1980. Topographic organization of the orientation column system in the striate cortex of the tree shrew (*Tupaia glis*): I. Microelectrode recording. J Comp Neurol 192:531–547.

Insausti R, Herrero MT, Witter MP. 1997. Entorhinal cortex of the rat: cytoarchitectonic subdivisions and the origin and distribution of cortical efferents. Hippocampus 7:146–183.

Jerison HJ. 1973. Evolution of brain and intelligence. New York: Academic Press.

Kaas JH. 1980. A comparative survey of visual cortex organization in mammals. In: Ebbesson SOE, editor. Comparative neurology of the telencephalon. New York: Plenum Press. p 483–502.

Kaas JH. 1988. Why does the brain have so many visual areas? J Cogn Neurosci 1:121–135.

Kaas JH. 1997. Theories of visual cortex organization in primates. In: Rockland KS, Kaas JH, Peters A, editors. Cerebral cortex. New York: Plenum Press. p 91–125.

Kaas JH, Krubitzer LA. 1991. The organization of extrastriate visual cortex. In: Dreher B, Robinson SR, editors. Neuroanatomy of the visual pathways and their development. London: MacMillan. p 302–323.

Kaas J, Hall WC, Diamond IT. 1970. Cortical visual areas I and II in the hedgehog: relation between evoked potential maps and architectonic subdivisions. J Neurophysiol 33:595–615.

Kaas JH, Hall WC, Diamond IT. 1972. Visual cortex of the grey squirrel (*Sciurus carolinensis*): architectonic subdivisions and connections from the visual thalamus. J Comp Neurol 145:273–305.

Kaas JH, Krubitzer LA, Johanson KL. 1989. Cortical connections of areas 17 (V-I) and 18 (V-II) of squirrels. J Comp Neurol 281:426–446.

Kirsch JAW. 1977. The classification of marsupials In: Hunsaker D, editor. The biology of marsupials. New York: Academic Press. p 1–50.

Krubitzer L. 1998. What can monotremes tell us about brain evolution? Philos Trans R Soc Lond B Biol Sci 353:1127–1146.

Krubitzer L. 2000. How does evolution build a complex brain? In: Bock GB, Cardew G, editors. Evolutionary development biology of the cerebral cortex. Chichester: John Wiley & Sons. p 206–219.

Krubitzer L, Kaas J. 1990. Cortical connections of MT in four species of primates: areal, modular and retinotopic patterns. Vis Neurosci 5:165–204.

Krubitzer LA, Kaas JH. 1993. The dorsomedial visual area of owl monkeys:

connections, myeloarchitecture, and homologies in other primates. J Comp Neurol 334:497–528.

Krubitzer L, Künzle H, Kaas J. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). J Comp Neurol 379:399–414.

Law MI, Zahs KR, Stryker MP. 1988. Organization of primary visual cortex (area 17) in the ferret. J Comp Neurol 278:157–180.

Malach R. 1989. Patterns of connections in rat visual cortex. J Neurosci 9:3741–3752.

Manger P, Sum M, Szymanski M, Ridgway SH, Krubitzer L. 1998. Modular subdivisions of dolphin insular cortex: does evolutionary history repeat itself? J Cogn Neurosci 10:153–166.

Martinich S, Rosa MG, Rocha-Miranda CE. 1990. Patterns of cytochrome oxidase activity in the visual cortex of a South American opossum (*Didelphis marsupialis aurita*). Braz J Med Biol Res 23:883–887.

Martinich S, Pontes MN, Rocha-Miranda CE. 2000. Patterns of corticocortical, corticotectal, and commissural connections in the opossum visual cortex. J Comp Neurol 416:224–244.

Montero VM 1981. Topography of the cortico-cortical connections from the striate cortex in the cat. Brain Behav Evol 18:194–218.

Montero VM, Bravo H, Fernández V. 1973a. Striate-peristriate cortico-cortical connections in the albino and gray rat. Brain Res 53:202–207.

Montero VM, Rojas A, Torrealba F. 1973b. Retinotopic organization of striate and peristriate visual cortex in the albino rat. Brain Res 53: 197–201.

Preuss TM, Beck PD, Kaas JH. 1993. Areal, modular, and connectional organization of visual cortex in a prosimian primate, the slow loris (*Nycticebus coucang*). Brain Behav Evol 42:321–335.

Ringo JL. 1991. Neuronal interconnections as a functions of brain size. Brain Behav Evol 38:1–6.

Ringo JL, Doty RW, Demeter S, Smiard PY. 1994. Time is of the essence: a conjecture that hemispheric specialization arises from interhemispheric conduction delay. Cereb Cortex 4:331–343.

Roe AW, Ts'o DY. 1995. Visual topography in primate V2: multiple representations across functional stripes. J Neurosci 15:3689–3715.

Rosa MG, Krubitzer LA 1999. The evolution of visual cortex: where is V2? Trends Neurosci 22:242–248.

Rosa MGP, Schmid LM, Krubitzer LA, Pettigrew JD. 1993. Retinotopic organization of the primary visual cortex of flying foxes (*Pteropus poliocephalus* and *Pteropus scapulatus*). J Comp Neurol 335:55–72.

Rosa MG, Casagrande VA, Preuss T, Kaas JH. 1997. Visual field representation in striate and peristriate cortices of a prosimian primate (*Galago garnetti*). J Neurophysiol 77:3193–3217.

Rosa MG, Krubitzer LA, Molnár Z, Nelson JE. 1999. Organization of visual cortex in the northern quoll, *Dasyurus hallucatus*: evidence for a homologue of the second visual area in marsupials. Eur J Neurosci 11:907–915.

Rowe M. 1990. Organization of the cerebral cortex in monotremes and marsupials. In: Jones EG, Peters A, editors. Cerebral cortex. New York: Plenum Press. p 263–334.

Sherk H. 1990. Functional organization of input from areas 17 and 18 to an extrastriate area in the cat. J Neurosci 10:2780–2790.

Sousa AB, Gattass R, Oswaldo-Cruz E. 1978. The projection of the opossum's visual field on the cerebral cortex. J Comp Neurol 177:569–587.

Swanson LW, Köhler C. 1986. Anatomical evidence for direct projections from the entorhinal area to the entire cortical mantle in the rat. J Neurosci 6:3010–3023.

Tancred E. 1981. The distribution and sizes of ganglion cells in the retinas of five Australian marsupials. J Comp Neurol 196:585–603.

Tusa RJ, Palmer LA, Rosenquist AC. 1978. The retinotopic organization of area 17 (striate cortex) in the cat. J Comp Neurol 177:213–235.

Tusa RJ, Rosenquist AC, Palmer LA. 1979. Retinotopic organization of areas 18 and 19 in the cat. J Comp Neurol 185:657–678.

Tyler CJ, Dunlop SA, Lund RD, Harman AM, Dann JF, Beazley LD, Lund JS. 1998. Anatomical comparison of the macaque and marsupial visual cortex: common features that may reflect retention of essential cortical elements. J Comp Neurol 400:449–468.

Van Essen DC. 1979. Visual areas of the mammalian cerebral cortex. Annu Rev Neurosci 2:227–263.

Vidyasagar TR, Wye-Dvorak J, Henry GH, Mark, RF. 1992. Cytoarchitecture and visual field representation in area 17 of the tammar wallaby (*Macropus eugenii*). J Comp Neurol 325:291–300.

Volchan E, Bernardes RF, Rocha-Miranda CE, Gleiser L, Gawryszewski, LG. 1988. The ipsilateral field representation in the striate cortex of the opossum. Exp Brain Res 73:297–304.

Wagor E, Mangini NJ, Pearlman AL. 1980. Retinotopic organization of striate and extrastriate visual cortex in the mouse. J Comp Neurol 193:187–202.

Walsh TM, Ebner FF. 1970. The cytoarchitecture of somatic sensory cortex in the opossum (*Didelphis marsupialis virginiana*): a Golgi study. J Anat 107:1–18.

Weller RE, Kaas JH. 1983. Retinotopic patterns of connections of area 17 with visual areas V-II and MT in macaque monkeys. J Comp Neurol 220:253–279.