Anatomical Comparison of the Macaque and Marsupial Visual Cortex: Common Features That May Reflect Retention of Essential Cortical Elements

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ABSTRACT

This study identifies fundamental anatomical features of primary visual cortex, area V1 of macaque monkey cerebral cortex, i.e., features that are present in area V1 of phylogenetically distant mammals of quite different lifestyle and features that are common to other regions of cortex. We compared anatomical constituents of macaque V1 with V1 of members of the two principal marsupial lines, the dunnart and the quokka, that diverged from the eutherian mammalian line over 135 million years ago. Features of V1 common to both macaque and marsupials were then compared with anatomical features we have previously described for macaque prefrontal cortex. Despite large differences in overall area and thickness of V1 cortex between these animals, the absolute size of pyramidal neurons is remarkably similar, as are their specific dendritic branch patterns and patterns of distribution of intrinsic axons. Pyramidal neuron patchy connections exist in the supragranular V1 in both the marsupial quokka and macaque as well as in macaque prefrontal cortex. Several specific types of aspinous interneurons are common to area V1 in both marsupial and macaque and are also present in macaque prefrontal cortex. Spiny stellate cells are a common feature of the thalamic-recipent, mid-depth lamina 4 of V1 in all three species. Because these similarities exist despite the very different lifestyles and evolutionary histories of the animals compared, this finding argues for a highly conserved framework of cellular detail in macaque primary visual cortex rather than convergent evolution of these features. J. Comp. Neurol. 400:449–468, 1998.  © 1998 Wiley-Liss, Inc.

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The aim of this study is to provide a basic anatomical framework to be used for models of function of the macaque monkey primary visual cortex, area V1. To lay a framework for such modeling studies, we first need to know what elements of the macaque V1 cortex may be essential for cerebral cortex as a whole and what special features may characterize mammalian visual cortex in general.

We now have a wealth of information concerning cell types and circuitry within the macaque area V1 (see Levitt et al., 1996). We wish to know how much of this anatomical organization represents the basic building blocks of mammalian neocortex rather than being specializations subserv-ing the particular needs of macaque vision. To identify elements that are likely to be basic, essential features of cerebral cortex and, as well, those features that may...
characterize fundamentals of visual cortex in particular, we have compared the intrinsic organization of primary visual cortex of two marsupials, the dunnart (Sminthopsis crassicaudata) and the quokka (Setonix brachyurus), with area V1 of the eutherian macaque monkey (M. mulatta, M. nemestrina, and M. fascicularis).

The marsupial and eutherian lines already appear distinct in fossil records from 135 million years ago, allowing ample time for evolutionary change to have occurred between these mammalian subclasses (Austad, 1988; Walton and Richardson, 1989). If marsupial and eutherian cortices share a common intrinsic element, such as a particular cell type or patterns of connectivity, then that feature could be the result of retention from the common ancestor or the product of convergent evolution. If there are commonalities in cortical neural organization between marsupials and macaques, which markedly differ in terms of lifestyle, size, and evolutionary history, it is more likely that they are retained elements of an ancient configuration, rather than being due to convergent evolution, and that the shared features are basic to neocortical function. The two marsupials were chosen because they effectively represent extremes in marsupial evolution. They are very different in size and have different lifestyles and degrees of binocular overlap. The dunnart belongs to order Polyprotodonta, has an adult body weight of 10–15 g, is a nocturnal carnivore and also prey to larger carnivorous mammals and birds, and has 110–140° of binocular overlap (Strahan, 1983; Dunlop et al., 1997). The quokka belongs to order Diprotodonta, has an adult body weight of 2.5–3.5 kg, is a crepuscular herbivore, and has a binocular overlap of 80°. For comparison, the adult macaque is a primate that weighs 4–10 kg, is primarily herbivorous, generally arboreal but also ground foraging, and has 130–140° of binocular overlap (Van Essen et al., 1984).

In this study, we examined cell types and specific patterns of axonal and dendritic aborizations, searching for common elements in the primary visual cortices of the dunnart, quokka, and macaque; we asked whether these same elements are also present in a quite different cortical area, area 46 of prefrontal cortex, of the primate. Our findings of common features in visual and prefrontal cortical organization between such disparate species suggest that these common anatomical features are highly conserved and essential for macaque visual cortex function, because they appear to be generally required constituents of cerebral cortex. Features present in both marsupial and macaque visual cortex, but not present in prefrontal cortex, suggest that they are required for visual cortex function but are not specific to macaque V1. Models of macaque visual cortex function based on real anatomy should include both sets of features.

MATERIALS AND METHODS

Animals and anesthesia

Dunnarts and quokkas were bred in captivity by Animal Services and the Department of Zoology of the Western University of Australia. All experimental procedures were approved by the proper governmental agencies and complied with the guidelines approved by the animal care committees at both London and Perth research facilities. For intraocular injections, animals were anesthetized by inhalation of halothane (1–3%, in a nitrous oxide/oxygen mixture 4:1). Terminal anesthesia was induced in dunnarts by intramuscular injection of Saffan (alphaxalone/alphadalone acetate, 0.9 mg/0.3 mg, respectively, per 10 g of body weight) and in quokkas by intraperitoneal injection of Valabarb barbiturate (0.9 g/kg body weight). Tissue was obtained from macaque monkeys of a variety of ages and species (M. mulatta, M. nemestrina, and M. fascicularis). These animals had all received a lethal dose of barbiturate anesthetic before transcardial perfusion with aldehydes. Full details of preparation of macaque material used for this study can be found in previous studies (Lund, 1973;...
Fig. 2. Edge of primary visual cortex contralateral to intraocular proline injection in the quokka: A: Darkfield photomicrograph showing termination of proline label (arrow). B: The same region in lightfield showing decrease in cell density (arrow) between layer 4 of primary visual cortex and the adjacent region. Primary visual cortex is to the left of the arrow in both A and B. Scale bar = 100 µm (applies to A,B).
Lund and Yoshioka, 1991; Lund and Wu, 1997). We will also make reference to cat and rat material prepared in a similar manner.

**Measures of primary visual cortex**

**Areal measures.** V1 in the dunnart and quokka was defined initially on the basis of distinct cellular (cresyl violet stain) and fiber (Galyas silver stain) architecture at the occipital dorsal pole of the brain. Animals (n = 2 dunnarts and 2 quokkas) were perfused by transcardial perfusion with 0.9% saline followed by 4% paraformaldehyde. Brains were sunk overnight in 30% sucrose, frozen, and sectioned coronally at 40 µm. The distinct fiber architecture of these cortical regions was also visible in

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**Fig. 3.** Camera lucida drawings of coronally sectioned visual cortex after intraocular injection of proline in the right eye of (A) dunnart and (B) quokka. Every section is shown in the serial reconstruction of the dunnart and every fifth section is shown in the quokka reconstruction. Regions in which proline label was detected in the cortical sections are denoted by dashed lines. The corresponding cortical regions are denoted by the shaded areas in the whole brain drawings. Scale bar = 5 mm in both A and B in relation to histological sections.
Golgi Rapid impregnations, allowing the borders of the region to be recognized in this material. Camera lucida drawings of the cell- and fiber-stained serial sections were used to reconstruct the boundaries and derive the area of V1.

Transneuronal transport of tritiated proline, injected into one eye, was used to confirm the boundaries of V1 in adult dunnarts and quokkas (n = 2 of each species) and to define the area of visual cortex receiving crossed and uncrossed visual pathways. The periocular region was coated with xylocaine, and the right eye was injected with L-[2,3,4,5,-3H] proline with a specific activity of 100 Ci/µmol by using a Hamilton syringe coupled with a 30-gauge needle. The proline had previously been concentrated by evaporation in a controlled stream of nitrogen gas through a fume hood. For dunnarts, 200 µCi was delivered in a 10-µl injection, and for quokkas, 1 µCi was delivered in a 20-µl injection. Animals were returned to their usual environment after recovery from anesthesia. After 2–3 weeks, animals were terminally anesthetized and perfused with 0.9% saline followed by buffered 4% formaldehyde solution (pH 7.4). Brains were removed, dehydrated,
and embedded in paraffin wax. Coronal sections (10 µm) were mounted on slides, defatted, and processed for autoradiography by using Ilford K2 emulsion. Slides were stored at 4°C in the dark for 6 weeks before developing with Kodak D-19 developer (Eastman Kodak, Rochester, NY) and subsequent counterstaining with cresyl violet. Sections containing autoradiographic label in layer 4 were drawn by using either a camera lucida attachment or an MDII microscope digitizer (Minnesota Datametrics, St. Paul, MN) and reconstructed as a map on the outline of whole brains (Fig. 1).

**Cellular distribution measures.** In addition, the relative distribution of neurons with depth was estimated as described below in each of the layers in area V1; these estimates were directed at establishing how each species distributed its total population of cells between the cortical layers rather than establishing any absolute cell numbers, packing densities, or volumes. In addition to the dunnart, quokka, and macaque, we also surveyed cresyl violet-stained tissue from other eutherian species, namely the cat and rat (this tissue was available to us from previous studies). For each species, three spatially separated columns, 25-µm wide, were drawn from pia to white matter in 40-µm-thick sections in anterolateral V1, this region is known in those species where it has been mapped to represent central binocular vision (see for example Montero et al., 1973; Tusa et al., 1978; Van Essen et al., 1984). Cells within the column were counted as well as those whose somas overlapped the vertical lines delineating the column. In addition, the proportion of neuropil committed to layers 2–4 and 5–6 was ascertained by expressing the linear depth of layers 2–4 and 5–6 as a percentage of total cortical depth. Furthermore, the relative cell density (excluding obvious non-neuronal and glial elements) to layers 2–4 and 5–6 was determined by expressing cell counts in these layers as a percentage of the total cell count from the top of layer 2 (because layer 1 was almost neuron free in all species) to white matter. This approach enabled us to determine whether the relative contribution of cell number and neuropil to different layers of primary visual cortex was the same or different in the eutherians or marsupials examined.

**Cellular morphology**

The morphology of nonspiny interneurons (generally γ-aminobutyric acid-containing, GABAergic, and presumed to be inhibitory) and spine bearing (excitatory) neurons, both pyramidal and stellate, was determined by using Golgi Rapid impregnations of juvenile dunnart (postnatal day 80; n = 6) and quokka (postnatal day 150; n = 8) brains. Measurements were also made in juvenile macaques (6 months old; n = 3) for comparison. Animals were perfused with saline followed by 4% paraformaldehyde and were stained by using a Golgi Rapid procedure used in previous studies of primate neocortex (see Lund, 1973). The morphology of neurons in the marsupial V1 was then compared with cell populations described in present and previous studies of macaque V1 (Lund, 1973, 1987; Lund and Yoshioka, 1991; Lund and Wu, 1997) and prefrontal areas 9 and 46 (Lund and Lewis, 1993).

**Intra-areal connectivity**

Although we have been able to use horseradish peroxidase and biocytin tracers to examine intra-areal connectivity in mammals (see Rockland and Lund, 1982, 1983; Yoshioka et al., 1996), we found it difficult to obtain small, localized injections of either of these tracers which would result in sufficient tracer transport within marsupial cortex. Due to this problem, we used instead the fluorescent dye, 1,1'-dioctadecyl-3,3',3''-tetramethylindocarbocyanine perchlorate (DiI). Small crystals of DiI were inserted into the superficial layers of paraformaldehyde-perfused brains of dunnart (postnatal day 80; n = 6) and quokka (postnatal day 150; n = 2) primary visual cortex. The brains were then stored in fixative for 1 week, 3 weeks, or 3 months. The visual cortex was then cut tangentially on a Vibratome at 50 µm, and serial sections mounted in glycerol were examined for lateral transport of the DiI label by using a rhodamine excitation filter. The 6–12% shrinkage of the Golgi sections compared with the DiI preparations was taken into account when comparing the dimensions of dendritic and axonal arbors and the spatial scale of the intra-areal stepping connections.
RESULTS

Areal extent and cellular and fiber architecture of the quokka and dunnart visual cortex

Figures 1-3 illustrate the extent of area V1 in the marsupial and eutherian cortices. In absolute size, our measurements show the surface area of the dunnart striate cortex to be substantially smaller at 5.6 mm² per hemisphere than that of the quokka, which measures 92 mm² per hemisphere (Figs. 2, 3). For comparison, the rat V1 region measures 7.1 mm² per hemisphere, the cat 380 mm², and the macaque approximately 1,200 mm². The dunnart V1 cortex, at 495 µm, is also much thinner than that of the quokka (1,100 µm) and rat (1,250 µm) striate cortex and is approximately a third the depth of the macaque striate cortex (1,592 µm; Fig. 4 and Table 1).

The dunnart has an interesting specialization in the form of a cell-sparse narrow “cleft” immediately above the small-celled layer 4 (Fig. 4). This cleft is clearly present up to the lateral border of the V1 region but is absent from the medial third of the area. Our transneuronal tritiated proline studies show that the V1 region containing the cleft receives binocular input, whereas the medial region lacking the cleft represents the monocular crescent receiving input from the contralateral nasal retina. From the transneuronal transport studies, it appears that the cleft region lies directly above the zone of thalamic terminations in layer 4. The cellular and fiber architecture of area V1 of the quokka resembles that of the cat V1 with a relatively narrow, small-celled layer 4 and rather ill-defined laminar boundaries in Nissl-stained material (Fig. 4).

Relative distribution of the V1 cell population to different laminae in marsupials and eutherians

As well as clear differences in overall number of neurons between pia and white matter in any common-diameter column of tissue taken from pia to white matter in V1, there are clear differences in the proportion of neurons that each species distributes to individual cortical laminae (Table 1). There is, however, no indication of an eutherian-marsupial split in terms of the percentage commitment of their neurons to different cortical layers. For example, the cellular distribution in the dunnart closely resembles that of the primate and cat in having a higher proportion of its neurons and neuropil devoted to layers 2-4 and fewer within layers 5 and 6. By contrast, the quokka resembles the rat most closely amongst the eutherians examined in having more cells and neuropil distributed to deep layers (5 and 6) than to superficial layers (2 through 4).

Organization of spine bearing neurons

Neuron varieties. Eutherian cortical neurons in past studies (Jones and Peters, 1984) have been divided into two major groups, i.e., spine-bearing (approximately 80% of cortical neurons) and spine-poor neurons (approximately 20% of cortical neurons). The spine-bearing neurons, composed of stellate and pyramidal forms, represent

Fig. 6. Camera lucida drawings of Golgi-impregnated spiny stellate neurons in layer 4 of (A) dunnart, (B) quokka, and (C) macaque striate cortex (Lund et al., 1977). Scale bar = 50 µm (applies to A–C).
the excitatory neurons of neocortex. Within the cortex, the pyramidal neurons generally make both intra-areal and inter-areal projections, but a small proportion project solely intra-areally. The stellate form is found in primary sensory areas and projects mainly intra-areally and only occasionally between areas.

The spine-poor neurons are almost all GABAergic and usually project locally within cortical areas, rarely between them or out of cortex. Distinct subclasses of these interneurons are defined on the basis of clear differences in axonal morphology as described below in a separate result subsection.

Pyramidal neurons. The general form of the pyramidal neurons is similar across species; the exception is in the quokka in which the pyramidal neuron population divides into two ascending trunks rather than remaining as a single rising apical dendrite. Interestingly, the small size of the dunnart striate cortex has not led to a corresponding overall miniaturization of its constituent neurons. The lateral spread of the dendrites of layer 3 pyramidal neurons of the dunnart averages 245 µm. The lateral spread of the dendrites of layer 3 pyramidal neurons of the macaque averages 240 µm, and of the quokka average 285 µm. For comparison, the lateral spread of rat layer 3 pyramidal dendrites measure 265 µm and those of the cat measure 311 µm. It would, therefore, take only approximately 11 layer 3 pyramidal neuron dendritic field widths to bridge the mediolateral extent of the dunnart V1 region. Layer 6 of both quokka and macaque contains many small pyramidal cells with basal dendritic fields of lateral spread approximately 250 µm; the mean basal dendritic arbor spread of layer 6 pyramids in the dunnart is only 140 µm, thus, generally smaller by a half than those found in the quokka or macaque.

The small to medium-sized pyramidal neurons in layer 6 of dunnart V1 show very precise laminar-specific dendritic arbor distributions, which compare closely with those seen in macaques. Each layer 6 pyramidal neuron has apical dendritic branching in a subset of the overlying layers, e.g., within layer 4, within the cell sparse cleft, or within the overlying layers 2/3. These lamina-specific arbor patterns are also characteristic of the apical dendrites of layer 6 pyramidal neurons in the macaque V1 (see Fig. 5). In the macaque, they are accompanied by equally specific recurrent axon collateral distribution from the same cells to different overlying layers and specific patterns of feedback to different divisions of the thalamic lateral geniculate nucleus that provides the region with its primary input (Lund et al., 1975; Fitzpatrick et al., 1994a; Wiser and Callaway, 1996). The axons of the dunnart layer 6 pyramids did not impregnate, but it is probable that they send recurrent collaterals to overlying layers in patterns as specific as their apical dendritic arbor.

In addition, there are similarities between the marsupials and the macaque in the characteristics of their largest V1 pyramidal neurons. The layer 5 pyramidal neurons of the dunnart are among the largest cells in its area 17.
diameter of the basal dendritic arbor is 350 µm, and the basal dendrites reach down through layer 6 to the white matter, whereas the apical dendritic arbors reach into the superficial layers and include layer 1. In the quokka, large pyramidal neurons in layer 5 have dendrites that sweep down in wide, spreading arbors 700 µm in diameter through layer 6 and into the white matter. These pyramids resemble the giant pyramidal neurons (Meynert cells) with somata lying at the border of layers 5 and 6 in macaque whose basal dendrites have a very similar wide and down-sweeping arbor form, creating dendritic fields averaging 770 µm in diameter (Lund, 1980).

Spiny stellate neurons. In both the dunnart and the quokka the small-celled, thalamic recipient layer 4 of area V1 has a substantial population of spiny stellate neurons (Fig. 6). The dendrites of the spiny stellate neurons in layer 4 spread laterally approximately 200–225 µm in the dunnart and 204–219 µm in the quokka. This dendritic spread is similar to that of the spiny stellate neurons of the macaque thalamic recipient layer 4C (190–210 µm). Larger spiny stellate neurons are present in the macaque's layer 4B whose dendrites spread between 305 and 348 µm (see Lund, 1973); this layer does not receive direct thalamic afferents. In the marsupials, we have found spiny stellate...
neurons to be of fairly uniform size, without impregnations of larger varieties.

The proportion of spiny stellate neurons to pyramidal neurons in layer 4 seen in Golgi Rapid preparations varies between species and between divisions within layer 4. In dunnart Golgi impregnations, spiny stellate neurons considerably outnumber pyramidal neurons in layer 4. The quokka Golgi impregnations have a somewhat higher proportion of pyramidal neurons to spiny stellate neurons than do those of the dunnart and, in this respect, they resemble Golgi impregnations of layer 4 of the eutherian cat area V1. In macaque V1 Golgi impregnations, almost all cells of layer 4C are spiny stellate neurons; in another eutherian, *Tupaia glis*, a tree shrew, this is also true except for one narrow stratum of pyramids in the middle of layer 4 (Lund et al., 1985). As one crosses the border from V1 into the presumed area V2 of the quokka and dunnart (lying immediately lateral and anterior to V1) the small stellate spine-bearing cells in V1 are replaced in the Golgi impregnations by small pyramidal neurons in layer 4. This transition in cell type is also a characteristic of the transition from V1 to V2 in eutherians, and these small pyramids in layer 4 are also a characteristic of eutherian granular association cortex (Lund, 1984).

### Intrinsic lateral connectivity of superficial layer pyramids

After DiI label in quokka V1, we observed evidence for wide-spread pyramidal neuron lateral connectivity in layers 2–3, with punctate zones of terminals and retrograde cell label similar to those found in V1 (Rockland and Lund, 1983; Lund et al., 1993) as well as in other areas of the primate cortex (Fig. 7). In contrast, similar periodic patterns were not observed in the dunnart by using the same technique. The average diameter of the patches of terminals in the quokka is 283 µm, closely comparable to the mean diameter of 319 µm (285 µm before adjustment for differential shrinkage) for single, superficial layer pyramidal dendritic fields. A similar correspondence has been previously recognized for patchy connections in superficial striate cortex of the eutherian species studied to date (Luhmann et al., 1986; Lund et al., 1993). Moreover, in some sections of quokka V1, the patchy terminal zones seems to be arranged in beaded, stripe-like patterns (Fig. 7).

### Interneuron populations

We restricted our attention to the sparsely spined cells that lay in the superficial layers of the marsupials (deeper layer interneurons were not impregnated in sufficient numbers to allow adequate analysis); furthermore, because more Golgi material was available for study in the quokka than in the dunnart, our chances of finding specific interneuron types for comparison with those in macaque was greater in this species than in the dunnart. Because of the vagaries of the Golgi method, the absence of a particular cell type in Golgi impregnations cannot be taken as an indication of its absence in the actual tissue; we are here concerned more with classification of cell types found in the species examined (Fig. 8). The superficial layer interneurons are of particular interest in that in the macaque and cat, at least, some forms appear to have particular spatial scale in relation to the pyramidal neuron connective lattice in the same layers (see Lund et al., 1993; Lund and Wu, 1997).

#### Wide arbor basket neurons.

These neurons are present in the superficial layers of both the dunnart and quokka (Fig. 9). In the quokka, wide arbor basket neurons lie deep in layer 3 and their stout axons generally arise from the superficial aspect of the cell body; the axon's extensive lateral arbor can spread up to 466 µm from the soma, with largest observed total arbor diameter of 855 µm; the diameters of the basket neurons' dendritic fields measure approximately 315 µm. In the macaque striate cortex, similar wide arbor basket neurons are found in layer 4B and deep layer 3; in the cat, they occur in upper layer 4 and deep layer 3. These wide arbor basket cells in macaque V1 have axonal field diameters of 750–800 µm and dendritic field diameters of approximately 350 µm. In the dunnart, the somata of wide arbor basket cells lie in the cleft region or deep in layer 3. The average diameter of their dendritic fields is 250 µm. Their axons do not
Fig. 9. Camera lucida reconstructions of Golgi-impregnated wide arbor basket neurons observed in (A) dunnart, (B) quokka, and (C) macaque striate cortex (Lund and Yoshioka, 1991). Dendritic representation is off-set to the left of the axonal representation of each neuron. Arrowheads indicate the borders of the cortical laminae. Scale bar = 50 µm (applies to A–C).
appear to be evenly distributed around the soma, suggesting the arbor may have radial arms of terminal distribution with gaps between. Their axons generally spread furthest within the cleft region. The average unilateral axon spread from the soma was 249 µm, which, if one assumes that the axon field is symmetric, would produce a potential axonal field of 498 µm. The largest axonal spread observed for wide arbor basket cells in the dunnart was 519 µm, indicating that the absolute cortical range of basket cell inhibition in the dunnart is approximately two-thirds of that in the quokka and macaque. The rat has a similar scale of basket neuron axon spread to pyramidal dendritic spread as the dunnart; rat basket neuron axon spread has a mean of 550 µm and a mean pyramidal neuron dendritic spread of 265 µm in layers 2/3.

**Medium arbor basket neurons.** These cells are present in the dunnart, and although their axons have been observed in the quokka, their cell bodies were not impregnated. These basket neurons are also present in macaque V1. They lie in layer 2 and upper layer 3 and have an axon that arises from the white matter side of the soma that forms stout recurrent trunks that rise and spread to form an axon arbor within upper layer 3 and layer 2 (Fig. 10). In the dunnart, axon field diameters range from 375 to 400 µm; in the quokka, they spread up to 544 µm, and they reach 500 to 700 µm in the macaque. Dendritic fields measured in dunnart extend up to at least 170 µm, and they measure 125–150 µm in the macaque. The dendritic fields of these cells were not adequately impregnated in our quokka Golgi preparations.

**Robust columnar axon cells.** These cells have been identified in the quokka and are a frequent component of the primate superficial layer neuropil in V1 (Fig. 11). They lie in layers 2/3 with a robust axon trunk arising from the superficial aspect of the soma that rises toward the pia, emitting short lateral side branches with large terminal boutons. Cellular dimensions are similar in the quokka and primate with columnar axon arbor widths of 217 µm and 220–350 µm, respectively, and associated dendritic field diameters of 219 µm and 80–250 µm. These cells were not impregnated in our dunnart Golgi material.

**Double-bouquet neurons.** These neurons are present in both dunnart and quokka, and their narrow columnar axons have similar characteristics to similar cells in macaque V1 (Fig. 12). Total lateral spread of the axon fields and dendritic arbors average, respectively, 118 µm and 83 µm in the dunnart, 232 µm and 132 µm in the quokka, and 125 µm and 100 µm in the macaque area V1.

**Neurons with simple beaded axon arbors.** Similar cells are found in this category in both quokka and primate V1 (Fig. 13). The dendritic fields of these cells in the quokka, ranging from 233- to 274-µm across, are more than twice those observed in the macaque where they tend to be vertically oriented with the diameter of the field averaging 100-µm across. The diameter of the axon field of these cells ranged between 200 and 250 µm in the macaque and 170 and 256 µm in the quokka. These cells were not observed in our dunnart Golgi impregnations.

**Chandelier neurons.** These neurons are identifiable by their characteristic axon arbors that contact the initial segments of pyramidal neurons (Somogyi et al., 1982) and are found across many cortical areas in a variety of eutherians. Chandelier neurons are present in both dunnart and quokka visual cortex, although we were unable to reconstruct complete cells in the dunnart. They are particularly evident amongst the small pyramidal neurons of layer 4 in area V2 in the quokka and their axons are seen in a similar region of dunnart cortex. The best examples in the quokka were found in V2 cortex where they had dimensions similar to those of macaque V2 chandelier cells (Fig. 14). The average axon field of a chandelier cell in quokka cortex measured 231-µm across, and the dendritic fields measured approximately 105-µm.
across. In macaque V1, the average axon field measures 150-µm across, and the average dendritic field measures 100-µm across.

Layer 4 type beta 3 neuron. Among the interneuron populations, only a few classes restrict their arbor entirely to layer 4 itself. One of these is the type beta 3 neuron (Lund, 1987); this neuron has an axonal field in the macaque that ranges between 160- and 190-µm wide and a dendritic arbor spread that measures approximately 150-µm across. In the quokka layer 4, a commonly occurring interneuron resembles the primate layer 4C beta 3 neuron (Fig. 15). This cell has an axon field of 160- to 170-µm across and a dendritic field 150- to 160-µm across in the quokka. A similar cell type has also been identified in the dunnart, with axon fields measuring between 92- and 142-µm across accompanied by dendritic fields ranging between 146- and 179-µm across.

Layer 1 neuron. The cell sparse layer 1 of the adult primate has only one cell type that occurs with any frequency. This neuron has a characteristic fine, densely

Fig. 11. Camera lucida drawings of Golgi-impregnated robust columnar cells observed in quokka (A) and macaque (B) striate cortex (Lund and Yoshioka, 1991). For clarity, dendritic fields have been off-set to the right of their associated axonal fields, as indicated by large arrowhead in A, which links the quokka robust columnar dendritic field with its associated axonal field. Small arrowheads indicate the borders of cortical laminae. Scale bar = 50 µm (applies to A,B).
ramifying axon (see Lund and Wu, 1997). The axon field of these cells spreads laterally 300–350 µm, slightly wider than the dendritic field of the cell that spreads 250–300-µm across. Like the primate, a single cell type was impregnated in layer 1 of our quokka tissue (Fig. 16). Although slightly smaller in scale than the primate's layer 1 cell, this quokka cell type had similar features with an axon field averaging 233-µm across and a dendritic field of approximately 113-µm across. These neurons were not impregnated in our dunnart material.

DISCUSSION

It is clear from previous work (Krubitzer, 1995) that there has been considerable conservation of cortical areal characteristics. This study has carried such a comparison to a cellular level and has found considerable similarity of neuron form and connectional architecture between the marsupials and macaque monkey V1. These similarities exist despite the evolutionary divergence of these animals from a common ancestral stock many millions of years ago and, as indicated in the introduction section, despite having very different lifestyles and a 200-fold difference in surface area of V1. We suggest, therefore, that commonalities reflect conservation of ancient structural elements essential to area V1 function in the macaque.

Common neuronal elements

Pyramidal neurons. The pyramidal neuron is certainly the principal constituent of both the eutherian and marsupial cortex; but it is clear that modest variation in its form exists. An example is the prevalence of pyramids with double apical dendrites in the quokka. By comparison, in the other species examined there is generally a single process, particularly in the monkey where bifurcated apical dendrites are rare. The presence of pyramids with double apical dendrites in the protherian echidna (Dann and Buhl, 1995) and lizard dorsomedial cortex (Ulinski, 1979) suggests that this pyramidal form is archaic, and perhaps the single apical dendrite, as seen in many mammalian species, is an adaptation from that form. Although the presence of double apical dendrites is infrequently represented in mammalian cortex today, its retention in some species and not in others suggests a selection pressure at play.

Fig. 12. Camera lucida drawings of double-bouquet neurons observed within the superficial striate cortex of (A) dunnart, (B) quokka, and (C) macaque (Lund and Wu, 1997) after Golgi impregnation. Dendritic fields are to the left of their associated axonal fields, as indicated by the large arrowhead in B, which links the quokka double-bouquet neuron's columnar dendritic field with its associated axonal field. Small arrowheads indicate the laminar boundaries. Scale bar = 50 µm (applies to A-C).
Spiny stellate cells and their presence in mid-depth, thalamic recipient cortical laminae. The presence of spiny stellate neurons in thalamic recipient layer 4 appears to be a basic characteristic of primary visual cortex in at least terrestrial mammals. Their presence in layer 4 of other primary sensory regions of cortex has also been noted (see Lund, 1984). These neurons project primarily to the more superficial layers (Lund et al., 1979; Fitzpatrick et al., 1985). The proportion of spiny stellate neurons to pyramidal neurons with somata within layer 4 of visual cortex varies between species. The absence of layer 4 pyramidal neurons with apical dendrites entering the superficial layers may allow sharper differences in physiologic properties to exist between layer 4 and the superficial layers (as in the macaque monkey; see for example Ringach et al., 1997), because the thalamic recipient pyramidal neurons in layer 4 can sample activity in the superficial layers by means of their apical dendrites (as in cat, where neurons of layer 4 share most of the properties shown by neurons in more superficial layers). We would predict on this basis that the dunnart layer 4 cells (largely spiny stellate neurons) are likely to differ in their physi-
ologic properties from the superficial layer cells, whereas quokka layer 4 cells (including many pyramidal as well as spiny stellate neurons) should show a closer match in properties to the neurons in the superficial layers of its area V1.

Common sparsely spined interneuron types. We have demonstrated in previous studies that widely separated areas of the primate cortex (V1 and prefrontal areas 9 and 46) have common forms of interneurons (Lund and Yoshioka, 1991; Lund and Lewis, 1993). The present study shows that many of these same cells can be readily recognized in mammals of widely disparate phylogeny and behavior, arguing for an essential role throughout mammalian cortical evolution. The superficial layers of cortical area V1 in the dunnart, quokka, and macaque consistently contain wide and medium arbor basket cells, double-bouquet (or bipolar) cells, chandelier cells, and, at least in the quokka and macaque, layer 1 cells and robust columnar cells. These neuron types are also found in the primate prefrontal cortex, suggesting that they are universal cortical elements. We suggest, therefore, that these cell types represent basic key elements of the mammalian neocortex as a whole.

The several different varieties of layer 4 interneuron types in macaque V1 (see Lund, 1987), and to a lesser extent in V1 of other eutherians such as cat (Lund et al., 1979) and tree shrew (Lund et al., 1985) were not apparent in our marsupial Golgi impregnations. Only the 4β-3 cells of the primate were detected in layer 4 of the dunnart and quokka, suggesting at least this variety may play an essential role. However, because of the limited availability of Golgi impregnations of the marsupial material, as well as the variable nature of Golgi impregnations, we are not able at this time to determine whether any more varieties of V1 layer 4 interneuron are present in the dunnart and quokka.

Conservation of cortical microcircuitry

Projections of superficial pyramidal neurons. The presence of patchy pyramidal neuron connectivity in the
superficial layers of the quokka as well as V1 and other
cortical areas of the macaque (see Rockland and Lund,
1983; Juliano et al., 1990; Huntley and Jones, 1991; Lund
et al., 1993; Malach et al., 1994) and its general presence in
the neocortex of other eutherians (Rockland and Lund,
1982; Ts’o et al., 1986; Matsubara and Phillips, 1988; Boyd
and Matsubara, 1991) suggests that this connectional
strategy is also an ancient and key component of neocorti-
cal anatomical organization. This organization may contrib-
ute to the sharpening of functional tuning by means of
recurrent connections of cortical circuitry (Douglas et al.,
1995), because pyramidal patch systems are thought to
link cortical points that are functionally similar, for ex-
ample joining points having similar orientation prefer-
ences in the striate cortex (Malach et al., 1993; Fitzpatrick
et al., 1994b; Fitzpatrick, 1996; Yoshioka et al., 1996).

There is a close match in scale between the lateral
spread of the dendritic arbor of individual layer 2–3
pyramidal neurons and the pyramidal neuron lateral axon
collateral connectional patch diameter in eutherian V1
and across different areas of the macaque cortex, where
size of terminal patch and pyramidal neuron size co-vary
and match in size (Lund et al., 1993). The scales of
pyramidal neuron dendritic field and connectional patch
also match well in quokka. The functional implications of
the match of pyramid size to the size of the patchy terminal
fields is uncertain, yet is of considerable interest in connec-
tional theories of how cortex functions and how particular
functions are distributed across the cortical sheet (for
discussion see Lund et al., 1993).

These superficial patchy projections are apparently not
invariably present, as evidenced by their absence in the rat
(Tyler and Lund, 1996) and dunnart, the eutherian and

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Fig. 15. Camera lucida drawings of layer 4 β-3 neurons observed in
the striate cortex of (A) dunnart, (B) quokka, and (C) macaque (Lund,
1987). Dendritic fields for each neuron are to the left of the axonal
fields, and arrowheads indicate the borders of the indicated cortical
laminae. Scale bar = 50 µm (applies to A–C).

Fig. 16. Camera lucida reconstructions of Golgi-impregnated layer
1 neurons of (A) quokka and (B) macaque striate cortex (Lund and Wu,
1997). For each neuron, dendritic fields are located to the left of the
respective axonal fields. Arrowheads indicate the limits of the
indicated cortical laminae. Scale bar = 50 µm (applies to A,B).
The pyramid neuron may relate to the specific function of their largest layer 5/6 pyramids in comparison with other cortical elements. This finding suggests that the functional dynamics of the slighlty larger size than those of the macaque monkey V1. pyramidal neurons of the dunnart V1 are of similar or even remarkably little in absolute size. For example, the layer 3 depth of V1 among the species examined, but the individual pyramidal cells of the superficial cortex vary surprisingly little in absolute size. For example, the layer 3 pyramidal neurons of the dunnart V1 are of similar or even slightly larger size than those of the macaque monkey V1. This finding suggests that the functional dynamics of the pyramidal neuron may need it to be of particular size, either for appropriate biophysical function or to be in scale with other cortical elements. Interestingly, the pyramid neuron can vary considerably in size within a species, as evidenced by comparing the size of the largest pyramids, in layer 5 or 6, with those of layer 3 in any species examined. The dunnart shows a 0.42-fold, the quokka shows a 2.4-fold, and the macaque shows a 3.2-fold increase in basal dendritic field diameter in their largest layer 5/6 pyramids in comparison with their layer 3 pyramids. Within specific layers, the scale of the pyramid neuron may relate to the specific function of the cell, as is the case in the macaque in which the giant pyramidal cells of layer 6 (Meynert cells) have different projections than do the smaller pyramid cells of the same layer. In the macaque these large (770-µm dendritic diameter) layer 6 pyramids project to area MT and superior colliculus (Lund et al., 1975; Fries et al., 1985), whereas smaller (200- to 300-µm dendritic spread) layer 6 pyramids project to lateral geniculate nucleus and claustrum (Lund et al., 1975; Fitzpatrick et al., 1994a).

Within single species, similar layers in different areas display different pyramidal neuron sizes such as in monkey motor cortex where layer 3 pyramidal neurons are almost twice the size of those within striate cortex layer 3. The smallest pyramids in any species are generally those of the "granular" layer 4 of cortical association regions including granular association prefrontal cortex (e.g., 160- to 180-µm dendritic diameter in macaque V2). Moreover, some species, for instance the cat, can have pyramidal neurons all across the cortex almost 50% greater in size than pyramids in the primate or marsupials examined here. This difference leaves open to debate the factors that determine the dendritic arbor size of pyramid neuron populations and the absolute limits of the cortical pyramidal neuron size range. As with the pyramidal neurons, the absolute dendritic field sizes of the spiny stellate neurons of thalamic recipient layer 4 vary little between marsupials and eutherians. There is, however, one population of spiny stellate neurons in macaque layer 4B, not postsynaptic to thalamic input, that has large dendritic fields spreading 325–375 µm, almost twice the distance of those in layer 4C. This finding reveals that it is possible for spiny stellate neurons to differ in size and that different constraints may determine their dimensions.

The absolute size of the sparsely spined interneurons and their axon fields in V1 differs little between V1 of different species except for the spread of the axons of the large basket neurons. In the quokka, the basket neuron axon spread is approximately three times the dendritic arbor size of the local pyramidal neurons and connectional patch size; this relationship has also been observed for V1 and prefrontal cortex of the macaque and is also true for the relative scale of giant basket neurons, patch size, and pyramidal neuron arbor size of the cat V1 superficial layers. In the tree shrew, we have found basket neuron axons to reach up to 370-µm laterally from their somata (therefore, with a potential total spread of 740 µm), and this value is again approximately 3 times the mean spread of their superficial layer pyramidal neuron dendrites (244 µm) or connectional patch size (230 µm). These matches in scale between basket neuron axon fields and pyramidal dendritic arbors suggest a functional relationship between these anatomical features and support a suggestion we made earlier (Lund et al., 1993, 1995), i.e., that the basket neuron axon may create an inhibitory field around active cortical points. These inhibitory fields could then prevent pyramidal neurons' lateral axon collaterals establishing terminals within the field of suppressed activity; during maturation of the connectional system, this could lead to the development of a spaced patch system of connections. An important scaling factor could be the absolute arbor size of the basket neuron, measuring three times that of the single pyramidal neuron dendritic arbor (see Lund et al., 1993). The narrower axon fields of dunnart and rat basket neurons may preclude development of stepping connections in the striate cortex of these species and...
indicate that different scaling factors can exist for cortical inhibition.

Species-specific variations

A basic organizational plan of V1 cortical organization that has been preserved, as evidenced by common neuronal elements and microcircuitry, has not been immune to independent changes within the eutherian and marsupial lines. Differences in overall V1 area, the depth and stratification of cortex, the percentage of cells in individual cortical lamina in V1, the proportion of pyramidal neurons to spiny stellate neurons in layer 4, the stratification of layer 4 in relation to incoming afferents and with accompanying elaboration of interneuron and spiny cell circuitry projections within layer 4 and from it to other layers, and the precise patterns of stratification of the processes of layer 6 pyramids in relation to the overlying layer 4 vary among species. These variations suggest that species-specific differences in cortical functional circuitry may be built on to a basic framework of cortical organization.

It is likely that the scale of functional representation in the input layers is determined by the thalamic axon arbor size and overlap factor set against the spread of the postsynaptic neurons’ dendrites, both excitatory and inhibitory; these elements of scaling will determine patterns of function postsynaptically (see Lund et al., 1995; Bauer et al., 1998). Anatomical scale of presynaptic and postsynaptic elements is likely to be equally important in functions generated by further intracortical relays such as those discussed above.

CONCLUSIONS

The presence of common connectional strategies and morphologic classes of both spine-bearing neurons and interneurons in V1 of marsupials and macaque, together with the occurrence of nearly all these elements in macaque prefrontal cortex, suggests that these are highly conserved, essential anatomical features of cerebral cortex as a whole, not just of visual cortex. It would seem important, therefore, that this fundamental microstructure, with suitable scaling, must be incorporated in developing models of cortical functional organization, including primary visual cortex.

LITERATURE CITED


