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Organization of visual cortex in the northern quoll, *Dasyurus hallucatus*: evidence for a homologue of the second visual area in marsupials

Marcello G. P. Rosa,[1] Leah A. Krubitzer,[1,2] Zoltán Molnár[3] and John E. Nelson[4]
[1]Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, The University of Queensland, QLD 4072, Australia
[2]Center for Neuroscience, University of California at Davis, Davis, CA 95616, USA
[3]University Laboratory of Physiology, Oxford University, Parks Road, Oxford OX1 3PT, UK
[4]Department of Biological Sciences, Monash University, Clayton, VIC 3168, Australia

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Abstract

Two visual areas, V1 and V2 (first and second visual areas), appear to be present in the posterior neocortex of all eutherian mammals investigated so far. However, previous studies have not established whether an area homologous to V2 also exists in metatherian mammals (marsupials). Using electrophysiological techniques, we mapped the visual receptive fields of neurons in the striate and peristriate cortices of the northern quoll, an Australian marsupial. We found that neurons in a 2-mm-wide strip of cortex rostrolateral to V1 form a single, relatively simple representation of the complete contralateral hemifield. This area resembles V2 of eutherians in several respects: (i) neurons in the medial half of the peristriate area represent the lower visual quadrant, whereas those in the lateral half represent the upper visual quadrant; (ii) the vertical meridian of the visual field is represented adjacent to V1, while the visual field periphery is represented along the lateral and rostrolateral borders of the peristriate area; (iii) there is a marked anisotropy in the representation, with a larger magnification factor parallel to the V1 border than perpendicular to this border; and (iv) receptive fields of multiunit clusters in the peristriate cortex are much larger than those of cells in V1 at comparable eccentricities. The cortex immediately rostral and lateral to V2 did not respond to visual stimulation under our recording conditions. These results suggest that V1 and V2 together form a 'core' of homologous visual areas, likely to exist in all therian mammals.

Introduction

The visual cortex of eutherian mammals consists of multiple areas which can be distinguished on the basis of criteria, such as connections, cortical architecture, visuotopy and neuronal response properties. There are obvious variations regarding the size, complexity and diversity of areas among species belonging to different mammalian orders (e.g. Krubitzer, 1995). At one extreme, the small neocortex of insectivores, e.g. hedgehogs and tenrecs, is reported to have only two or three visual areas (Kaas *et al*., 1970; Krubitzer *et al*., 1997). At the other extreme, the primate cortex may have more than 30 such areas (Rosa, 1997). In spite of these differences, some unifying features are likely to exist that are common to all eutherian mammals. For example, it has been argued that two areas, V1 and V2 (first and second visual areas), form a 'core' of homologous fields which can be recognized in every eutherian (Kaas & Krubitzer, 1991; Rosa *et al*., 1994). Because of their widespread distribution, it is reasonable to propose that these areas were already present in the first eutherians.

The present study tests the hypothesis that an area homologous to V2 also exists in metatherian mammals (marsupials). Although many previous studies have investigated the organization of V1 in marsupials with electrophysiological techniques (e.g. Rocha-Miranda *et al*., 1976; Sousa *et al*., 1978; Volchan *et al*., 1988; Vidyasagar *et al*., 1992), the organization of the cortex rostrolateral to V1, in the expected location of V2, remains unknown. Both Sousa *et al*. (1978), in the opossum, and Vidyasagar *et al*. (1992), in the wallaby, reported that neurons in this region are responsive to visual stimulation. However, both studies also noted that neuronal responses were often difficult to elicit, and that cells had quite specific stimulus requirements (e.g. stimuli much larger and faster moving than those that optimally drive V1 cells). Perhaps because of the difficulty in obtaining reliable responses, the visuotopic organization of rostrolateral peristriate cortex in marsupials has never been determined. In addition, anatomical investigations of this problem have not been particularly helpful. Although the projections of V1 to peristriate areas are reported to follow a rough mediolateral gradient, as would be expected if V2 were present, they are also widespread, making the interpretation of results difficult (e.g. Benevento & Ebner, 1971; Crewther *et al*., 1984). Indeed, a study of intra- and interhemispheric connections of V1 in the mouse opossum (Bravo *et al*., 1990) argued against the existence of a single area homologous to V2 in rostrolateral peristriate cortex. Instead, these authors proposed the existence of multiple areas in the expected location of V2.

Thus, it is not clear whether the extrastriate cortex in extant marsupials resembles that observed in eutherians with simple brains (e.g. insectivores), or has a different organization. Of particular

Correspondence: M. G. P. Rosa, as above. E-mail: M.Rosa@vthrc.uq.edu.au

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importance is whether the cortex rostrolateral to V1 comprises multiple visual field representations (and therefore multiple areas), or is a single, elongated area, which represents the contralateral hemifield in a systematic manner. Only if the latter hypothesis is supported could one argue that a homologue of V2 exists in marsupials. We have addressed this issue by means of intracortical electrophysiological recordings in a nocturnal, carnivorous marsupial, the northern quoll.

Materials and methods

Anaesthesia and surgical preparation

Four adult female quolls (*Dasyurus hallucatus*, also known as the 'native cat'), weighing between 600 and 800 g, were used in acute recording sessions. The animals were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Nembutal, Boehringer Ingelheim, Artarmon, NSW, Australia, 50 mg/kg). Injections of dexamethasone (Dexadreson, Intervet, Boxmeer, Holland, 0.4 mg/kg i.m.) and atropine sulphate (Apex Laboratories, St Marys, NSW, Australia, 0.15 mg/kg i.m.) were administered immediately thereafter. Throughout the surgery, additional intraperitoneal doses of sodium pentobarbitone (10 mg/kg) were used to maintain a deep level of anaesthesia, which was evaluated by monitoring the leg withdrawal and corneal reflexes. Additional doses were usually required at 30 min intervals for the first 1–2 h of the experiment, after which the animal's anaesthetic level stabilized.

After being tracheotomized, the animal was placed on a thermostatically controlled heating pad, with its head positioned in a stereotaxic frame. Craniotomies 7–10 mm in diameter were made over the right hemisphere, allowing access to the full extent of V1 and adjacent areas. An acrylic well was constructed around the craniotomy, being secured to the skull by orthopaedic screws. A rod attached to an adjustable arm (mounted on the stereotaxic frame) was positioned over the frontal midline, and fixed to the acrylic well. This arrangement allowed the head to be supported without the need for stereotaxic bars, and offered an unhindered field of vision. The well was then filled with silicone oil, and a picture of the cortical surface was taken for plotting of electrode penetration sites.

After all surgical procedures were finished, the animal was allowed to breathe a gaseous anaesthetic mixture (0.3–0.5% halothane, in a 70% N_2O/30% O_2 mixture), which was used for maintenance throughout the recording period. As the animals were not paralysed, the level of anaesthesia was monitored by testing the withdrawal reflexes. In addition, a virtual oscilloscope system (MacLab 8, Analog Digital Systems, Castle Hill, NSW, Australia) was used to monitor the electromyographic and electrocardiographic activity. A thermostatically controlled blanket was used to maintain the animal's temperature near 38 °C.

Protection of the cornea and control for eye movements

To stabilize the position of the left eye (contralateral to the experimental hemisphere), a stainless steel ring was sutured to the sclera. This ring was connected to a thin metal rod, which was then cemented to the head holder, immobilizing the eye (Allman & Kaas, 1971). Fiducial permanent ink marks, made on the sclera prior to suturing the ring, were used to ensure that the horizontal meridian of the eye was not rotated relative to its 'resting' position (i.e. with relaxed extraocular muscles) during the process of cementing the ring. The eye ring was found to stabilize the position of the eye throughout the experiments (within 1–2 °, as evaluated by repeatedly plotting the neuronal receptive field of a site in the central representation of V1). Although stabilization of the eye was not perfect, the margin of error is small relative to the size of cortical receptive fields in the quoll; thus, it does not affect the conclusions of the present study. On the other hand, eye stabilization with a metal ring enables a much easier assessment of the animal's level of anaesthesia, by obviating the need of muscle relaxants. Atropine (1%) and phenylephrine hydrochloride (10%) eye drops (Chauvin Pharmaceuticals, Harold Hill, Essex, UK) were used to produce mydriasis and cycloplegia, and a thin layer of silicone oil (350 CSI) was used to protect the cornea from desiccation. The positions of the vertical and horizontal meridians of the visual field were estimated based on the relationship between the blind spot, the peak ganglion cell distribution, and the visual streak in the quoll retina (Harman *et al*., 1986). The location of the centre of the blind spot was projected onto the hemispheric screen using a reversible ophthalmoscope.

Electrophysiological recordings: equipment and procedures

Low impedance tungsten-in-glass microelectrodes (0.8 MΩ at 1 kHz; Microprobe, Clarksburg, MD, USA) were inserted following a trajectory nearly normal to the cortical surface. Amplification and filtering of the electrophysiological signal was achieved via an AM Systems Model 1800 Microelectrode AC amplifier (AM Systems, Everett, WA, USA) and a 50 Hz eliminator (HumBug, Quest Scientific, Vancouver, Canada). Because the emphasis of the experiments was on plotting the neural receptive fields at as many sites as possible, rather than studying response properties in detail, we made no effort to isolate single units at each point. Instead, we relied on the use of loudspeakers and an oscilloscope to monitor the responses, and plotted the receptive fields of multiunits as well as field potentials. To characterize the visuotopic organization, luminous white spots (5–15 ° in diameter) and bars (typically 15–20 ° long, 2 ° wide) were moved on the surface of a translucent hemispheric screen 40 cm in diameter, via a hand-held projector. The bars were used when the cell cluster was deemed to be orientation or direction biased; otherwise, the spots were preferred. The screen was centred on the contralateral (left) eye, and positioned in such a way as to afford stimulation of 100 ° of the visual angle in the contralateral hemifield, at the level of the horizontal meridian. Electrolytic lesions (5 μA, 10 s) were placed in many penetrations, in order to allow histological location of the recording sites.

Histology

At the end of the experiment, reference probes (100 μm in diameter) were placed in selected cortical sites, in order to generate fiducial marks for alignment of histological sections. The animal was then given a lethal dose of sodium pentobarbitone (200 mg/kg, i.p.) and perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, and 4% paraformaldehyde/10% sucrose in phosphate buffer. Once the brain was removed from the skull, the cortex was dissected and flat-mounted between glass slides, according to previously described procedures (Huerta *et al*., 1987; Rosa *et al*., 1988). Alternate series were stained for myelin (Gallyas, 1979) and cytochrome oxidase (Wong-Riley, 1979). By aligning the blood vessels, probe marks and tissue outlines throughout the entire series of sections, results from electrophysiological recordings were superimposed on histologically processed tissue, and in this way were correlated with architectonic boundaries.

Results

The main conclusions of the present study are summarized by Fig. 1. As described previously (Krubitzer, 1995), in the quoll the 'primary'

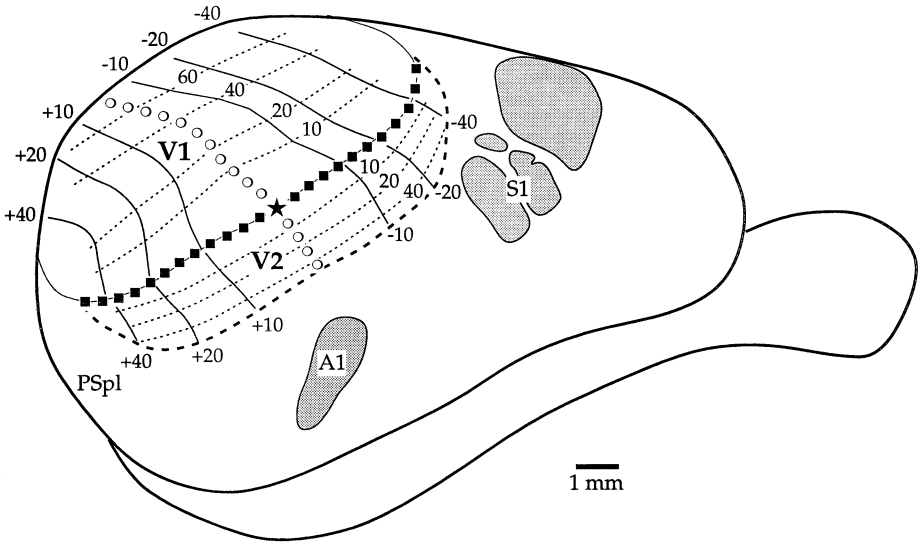

FIG. 1. Summary view of the cortical organization of areas V1 and V2 in the quoll. Dorsolateral view of the right cerebral hemisphere of a quoll, where the locations of the putative area A1 and S1, and the visuotopic organization of V1 and V2, are indicated. The representation of the area centralis is indicated by the star, that of the vertical meridian (together with a narrow strip of ipsilateral hemifield) by black squares, and that of the horizontal meridian by the white circles. Thin dotted lines are isoazimuth lines, and thin continuous lines are isoelevation lines. The border between V1 and V2 is based on both myeloarchitecture and physiological recordings, whereas the rostral border of V2 (coarse dashed line) is based solely on physiological recordings. Area S1 is formed by islands of high myelination, which interdigitate with a less myelinated somatosensory area (caudal somatosensory field); the putative A1 has uniformly high myelination. PSpl: posterolateral peristriate area.

sensory cortical areas (somatosensory, S1, and visual, V1, as well as the presumptive auditory cortex, A1) can be easily delimited in flat-mounted preparations stained for cytochrome oxidase or myelin. Of particular relevance for this study is the fact that the lateral border of V1 is sharply defined (Fig. 2), by virtue of this area staining very darkly in comparison with the adjacent cortex in both preparation types. This allowed an unambiguous assignment of recording sites to either striate or peristriate cortex. The visuotopy of V1 yielded no surprises, resembling that described for other marsupials (Sousa *et al*., 1978; Vidyasagar *et al*., 1992; Beck *et al*., 1996) as well as plesiomorphic eutherians, e.g. the hedgehog (Kaas *et al*., 1970). Either bars or moving circles were effective in eliciting visual responses from V1 cells, and the receptive fields had sharply defined borders.

Confirming a previous report (e.g. Sousa *et al*., 1978), we found that flashing or stationary patterns and bars are not effective in eliciting responses from cells in the cortex adjacent to V1. However, by using large (> 5 °) moving circles, clearly defined receptive fields could be plotted on the basis of either multiunit activity or field potentials. A particularly effective way of delimiting the receptive fields was by introducing quick, low amplitude ('jerky') movements of the stimulus. By sampling a large number of closely spaced recording sites in this way, we were able to determine that the receptive fields of neurons in the peristriate cortex of the quoll form a single representation of the visual field, similar to area V2 of eutherians. As shown in Fig. 1, V2 in the quoll forms a strip 1.5–2 mm wide and 8–9 mm long, bordering the representation of the vertical meridian in V1. The lower quadrant of the contralateral hemifield is represented rostral and medially in V2, and the upper quadrant caudal and laterally, each receiving a roughly equal representation (in terms of cortical surface) within V2. Crossing V2 from its border with V1 to its rostrolateral border results in a systematic displacement of receptive fields towards the visual field periphery. However, the visual field representation summarized in Fig. 1 is schematic in at least two ways. First, because of the large receptive fields and low magnification factor, imaginary 'lines' in the visual field, e.g. the horizontal meridian, do not project to discrete 'lines' of representation, but to 1–2-mm-wide strips of cortex. Second, while the caudomedial border of V2 always corresponds to the representation of the vertical meridian, not every sequence of recording sites across V2 results in receptive fields that reach the far periphery of the visual field. Thus, field discontinuities similar to those reported in other mammals (e.g. Albus & Beckmann, 1980) are likely to form the rostrolateral border of V2.

Neurons at sites immediately rostral to the lower quadrant representation in V2 did not respond to the visual stimuli we employed, but in some cases yielded responses to taps to the forepaw skin. This region probably corresponds to the caudal somatosensory field described in the quoll (Krubitzer, 1995). In contrast, recording sites rostral and lateral to the representation of the horizontal meridian and upper quadrant were not obviously responsive to either somatosensory or visual stimuli.

Figure 3 illustrates the data collected in one animal (NC12). The correlation between recording sites and receptive field centres of V1 neurons (indicated by the black circles) demonstrates the presence of a first-order representation of the visual field. The lower quadrant is represented rostrally in V1 (e.g. Fig. 3, fields 1–12), the upper quadrant caudally (e.g. fields 30, 36 and 40–42), the vertical meridian laterally (e.g. fields 12, 21, 29, 35, 39, 40 and 42) and the visual field periphery medially (e.g. fields 1–4). A separate representation of the visual field is present in V2 (recording sites indicated by white circles). The representation of the area centralis in V2 (fields 57 and 64) is immediately adjacent to that in V1 (field 39). Moreover, the locations of the representations of the upper (e.g. fields 64, 68 and 74–75) and lower (e.g. fields 44–47 and 52–56) quadrants in V2 parallel those in V1. However, there is an increase in receptive field size, as well as a reversal in the receptive field sequence, as the V1/V2 border is crossed (compare, e.g. fields 30–35 in V1 and 52–56 in V2). The azimuth of neuronal receptive fields in V2 increases gradually with distance from the V1 border, with the extreme periphery of the visual field being represented at the rostrolateral border of V2.

These observations are summarized in Fig. 4, which illustrates the visuotopic organization of V1 and V2 in this animal. As V2 neuronal receptive fields are larger than those of V1 (Fig. 3), it is far more difficult to draw idealized 'isoazimuth' and 'isoelevation' lines in the cortex for V2. Nonetheless, the representation of the visual field in this area is quite regular, without any evidence of repetition beyond that expected on the basis of the point-image size (McIlwain, 1976). As shown in Figs 1 and 4, the representation of the visual field in V2 differs from that in V1 in that it is markedly anisotropic. Thus, a similar distance in the visual field, in degrees, is represented by a distance roughly twice as long in the cortex parallel to the V1/V2 border as perpendicular to this border. For example, compare the

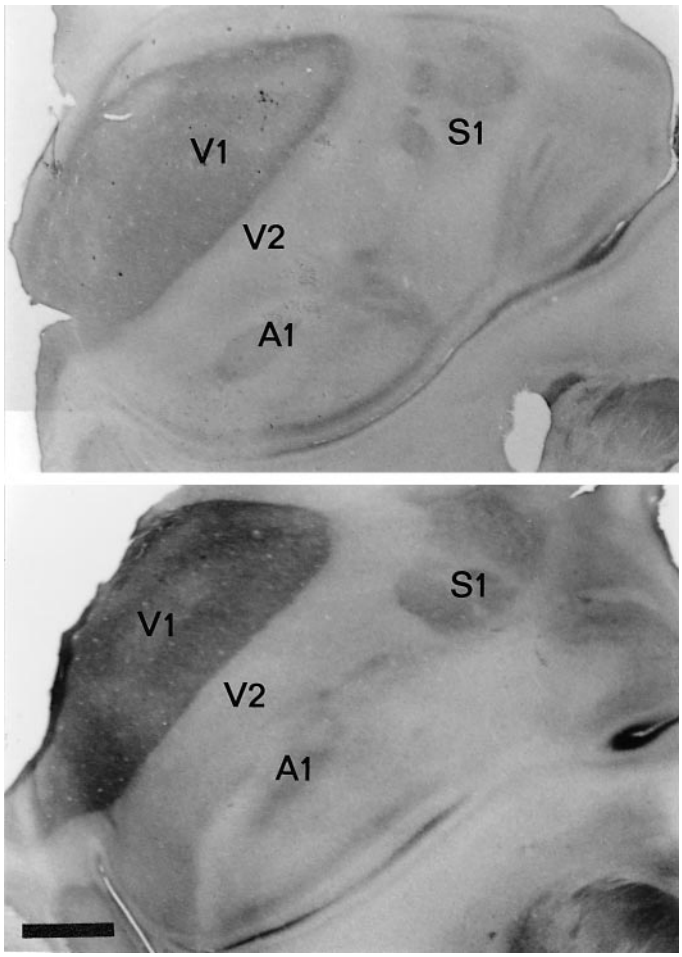

FIG. 2. Cytochrome oxidase (upper) and myelin (lower) architectures of the neocortex in quolls. Medial is to the top, and caudal is to the left of each panel. These are low-power digital images of sections cut tangential to the surface of the cortex, captured via a CCD camera and Scion Image 1.55 software. The clear demarcation of the boundary between areas V1 and V2 is illustrated, as well as the locations of S1 and the putative A1. Scale bar (bottom left) = 2 mm.

distance between the representations of the horizontal meridian (circles) and the + 10 ° isoelevation line, with that between the representations of the vertical meridian and the 10 ° isoazimuth line (Fig. 1).

The receptive fields of V2 clusters in case NC12 demonstrate a complete (or nearly so) representation of the lower visual quadrant, but do not extend into the periphery of the upper visual quadrant. This observation merely reflects the incomplete sampling of the caudal sector of V2 in this particular animal. To prove this point, we illustrate data from another animal (NC13), in which we concentrated recordings in the caudal sector of V2 (Fig. 5). An extensive representation of the upper contralateral quadrant was observed. Although we were unable to stimulate neurons representing the extreme periphery of the visual field (> 100 °), the data shown in Figs 3 and 5 indicate that the representation of the visual hemifield in V2 is at least as complete as that in V1, including eccentricities of up to 100 ° along the horizontal meridian.

In many mammals, V2 forms a second-order representation of the visual field, usually including field discontinuities along the visual field periphery (see Rosa *et al*., 1994 for a review). For example, in monkeys, the discontinuity corresponds to the representation of the horizontal meridian, whereas in cats, flying foxes and prosimians, the discontinuities usually occur along the peripheral representation of the lower quadrant (Albus & Beckmann, 1980; Rosa *et al*., 1997). In some cases (e.g. Fig. 3, sites 52–56), cells at the lateral border of V2 had receptive fields away from the visual field periphery; thus, small field discontinuities are likely to exist. However, the present sample was not sufficient to reveal whether the field discontinuities occur in constant locations, as in primates and flying foxes, or vary between individuals, as in cats.

Discussion

The main result of the present study is that a visual area, forming a single complete representation of the visual field, exists in the peristriate cortex of a marsupial. The similarities in location, shape, visuotopic organization and myeloarchitecture suggest that this area is a homologue of area V2 of eutherian mammals (e.g. Kaas *et al*., 1970, 1989; Hall *et al*., 1971; Tiao & Blakemore, 1976; Rosa *et al*., 1994). Our results, together with those of previous studies that observed visually responsive neurons in the peristriate cortex of marsupials and monotremes (e.g. Lende, 1969; Magalhães-Castro & Saraiva, 1971; Sousa *et al*., 1978; Krubitzer *et al*., 1995; Beck *et al*., 1996; Krubitzer, 1998) indicate that a homologue of V2 probably existed in the 'stem' mammalian group that gave rise to both metatherians and eutherians. According to this hypothesis, differences between species, e.g. the expansion and subdivision of V2 into stripe-like compartments in primates (Tootell *et al*., 1983; Rosa, 1997), or the reduction in size of V2 in rats (where it is likely to correspond to the 'lateromedial area', LM; Montero, 1993; see detailed discussion below), reflect modifications of a basic plan. The ubiquity of the V1/V2 organization among eutherian mammals has also been argued from the point of view of developmental mechanisms of formation of thalamocortical connections (Adams *et al*., 1997).

Is V2 present in all therian mammals?

The existence of a homologue of area V2 in some eutherian mammals has been questioned, particularly with reference to work in rodents. The lack of a V2 homologue in such a numerous group of animals would create problems for the idea that V2 is part of a shared 'core' group of visual areas, present in early eutherians, and likely to be inherited by all extant eutherian species. However, as discussed in detail previously (Rosa *et al*., 1994), the case against a V2 homologue in rodents is not compelling. The main argument is based on the fact that multiple visual field representations have been described in the cortex lateral to V1 of rats and hamsters, where only V2 was expected to exist (e.g. Montero *et al*., 1973; Olavarria & Mendez, 1979; Espinoza *et al*., 1992). These areas were also found to have different patterns of connections (Coogan & Burkhalter, 1993). Yet, other studies have failed to replicate the observation of multiple visual field representations in the lateral extrastriate cortex. Instead, they support the existence of V2 in rodents (including rats and hamsters), based on either physiological (e.g. Hall *et al*., 1971; Tiao & Blakemore, 1976; Wagor *et al*., 1980; Sereno *et al*., 1991; Paolini & Sereno, 1998) or anatomical (e.g. Kaas *et al*., 1989; Malach, 1989) data. Given the small size of the proposed 'areas' surrounding V1 (in some cases, 1 mm^2 or less), it is not surprising that the data are subject to different interpretations.

Perhaps more relevant for the present discussion is the fact that, even if future studies come to support the idea that the cortex lateral to V1 is formed by multiple areas, this does not logically

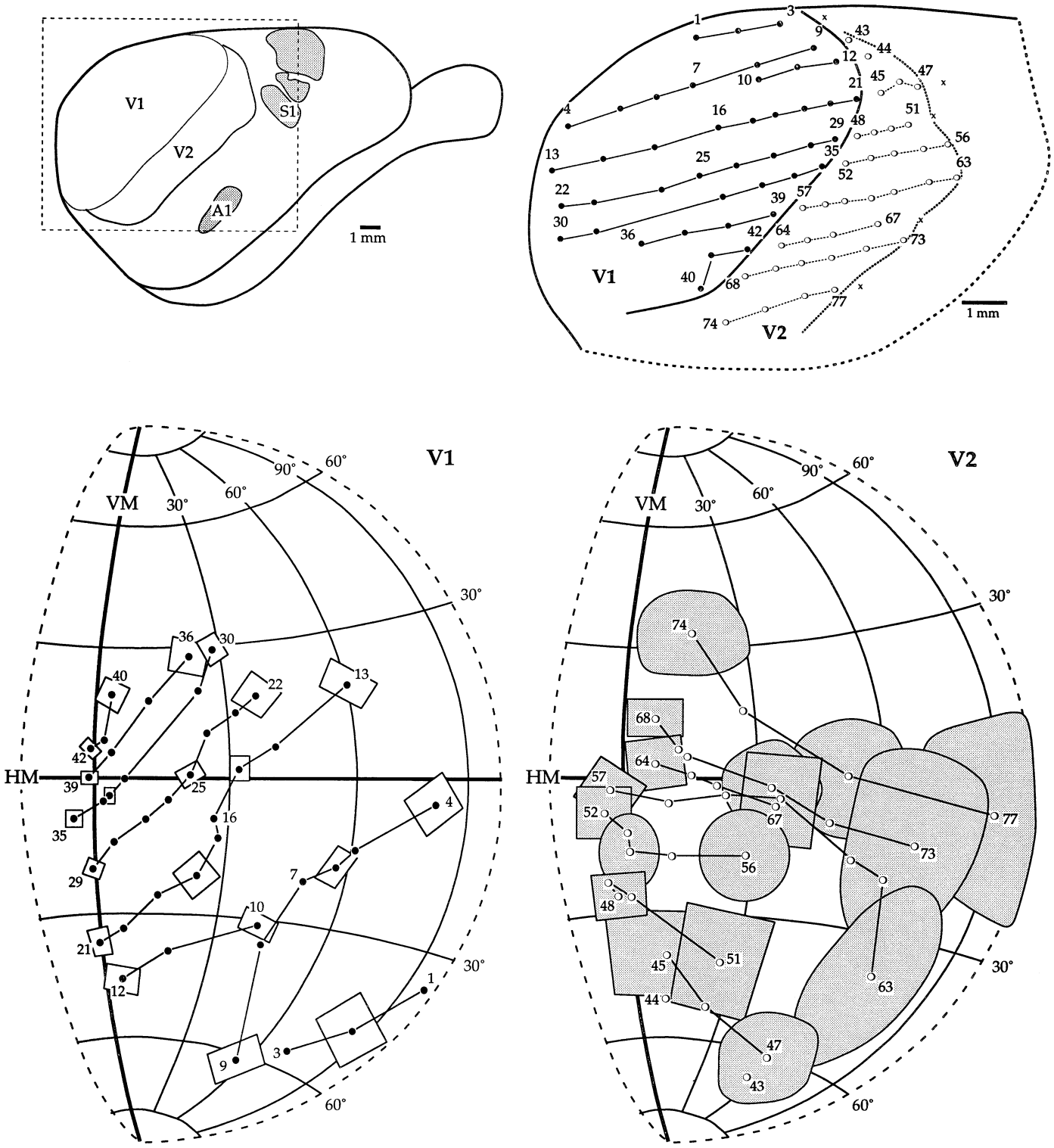

FIG. 3. Recording sites and receptive fields obtained from animal NC12. Top right: recording sites, indicated on a view of the surface of the occipitoparietal cortex [the approximate region illustrated is shown by the dashed box on the insert (top left)]. The map of recording sites is based on a digital image taken from a perspective normal to the cortical surface. Recording sites in V1 are indicated by black circles, and those in V2 by white circles. Recording sites where no visually evoked activity could be elicited are marked by a small 'x'. For the purposes of illustration, the recording sites within each area were joined in sequences (e.g. 1–3). Rostral is to the right, and medial is upwards. The coarse dashed line indicates the rostral and lateral limits of the craniotomy, and the solid line marks an architectonic boundary. Bottom, left and right: diagrams of the visual field of the animal, according to an equatorial azimuthal projection, showing the location of the receptive fields of V1 (left) and V2 (right) neurons. In order to illustrate azimuths up to 100 °, in this illustration the vertical meridian of the visual field was rotated by 10 °, forming an arch. Abbreviations: HM: horizontal meridian; VM: vertical meridian.

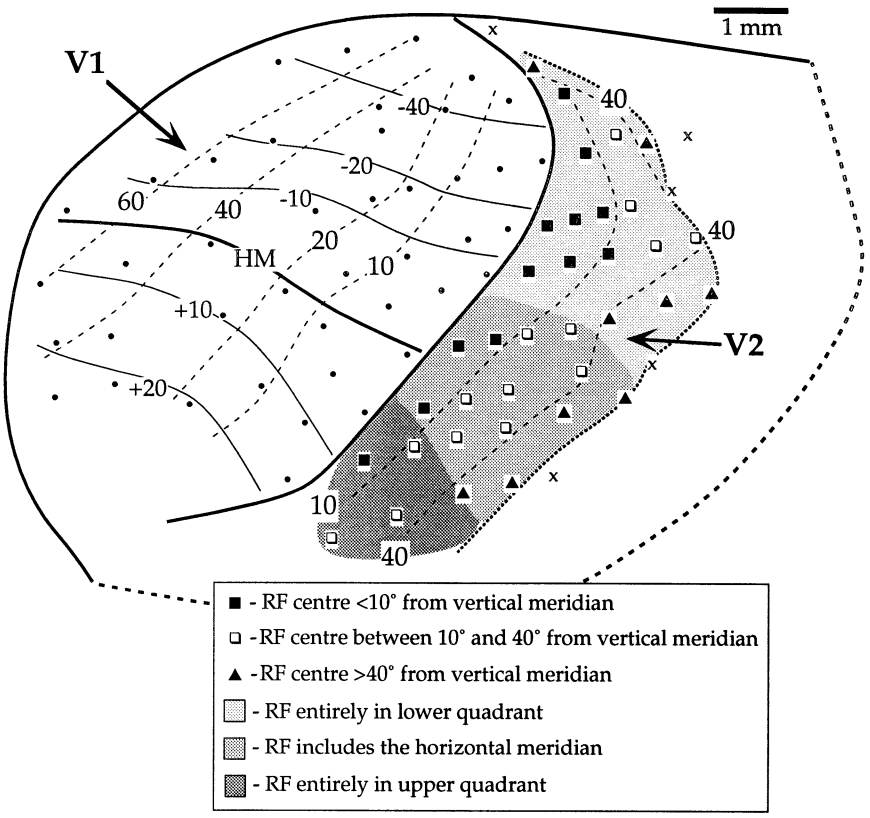

FIG. 4. Summary of visuotopic representation in V1 and V2 of animal NC12. This representation of a dorsolateral view of the occipitoparietal cortex is the same as that illustrated in Fig. 3 (top right). The recording sites in V1 (small dots) were used as a guide to interpolate isoazimuth and isoelevation lines (thin dashed and thin continuous lines, respectively). The recording sites in V2 are indicated by different symbols, according to their location relative to the vertical meridian (black squares, receptive fields with centres within 10 ° of the vertical meridian; white squares, receptive fields with centres more than 10 °, but less than 40 °, from the vertical meridian; black triangles, receptive fields with centres more than 40 ° from the vertical meridian). Moreover, V2 was divided into three regions (different shades of grey) according to whether the receptive field was entirely located in the lower quadrant, included the horizontal meridian, or was entirely located in the upper quadrant. As shown by this figure, the cortex rostrolateral to V1 forms a single visuotopic map.

lead to the conclusion that V2 is absent in rodents. It has been argued (Sanderson *et al*., 1991; Montero, 1993; Rosa *et al*., 1994), on the basis of visuotopic organization and laminar patterns of connections with other visual areas, that one of the proposed areas of rodent cortex (LM) is likely to be the homologue of V2. Like V2, LM forms an elongated belt adjacent to the representation of the area centralis in V1 (Olavarria & Mendez, 1979; Espinoza & Thomas, 1983), and alone it forms the second stage in the proposed hierarchy of visual areas in the rat (Coogan & Burkhalter, 1993). Thus, while V2 may have been reduced in size in some species, this hardly constitutes a valid argument against homology. Of course, one expects variation among mammalian species, related to ecological niche or degree of complexity of cortical organization. However, the study of brain evolution demonstrates that, while 'new' structures have been added to the cortex in specific mammalian lineages (e.g. Preuss & Goldman-Rakic, 1991), 'old' structures still remain functional. For example, even in 'blind' species of rodents adapted to underground living, the primary visual pathway (including visual cortex) remains anatomically distinct, and similar, in terms of connections, to that existent in other mammals (Herbin *et al*., 1994). We would like to argue therefore that even though it is possible that V2 has been greatly modified in some rodent lineages, this is not a valid argument against the notion of it being part of the common 'core' of visual areas present in all eutherians. Phylogenetic (e.g. Robinson *et al*., 1997) analyses suggest that rats and hamsters are both part of the superfamily Muroidea, a highly derived rodent group in comparison with, e.g. squirrels (Sciuroidea), which are known to have a well-defined V2. Thus, if confirmed, the multiplicity of small areas around V1 in addition to LM (V2) in rats and hamsters will most likely reflect a specialization of some rodent lineages.

V2 in marsupials: modules or multiple areas?

An aspect of V2 organization common to many eutherians is the existence of some type of modular organization, which can be detected on the basis of cytochrome oxidase reactivity, myelin stains or patterns of connections (e.g. Tootell *et al*., 1983; Anderson *et al*., 1988; Kaas *et al*., 1989). Although none of these criteria has revealed a periodic structure in quoll V2, there are indications that, at least in some marsupials, V2 is subdivided into modules (Martinich *et al*., 1990). This observation, together with the present results, suggests that the results of the anatomical study of Bravo *et al*. (1990), in which multiple foci of callosal and interareal connections were observed in the peristriate cortex of the mouse opossum, reflect a modular organization within V2, perhaps imposed by callosal connections, rather than separate visual areas. Note that a pattern of acallosal 'islands' contained within callosal 'bridges' also exists in V2 of cats and monkeys, areas which undoubtedly contain a single global visuotopic map (Sanides & Albus, 1980; Boyd & Matsubara, 1994; Olavarria & Van Sluyters, 1995; Olavarria & Abel, 1996). In addition, it is well known that V1–V2 connections are often patchy (e.g. Livingstone & Hubel, 1983), and Malach (1989) explained the patchiness of projections from V1 to the prestriate belt immediately lateral to it in rats as a consequence of modularity within V2. A similar rationale may apply to the mouse opossum.

In summary, while it is conceivable that multiple small extrastriate areas do exist in some species of marsupials, the anatomical

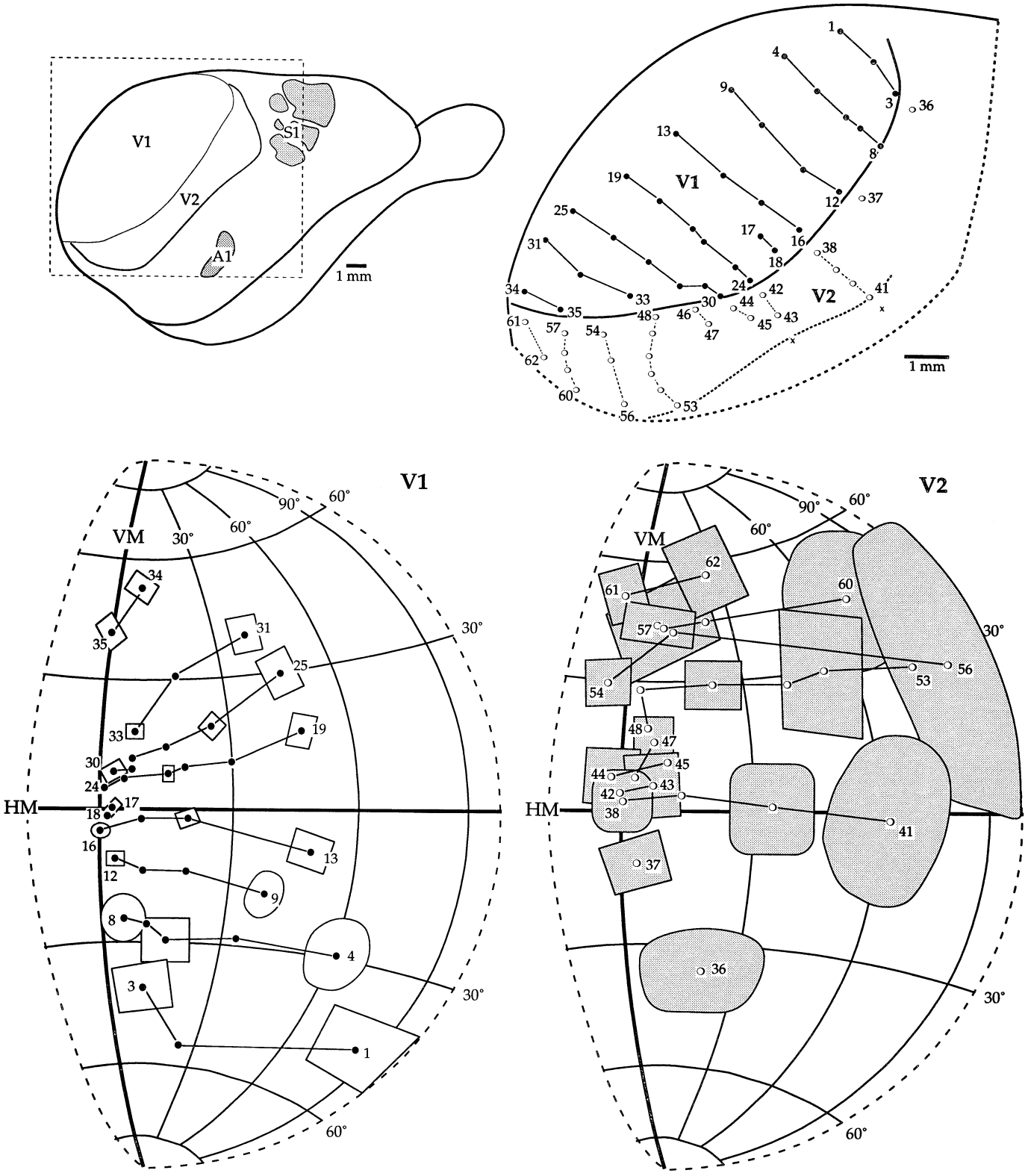

FIG. 5. Recording sites and receptive fields obtained from animal NC13. Conventions as for Fig. 3.

evidence presented by Bravo *et al*. (1990), when considered alone, is inconclusive, being equally consistent with either the hypothesis we favour (modular organization) or the presence of multiple minute areas. On the other hand, the present electrophysiological evidence leaves no doubt of the presence of V2 in a marsupial, and is consistent with the conclusions of anatomical and physiological work in the opossum and brushtailed possum (Haight *et al*., 1980; Crewther *et al*., 1984; Martinich *et al*., 1990, 1992), which

found no evidence of additional visual areas adjacent to V1. Thus, the hypothesis of multiple areas immediately lateral to V1 is clearly less parsimonious than that of a V2 which is divided into modules.

Are there other visual areas in marsupials?

The conclusions above do not imply that other visual areas do not exist in marsupials, only that they are unlikely to be adjacent to V1 in the lateral cortex. While V2 in the quoll is quite expansive, we found no evidence of other visually responsive areas. Moreover, according to our data (Fig. 1), there is little room for additional visual areas in the quoll. The rostral border of V2 is near the caudal border of the somatosensory cortex, and, in fact, we observed neurons responding to somatosensory stimulation immediately rostral to the medial portion of V2 (e.g. the site marked with an 'x' just rostral to site 47, in Fig. 3). In addition, while we have not recorded from the heavily myelinated cortex lateral to the caudal limit of V2 (posterolateral peristriate area [PSpl] of Benevento & Ebner, 1971), a previous study using evoked potentials in the opossum was unable to obtain any visual responses in this region, even though neurons in the remainder of the peristriate cortex were found to be responsive to flashes (Sousa *et al*., 1978). We therefore conclude that the only likely location of additional cortical visual areas in the quoll is in the narrow strip of cortex interposed between V2 and the putative primary auditory area. Anatomical work in other species has suggested that the areas in this region may, in fact, be visual in function (e.g. Haight *et al*., 1980). However, as areas devoted to different sensory modalities are known to be connected in some cases (e.g. Olavarria & Montero, 1984), this requires confirmation with physiological techniques. In the present study, we have sampled this region in two cases, but were unable to elicit any visual responses. This could simply result from the fact that the visual stimuli used in this study were inappropriate to activate cells in these areas, or that the visual responses therein are more strongly affected by anaesthesia.

Benevento & Ebner (1971) have suggested that the cortex of *Didelphis* shows two cytoarchitectonically distinct parallel bands, areas PScm and PScl (medial and lateral subdivisions of the central peristriate area), in the approximate location of V2. A study of V1 connections in this species has suggested that PScm and PScl could correspond to V2 and V3 (third visual area) of eutherians (Martinich *et al*., 1992). There is, to our knowledge, no detailed account of the cytoarchitecture of the peristriate cortex in the quoll. However, the physiologically defined V2 seems to encompass the regions of both PScm and PScl. It is possible that, rather than two functional areas, the cytoarchitectural distinction between PScm and PScl reflects a difference between the binocular and monocular representations in V2, similar to the distinction described in V1 of rodents (e.g. Hall *et al*., 1971; Sereno *et al*., 1991; Paolini & Sereno, 1998).

Functional characteristics of marsupial V2

V2 neurons in the marsupial appear to be unique in that responses are difficult to elicit, and depend on quite specific stimulus parameters. The different types of stimuli required to activate V1 and V2 neurons in the quoll suggest that, as in the cat (Tretter *et al*., 1975), these areas analyse parallel aspects of vision. In particular, the requirement for large and fast-moving stimuli for activation of V2 cells suggest that this area is involved with low-acuity spatial aspects of vision, while V1 may have a role in shape analysis and high-acuity vision. In other species, the parallel organization of V1 and V2 has also been suggested on the basis of V2 cells retaining visual responsiveness after destruction or temporary inactivation of V1 (for a recent review, see Funk & Rosa, 1998).

One of the points unresolved by the present study is the exact configuration of the field discontinuities that are likely to exist along the rostrolateral border of V2. Although the present data suggest that such field discontinuities exist, they have not been mapped in detail, and it is unknown whether or not they occur in a reproducible fashion between individuals. For the purposes of the present study, it has been sufficient to note that the visuotopy along the rostral border of V2 in quolls does not differ from that observed in eutherians with simple visual cortices, e.g. rabbits and squirrels (Hall *et al*., 1971; Hughes, 1971). Nonetheless, given its relevance for the interpretation of the evolutionary relationships among mammals (as detailed above), it would be interesting to know if there is a correlation between field discontinuities in the V2 visuotopic map and anatomical connections, as has been demonstrated in the cat (Sanides & Albus, 1980).

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Abbreviations

LM, lateromedial area; PScl, lateral subdivision of the central peristriate area; PScm, medial subdivision of the central peristriate area; PSpl, posterolateral prestriate area; V1–V3, first–third visual areas.

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