Hierarchical Development of the Primate Visual Cortex, as Revealed by Neurofilament Immunoreactivity: Early Maturation of the Middle Temporal Area (MT)

It has been suggested that the development of the cerebral cortex reflects its hierarchical organization, with the primary sensory areas being the first to reach structural and functional maturity, and higher-order association areas being the last. In the present study, we labelled the cortex of New World marmoset monkeys of late fetal and early postnatal ages with an antibody to non-phosphorylated neurofilament, a marker of structural maturation of a subset of pyramidal cells. Supporting the concept of hierarchical maturation, we found that at birth labelled cells were found in the primary visual, auditory and somatosensory areas, but not in most other cortical fields. The exception was visual area MT, which revealed an infragranular pattern of labelling comparable to the one observed in the primary areas, as well as some supragranular staining. In MT, an adult-like pattern of labelled cells, including both supragranular and infragranular layer neurons, emerged within the first postnatal month. In comparison, the development of other extrastriate areas was delayed, with the first signs of neurofilament staining not present until the third week. The present results support the concept of MT as another primary visual area, an idea previously advanced on the basis of functional and anatomical evidence.

Keywords: cortical maturation, extrastriate cortex, callithrix, primary sensory field, SMI=32, V5

Introduction

The cortical networks responsible for sensation comprise primary fields for vision (V1, the striate cortex, or area 17), touch (S1, corresponding to cytoarchitectural area 3b) and hearing (the auditory 'core', which includes A1 and a rostral auditory field, R), as well as a number of higher-order sensory and association areas. While the primary areas appear to be present in every mammal, higher-order areas vary between species, forming the majority of the mass of six-layered homogenetic isocortex that has experienced marked expansion during primate evolution (Rosa and Tweedale, 2005). Presently, much is known about the developmental mechanisms that lead to the establishment of the boundaries and topographic organization of the primary areas (e.g. O'Leary, 1989; Rubenstein and Rakic, 1999). However, our knowledge of the steps involved in the formation of higher-order and association areas remains far more incomplete.

Similar to the primary sensory areas, it is likely that the establishment of areal boundaries in higher-order sensory cortices depends mainly on events happening prenatally, involving a combination of genetic determination and interactions with axons arising from non-cortical areas of the brain (O'Leary, 1989; Donoghue and Rakic, 1999; Sur and Leamey, 2001). However, it is also acknowledged that these areas undergo extended periods of postnatal maturation. For example, in the visual system certain perceptual abilities do not develop until well into adolescence (Kovacs, 2000), and it is reasonable to expect that this gradual emergence reflects postnatal changes in the molecular and cellular characteristics of the visual cortex. Indeed, a previous investigation using 2-deoxyglucose autoradiography to measure metabolic activity in young macaque monkeys revealed that the functional maturation of hierarchically higher visual cortical areas, particularly in the temporal lobe, is delayed relative to that of V1 (Distler et al., 1996). Moreover, the same study concluded that the development of the ventral stream areas (i.e. those involved in object identification on the basis of cues such as shape, colour and texture) occurs over a longer time course in comparison to that of dorsal stream areas (involved in the analysis of motion and spatial relationships). Evidence in support of a hierarchical maturation of the ventral stream has also been obtained by the analysis of the laminar distributions of parvalbumin- and calbindin-immunoreactive cells in the macaque (Conde et al., 1996).

The present study is focused on the perinatal development of the extrastriate cortex, with special emphasis on middle temporal area (MT, also known as V5). MT is a subdivision of the dorsal stream found in all primates studied so far, which has a crucial role in the processing of motion (Allman and Kaas, 1971; Zeki, 1974). Establishing the timeline of the structural maturation of MT is important in light of the suggestion that this area may be included within one of the few regions of the human cortex that are already myelinated at birth (Flechsig, 1901; Watson et al., 1993; Ammene et al., 2004) and the evidence that dorsal stream areas may develop ahead of ventral stream areas (Conde et al., 1996; Distler et al., 1996). Given the advantages represented by the lissencephalic cortex of marmosets, including location of MT on the exposed surface of the temporal lobe and the relative immaturity of these animals at birth [the eyes open on embryonic day (ED) 142 of a 145 day gestation], the study of the postnatal development of the marmoset cortex has the potential to clarify substantially the sequence of events leading to the maturation of the primate visual cortex. The visuotopic organization of the marmoset extrastriate cortex has been mapped in detail (for a review, see Rosa and Tweedale, 2005), allowing a precise correlation between patterns of labelling and areas.

Studies in adult monkeys have revealed a combination of anatomical and physiological features that makes MT unique among extrastriate areas. First, MT stands out as the specific cortical target of a small, but clearly defined retinal projection, relayed to the cortex through the medial nucleus of the inferior pulvinar (O'Brien et al., 2001). The neurochemical characteristics of this nucleus are suggestive of a lemniscal visual
pathway operating in parallel with the retinogeniculostriate pathway (Cusick et al., 1993; Gutierrez et al., 2000). These afferents, in combination with those relayed via the koniocellular layers of the lateral geniculate nucleus (Stepniewska et al., 1999; Sincich et al., 2004), may explain the fact that many neurons in MT remain visually responsive after lesions of V1, as well as the short latencies that characterize their responses (Rodman et al., 1989; Girard et al., 1992; Rosa et al., 2000). MT also stands out among extrastriate areas by virtue of its extent of myelination and atypically sharp histological boundaries, which can be revealed even by Nissl substance stains (Tootell et al., 1985). In all these aspects, MT resembles the primary visual cortex, rather than other extrastriate areas.

Neurofilament, a cytoskeletal triplet protein, comprises of heavy (200 kDa) medium (168 kDa) and light (68 kDa) subunits. The 'heavy' subunit has been shown to specifically emerge during the late phase of the morphological maturation of neurons and stabilization of connections (Carden et al., 1987; Liu et al., 1994; Kogan et al., 2000). A monoclonal antibody against non-phosphorylated epitopes of the heavy and medium subunits of neurofilament protein (SMI-32; Sternberger and Sternberger, 1983), specifically labels the perikarya and dendrites of a subset of pyramidal cells. This technique has been successful in allowing the architectonic subdivision of the adult visual cortex in a number of species (Hof and Morrison, 1995; Chaudhuri et al., 1996; van der Gucht et al., 2001; Bourne and Rosa, 2003; Boire et al., 2005), as well as the study of development of V1 in humans (Ang et al., 1991) and marmosets (Bourne et al., 2005). Here, we demonstrate that the perinatal development of MT, as revealed by SMI-32 immunohistochemistry, is synchronous with that of the classical primary sensory fields (V1, S1 and A1/ R). This result emphasizes the uniqueness of MT among extrastriate areas, and provides clues to understanding certain features of sensory development in humans.

Materials and Methods

Subjects and Tissue Preparation

Marmoset monkeys (Callithrix jacchus) were euthanased at PD 0 (2-5 h post partum), PD 3, PD 7, PD 14, PD 21, PD 28, PD 45, PD 66, PD 93 and postnatal month 22 (a sexually mature individual). Another two animals were obtained by Caesarean section and estimated to be of gestational age 130 (ED 130), based on prenatal growth curves (Hearn, 1983; Tardif et al., 1998). In captivity, juvenile marmosets are weaned between 40 and 120 days, reach puberty at ~9 months and are sexually and socially mature between 18 and 24 months (Clarke, 1994). Experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University Animal Ethics Committee. The animals were administered an overdose of the opiate sufentanyl citrate (0.05 mg/kg, i.m.). Following apnoea, they were transected and perfused with 0.1 M heparinized phosphate buffer (PB; pH 7.2, warmed to 37°C) containing 0.1% sodium nitrite, followed by 4% paraformaldehyde in 0.1 M PB at room temperature. Cerebral tissues were immediately removed and postfixed for 24 h in 4% paraformaldehyde containing 10% sucrose at 4°C. After cryoprotection with 30% sucrose, both hemispheres were sectioned at 40 μm using a cryostat, resulting in both coronal and sagittal series of sections from each animal. The sections were subsequently stored at ~20°C in a cryoprotective solution (50% 0.05 M PB, 30% ethylene glycol, 20% glycerol).

Immunohistochemistry and Histology

Every fourth section was processed in order to reveal the distribution of non-phosphorylated neurofilament protein (NNF), as detailed by Bourne et al. (2005). After 3 × 10 min washes in phosphate buffered saline (PBS; pH 7.2), free-floating sections were treated for 30 min with 0.3% hydrogen peroxide in 50% methyl alcohol, before being preincubated in a solution of 5% normal rabbit serum (NRS; Vector Laboratories, Burlingame, CA) in PBS containing 0.3% Triton X-100 (PBS-TX) for 1 h. Without rinsing, sections were transferred into PBS-TX containing the primary monoclonal antibody (SMI-32, 1:2000; Sternberger Monoclonals, Baltimore, MD) and 2% NRS for 16–18 h at 4°C. Sections were rinsed (3 × 10 min) with 0.1% Tween-20 (Sigma, St Louis, MO) in PBS and incubated for 1 h with a biotinylated rabbit anti-mouse secondary antibody (1:1500; DAKO A/S, Glostrup, Denmark). Following processing with avidin-biotin-horseradish peroxidase (1:200; Amersham Pharmacia Biotech, Little Chalfont, UK; for 1 h), immunoreactivity was revealed using a metal-enhanced chromogen, 3,3'-diaminobenzidine (DAB) and a stable peroxide buffer (Pierce Biotechnology Inc, Rockford, IL). Sections were then mounted, dehydrated in graded alcohols, defatted in xylene and coverslipped with DPX (BDH, Poole, UK). Negative and positive controls (adult tissue at the level of area MT) were performed routinely to ensure the constancy of labelling between immunohistochemical assays.

Cytochrome oxidase histochemistry was performed on free-floating sections, using a modification of the Wong-Riley (1979) method. First, sections were pretreated in 0.1% cobalt chloride solution at room temperature before being incubated at 37°C in 0.1 M PB containing a mixture of cytochrome C oxidase, catalase and DAB. Sections were reacted until layer 4 of V1 was clearly discernible from the infra/supra granular layers, or for a maximum of 2 h. Nissl substance labelling was achieved using a 0.05% cresyl violet solution. Sections were stained until layer 6 was discernible from other layers. Additional series of sections were kept from adult animals, and reacted for myelin using the Gallyas (1979) method. Because earlier results in our laboratory have demonstrated a lack of myelin staining in the cortex of marmosets throughout the first two months of life, this technique was not useful in tracing areal boundaries in newborn cases.

Analysis

Sections were examined under bright field microscopy using a Zeiss Axioplan imaging microscope. Low- and high-power photomicrographs (1300 × 1030 dpi) were taken on a Zeiss Axiocam digital camera connected to Axiovision software (v 3.1; Zeiss). Images were cropped and sized using Adobe Photoshop 8 and Illustrator 11.

Results

MT is One of Four Areas Showing NNF Reactivity in the Newborn Monkey

At birth (PD 0), many key events in the formation of the marmoset cerebral cortex have already occurred, as evidenced by the typical lamination visible in Nissl-stained material (~ED 130 animal, Fig. 5B; see also Fritschy and Garey, 1986). However, as shown in Figure 1, at this stage most areas of the cerebral cortex remain completely devoid of non-phosphorylated neurofilament protein (NNF) immunoreactivity. Clear immunolabelling is restricted to isolated clusters of pyramidal cells, which, with rare exceptions (see below), reside in the infragranular layers. Three of the labelled clusters are centred in the auditory 'core' (Fig. 1C), S1 (around the expected location of the hand/face representation boundary, Fig. 1D) and V1 (primarily in the foveal representation; Fig. 1A; see also Bourne et al., 2005). In addition, there is a fourth locus of dense NNF-immunolabelling, which can be seen on the lateral surface of the caudal part of the temporal lobe (Fig. 1B), extending approximately between the interaural (AP 0) plane caudally to the proximity of the tip of the lateral sulus rostrally. Reconstruction of serial sections reveals that this locus forms an oval ~4 mm long and <2 mm wide, with a major axis oriented posteroverentral to anterodorsal. On the basis of comparisons with series of coronal and sagittal sections, marker progression appears to advance from the rostral to the caudal surface of MT in the marmoset. An additional area of weak labelling is observed in the lateral extent of area MT (Fig. 1). The laminar distribution of immunoreactivity for NNF is shown in Figure 2.
parasagittal sections obtained in the course of this and other studies of the adult marmoset visual cortex (e.g. Rosa and Elston, 1998), this fourth locus has been found to correspond precisely to the location, shape and dimensions of area MT (Fig. 2). The NNF-demarcated early postnatal MT is separated from the auditory ‘core’ by cortex with different histological characteristics, which, on the basis of mapping studies in adult marmosets, is likely to contain the medial superior temporal visual area (MST) and the caudomedial auditory area (CM; Rosa and Elston, 1998; Kajikawa et al., 2005). Unlike MT and the auditory core, the CM/MST region does not label for NNF for the first two postnatal weeks (e.g. diamond symbol in Fig. 2A), although many labelled cells in these regions are already apparent by PD 28 (Fig. 2B).

MT Maturation in Comparison to the Classical Primary Sensory Fields

In V1, NNF-immunoreactivity emerges gradually during the first three postnatal months. Although immunolabelling of cell bodies is already apparent at birth (a band of cells located near the interface between layers 5 and 6), supragranular label (near the base of layer 3) does not become apparent until the second postnatal week (Bourne et al., 2005). A third, more superficial band of cells (in layer 3B) is first observed in the third or fourth postnatal week, although the numbers of labelled cells in this layer continue to increase for several months thereafter (Bourne et al., 2005). The present results demonstrate that a slightly different sequence of events occurs in A1 and S1, where the infragranular label in perinatal marmosets seems to concentrate in layer 5 itself, and the first supragranular labelled neurons have already become evident at the end of the first postnatal week (PD 7; Fig. 3G, L). In these areas, as in V1 (Bourne et al., 2005), an adult-like distribution of cell bodies is already evident by the end of the first postnatal month, with subsequent changes in laminar distribution of NNF labelling reflecting primarily an increase in the distribution of labelled neuropil (Fig. 3).

The present results demonstrate that the sequence of events in the maturation of MT is concomitant with, or even slightly accelerated to analogous events occurring in A1/R, S1 and V1. Similar to the primary sensory fields, cells showing strong label are already visible in the infragranular layers of MT at birth.

Figure 1. Immunohistochemical localization of NNF with monoclonal antibody SMI-32 in coronal sections of a neonatal (2 h post partum) marmoset cerebral cortex. Isolated expression was only observed in the primary sensory fields [V1 (A), the auditory ‘core’ (C) and S1 (D)], as well as in MT (B). The location of these sensory fields was determined on the basis of corresponding NNF-immunolabelled sections from adult animals. The insert illustrates the level of the sections relative to a lateral view of the neonatal marmoset brain. Scale bar = 1 mm.
However, in MT faintly labelled pyramidal cells can also be observed in layer 3, even at this stage (Fig. 3). Moreover, while cells in V1 gradually acquire their pyramidal morphology over the first few weeks of life, as revealed by NNF staining of the apical dendrite and perikaryon (Bourne et al., 2005), labelled neurons in MT already show heavily labelled, thick apical dendrites and clear defined pyramid-shaped cell bodies at birth. These signs of morphological maturation are evident in cells located both in layers 3 (Fig. 4A) and 5 (Fig. 4E). Pyramidal cells in the developing MT resemble those in V1 by undergoing an increase in the density of NNF immunolabelling of basal dendrites during the first postnatal month (Fig. 4).

The most obvious difference between the developmental pattern of NNF staining between MT and V1 refers to the timing of the appearance of labelled neurons in layer 6. While in V1 the sequence of expression of NNF is strictly inside-out (layer 6 first, top of layer 3 last), in MT cells located at the base of layer 6 are among the last cells to reveal labelling. Neurons with small NNF-labelled cell bodies in layer 6 first become evident at PD 7 (Figs 3B and 4I), at least a week after label is present in layers 3 and 5. In this respect, MT resembles the auditory ‘core’ areas, where a layer 6 band of labelled cells also emerges late in development (Fig. 3H,I). The laminar and morphological characteristics of the small layer 6 pyramids are analogous to those previously observed in the adult vervet monkey (Chaudhuri et al., 1996) and macaque (Hof et al., 1996). These differences between areas are unlikely to be due to methodological procedures and/or cross-reactivity to non-specific epitopes, as they emerge from comparisons involving sections from the same animals, which were processed simultaneously. Moreover, the laminar/areal patterns of labelling were consistent between individuals and hemispheres corresponding to an identical age, irrespective of whether the histological processing was done as part of the same or different experiments.

Late Maturation of Other Visual Areas

In comparison with MT and the primary sensory fields, the maturation of NNF-labelled cells in V2 and the ‘ventral stream’ extrastriate areas is not only delayed, but also suggestive of a sequential hierarchical pattern (Figs 5 and 6). NNF immunolabelling can first be detected in V2, near the V1 border, between the third and fourth postnatal weeks (Bourne et al., 2005; see also Fig. 5D). At PD 28 there are still relatively few labelled cells in cortex corresponding to the location of adult areas V3 and V4. The distribution of NNF-labelled cells gradually expands to encompass more rostral areas between the end of the first and second postnatal month (Fig. 5D,E), with further gains in the intensity of neuropil labelling even beyond the second postnatal month (Fig. 5F). By PD 45 there are very few NNF-labelled neurons, and virtually no neuropil staining in the inferior temporal cortex (cytoarchitectural area TE) and the adjacent parahippocampal cortex (areas 35 and 36), although at this age.
Figure 3. Comparison of the laminar distribution of NNF in the primary sensory fields A1 and S1 and in area MT at PD 0 (A, F, K), PD 7 (B, G, L), PD 45 (C, H, M) and in an adult (D, I, N). The appearance of NNF-immunolabelled cells in the supragranular layer of MT at PD 0 precedes that observed in A1 and S1. Laminar boundaries were determined in adjacent Nissl- (e.g. E, J, O) and cytochrome oxidase-stained sections. Scale bar = 200 μm.
near-adult levels of label are already evident in the posterior parietal cortex (Fig. 6). The laminar distribution of neurons in the inferior temporal area is not complete until the end of the third postnatal month. In addition, subsequent changes proceed throughout the first year of life, including an increase in neuropil labelling.

Discussion

Early Maturation of MT and the Concept of Hierarchical Maturation of Cortical Areas

The early development of MT in relation to other extrastriate areas was first suggested by Watson et al. (1993) on the basis of an anatomical comparison between the pattern of myelination in newborn human brains (Flechsig, 1901), and functional mapping of motion selectivity in adults, using positron emission tomography. As pointed out by Watson et al. (1993), the motion-selective 'V5 complex' (which encompasses MT and its satellite areas) is included within one of the few regions of the human cerebral cortex that are already myelinated at birth (Field 10 of Flechsig, 1901). The early maturation of MT is also compatible with research on the development of pattern motion selectivity and other presumed MT-mediated functions (Fine et al., 2003; Dobkins et al., 2004), as well as with the 2-deoxyglucose and histological evidence of an early maturation of the dorsal stream, as compared with the central stream (Conde et al., 1996; Distler et al., 1996; Kogan et al., 2000). The present results add significantly to these earlier observations by indicating an exact correspondence between area MT and one of the only four regions of cortex that already show NNF immunoreactivity in the newborn marmoset. Thus, while our observations confirm the fact that dorsal stream areas tend to develop ahead of ventral stream areas, they also suggest that a degree of hierarchical maturation also exists within the motion-sensitive dorsal stream areas. For example, the bridge of cortex between MT and the auditory 'core' (which includes MST, another subdivision of the 'V5 complex') and the adjacent fundus of superior temporal area (FST; Desimone and Ungerleider, 1986; Rosa and Elston, 1998) were both found to lack NNF immunolabelling at birth. However, even in the posterior parietal cortex, which is considered to include areas corresponding to the highest levels of the dorsal stream, the cellular distribution of NNF was adult-like already by PD 45.

Our results are also important in demonstrating that the biochemical maturation of MT progresses in approximate synchrony with that of classical primary sensory areas for vision, touch and hearing. These results are in agreement with the hypothesis that activity-dependent processes involving early-maturing circuits of neurons in both V1 and MT contribute to the early postnatal maturation of neuronal responses in other areas. In particular, it has been suggested that the well-defined visuotopic organizations and first-order visuotopic maps in these two areas could play a role in defining the topographic organization of adjacent cortices through interactions involving axonal projections or gap junctions (Rosa, 2002; Rosa and Tweedale, 2005). Our present observations suggest that the development of NNF immunoreactivity in the ventral stream cortices, resembles a 'wave' that propagates away from V1 in the sense that areas V2, V3, V4, and the posterior and anterior inferior temporal cortices become labelled in sequence (Figs 5 and 6). The faster time course of the maturation of the dorsal stream makes it more difficult to obtain a clear idea of the exact sequence of events. While recognizing that a study involving more time points during the first postnatal month will be necessary to clarify this issue, our results do indicate that MT develops ahead of MST, and that the maturation of the regions of posterior parietal cortex exposed on the dorsolateral surface of
the brain is slightly delayed relative to MST. For example, cellular label in MST is already dense in both supragranular and infragranular layers of PD 21 and PD 28 animals, while cells in the posterior parietal cortex are still relatively weakly labelled.

**Laminar Development of NNF Immunoreactivity in MT**

The sequence of laminar maturation of MT is distinct from that previously observed in V1, with NNF-immunolabelled cells first appearing near the base of layer 3 and in layer 5, followed after 1 week by labelled cells in layer 6. Given that no cortical label was observed in the ED 130 animal (Fig. 5A), our materials did not allow us to further clarify whether labelling in layer 3 of MT appears simultaneously with that in layer 5 or, as we deem...
more likely (based on the density of cellular label), at a slightly later stage. Layer 5 of MT is the main source of projections to the pulvinar complex, whereas most thalamocortical projections to this area terminate near the base of layer 3 (Rockland et al., 1999; Stepniewska, 2004). Thus, our observations are consistent with observations in V1, where it was found that the development of corticothalamic efferent connections occurs in approximate synchrony with the genesis of the afferent pathway from the LGN and with the onset of morphological differentiation of pyramidal neurons, as revealed by autoradiography (Shatz and Rakic, 1981). However, in macaque V1 these anatomical processes occur approximately midway through gestation. Even considering the fact that marmosets are relatively less developed at birth than macaques, these results indicate that the formation of connections significantly precedes the acquisition of NNF immunoreactivity.

The morphological characteristics of the NNF-labelled layer 6 cells in MT are similar to those of neurons forming projections to V1 (Doty, 1983), suggesting that the structural maturation of MT projections to V1 is relatively delayed, in comparison with that of the thalamocortical efferents. It is of particular interest that the first NNF-immunolabelled cells in layer 6 of MT occur at approximately the developmental same stage (between PD 3 and PD 7) as the first NNF-labelled cells at base of layer 3 in V1 (layer 4B of Brodmann’s nomenclature), which form the majority of the projection from this area to MT (Bourne et al., 2005). Thus, similar to thalamocortical and corticothalamic connections, the projections from V1 to MT and those from MT to V1 are likely to undergo this developmental stage in rough synchrony. Considering the traditional view that MT corresponds to a higher hierarchical level of processing in comparison with V1 (Maunsell and van Essen, 1983), this synchronous development contrast with the notion that 'feedforward' projections tend to mature ahead of 'feedback' connections (Kennedy and Burkhhalter, 2004). However, as argued below, it is also possible that V1 and MT both correspond to basal levels of the visual cortical hierarchy.

**Can MT Be Considered a Primary Sensory Area?**

Since Flechsig’s seminal studies of cortical myelogenesis over a century ago (Flechsig, 1901), evidence has been accumulating in support of a model whereby cortical areas develop in a hierarchical sequence, which starts with primary sensory areas and pathways to various extents. The balance of visual information relay to MT from different sources (e.g. the direct retinothalamic pathways, versus projections via V1) may change depending on the stage of development, and it is conceivable that in adult monkeys the geniculostriate pathway becomes functionally dominant. Nonetheless, as reviewed above in the Introduction, MT remains a somewhat unusual area, even in adult primates, in terms of its histology, physiology and anatomical connections (e.g. by receiving relatively direct inputs from the retina; O’Brien et al., 2001; Sincich et al., 2004).

Physiologically, there are also indications that V1 and MT process certain aspects of motion information in parallel (ffytche et al., 1995), and that MT can mediate conscious visual sensation even in the absence of V1, provided that stimuli of certain spatiotemporal characteristics are presented (Barbur et al., 1993; Sahraie et al., 1997; Zeki and ffytche, 1998). A degree of functional independence is corroborated by the studies of single-cell properties of MT after V1 lesions or deactivations in adult monkeys (Rodman et al., 1989; Girard et al., 1992; Rosa et al., 2000; Azzopardi et al., 2003), even though the responses of MT neurons become substantially weakened in the absence of V1 inputs and may be even abolished under some protocols (Girard et al., 1992; Collins et al., 2003). Whereas the concept of two primary visual areas might sound strange at first, it would probably not cause much reaction among most auditory neuroscientists, who have grown accustomed, over a quarter of a century, to the idea of multiple primary-like auditory ‘core’ fields operating in parallel (e.g. Reale and Imig, 1980).

Anatomically, MT has been traditionally seen as occupying the fourth or fifth level of a hierarchy of visual areas, constructed on the basis of laminar patterns of corticocortical connections (Felleman and Van Essen, 1991). While analyses of these types of data usually reveal good anatomical correlates of the likely levels of processing within a same hierarchical pathway (Vezoli et al., 2004), it is also the case that the relationship tends to break down when one analyses connections between areas that may not operate strictly in parallel. For example, contrary to the expectations of a hierarchical model, frontal lobe projections to various areas of the extrastriate cortex originate predominantly from supragranular layers (Rosa et al., 1993; Schall et al., 1995; Shipp et al., 1998; Barone et al., 2000; Vezoli et al., 2004).

Moreover, in the macaque it has been demonstrated that putative ‘feedback’ connections from MT change in character during postnatal development, with a gradual reduction of the number of supragranular neurons that engage in projections to caudal visual areas (Kennedy et al., 1989). Thus, the laminar patterns observed in the adult, while certainly significant in terms of understanding the mature primate visual system (Vezoli et al., 2004), may not necessarily reflect the functional organization of the developing cortex.

Primary sensory fields are thought to be part of an evolutionarily ‘old’ framework of cortical fields, reflecting a common plan of organization shared by most, if not all mammals (Rosa and Krubitzer, 1999). Although there is discussion as to whether or not MT exists in non-primate species (Kaas, 2002), several possible homologues have been suggested, including the carnivore lateral suprasylvian area. Adult cortical areas are the result of a complex interaction of genetic and epigenetic factors, and homologous fields may not look identical, either anatomically or functionally. Nonetheless, embryologic processes in vertebrates tend to be conservative, and neural structures that are quite different in the adult may reveal their homology through similarity in early embryonic stages. Thus, the present demonstration of a unique character of area MT in early development points to a way of clarifying aspects of the evolution of the visual cortex, as one would expect that its...
presumed homologues in other mammalian lineages would also be among the first cortical areas to mature.

Notes
The authors would like to thank Rowan Tweedale for making many suggestions that improved this manuscript, Prof. Jack Pettigrew for enthusiastic support and discussions, and Anderson Hind, Claire Warner and Yi Si for help in some of the experiments. Funded by research grants from the Australian Research Council and National Health and Medical Research Council. Equipment support from the Clive & Vera Ramaciotti and the ANZ Charitable Trust is gratefully acknowledged.

Address correspondence to Dr James A. Bourne, Department of Physiology, Monash University, Melbourne VIC 3800, Australia. Email: james.bourne@med.monash.edu.au.

References
O’Brien BJ, Abel PL, Olavarria JF (2001) The retinal input to calbindin-
D28k-defined subdivisions in macaque inferior pulvinar. Neurosci

O’Leary DD (1989) Do cortical areas emerge from a protocortex?
Trends Neurosci 12:400-406.

Reale RA, Igam TJ (1980) Tonotopic organization in auditory cortex of

analysis of pulvinocortical connections to several visual areas in the

Rodman HR, Gross CG, Albright TD (1989) Afferent basis of visual
response properties in area MT of the macaque. I. Effects of striate

implications for brain development and evolution. Braz J Med Biol
Res 35:1485-1498.

Rosa MGP, Elston GN (1998) Visuotopic organisation and neuronal
response selectivity for direction of motion in visual areas of the
caudal temporal lobe of the marmoset monkey (Callithrix jacchus): middle


Rosa MGP, Tweedale R (2000) Visual areas in lateral and ventral

Rosa MGP, Tweedale R (2005) Brain maps, great and small: lessons
from comparative studies of primate visual cortical organization.

of visual area MT in the Cebus monkey: possible homologies
between New and Old World monkeys. Vis Neurosci 10:827-855.

the middle temporal area of new world monkeys after lesions of

Cereb Cortex 9:521-523.

(1997) Pattern of neuronal activity associated with conscious and
unconscious processing of visual signals. Proc Natl Acad Sci USA 94:9406-9411.

Schall JD, Morel A, King DJ, Bullier J (1995) Topography of visual cortex
connections with frontal eye field in macaque convergence and
segregation of processing streams. J Neurosci 15:4464-4487.

the visual cortex of the fetal rhesus monkey. J Comp Neurol

through superior parietal cortex in the macaque monkey:
cortical connections of areas V6 and V6A. Eur J Neurosci 10:
3171-3193.

a direct geniculocortical input to area MT. Nat Neurosci 7:1125-1128.

Stepniewska I, Qi XH, Kaas JH (1999) Do superior colliculus projection
zones in the inferior pulvinar project to MT in primates? Eur J
Neurosci 11:469-480.

Sternberger LA, Sternberger NH (1983) Monoclonal antibodies
distinguish phosphorylated and nonphosphorylated forms of


gestational ages in the common marmoset (Callithrix jacchus) from

Tootell RB, Hamilton SL, Silverman MS (1985) Topography of
cytochrome oxidase activity in owl monkey cortex. J Neurosci
5:2786-2800.

van der Gucht E, Vandesaende F, Ackens L (2001) Neurofilament protein: a selective marker for the architectonic parcella-
tion of the visual cortex in adult cat brain. J Comp Neurol 441:
345-368.

Vezoli J, Falchier A, Jouve B, Knoblauch K, Young M, Kennedy H
(2004) Quantitative analysis of connectivity in the visual cortex:
extracting function from structure. Neuroscientist 10:476-482.

Watson JD, Myers R, Frackowiak RS, Hajnal JV, Woods RP, Mazzotta JC,
a combined study using positron emission tomography and magnetic

Wong-Riley M (1979) Changes in the visual system of monocularly
sutured or enucleated cats demonstrable with cytochrome oxidase


Zeki SM (1974) Functional organization of a visual area in the posterior
bank of the superior temporal sulcus of the rhesus monkey. J Physiol
236:549-573.