Cytochrome Oxidase ‘Blobs’ and Other Characteristics of Primary Visual Cortex in a Lemurid Primate, *Cheirogaleus medius*

**Key Words**
- Striate cortex
- Area 17
- Extrastriate cortex
- Middle temporal area (MT)
- Cytochrome oxidase
- Acetylcholinesterase
- Cat-301
- Stereophines
- Prosimians
- Primate evolution

**Abstract**
We recently obtained the brain of a rare lemurid primate, *Cheirogaleus medius*. The brain was not perfused before death, but rather fixed by immersion shortly thereafter. In both flat-mounted and transversely sectioned tissue, we were able to clearly demonstrate periodic zones of high cytochrome oxidase (CO) activity in the primary visual cortex, resembling the so-called ‘blobs’ described in many other primate species. Our results contrast with a previous report indicating that blobs are absent in *Cheirogaleus medius* and provide support for the view that blobs are an evolutionary specialization of primate visual cortex that evolved only once, early in primate history. In other aspects of architectonic organization, area V1 of this *Cheirogaleus* individual closely resembles that of other strepsirhine primates, such as *Galago*. We were able to identify additional divisions of cortex in this individual, including the middle temporal visual area (MT), auditory cortex, and the primary somatosensory area (S1 or area 3b). These observations indicate that valuable neuroanatomical information can, in favorable cases, be obtained from rare mammalian species that die of natural causes in captivity or which must be euthanized, even though the animals have not been perfused.

**Introduction**
Our understanding of mammalian brain organization is based on the study of relatively few taxa, mainly macaque monkeys, rats, and cats. The emphasis on these few taxa reflects the fact that the most powerful techniques for studying neural connectivity and brain function involve invasive procedures with live animals, followed by perfusion for optimal tissue fixation. Such procedures, however, are not conducive to the study of the many rare or endangered mammalian species, as well as to the study of species that are difficult to keep in captivity. Unfortunately, without information about a wide range of mammalian taxa, it is difficult to reconstruct in detail the course of mammalian brain evolution. The problem is particularly acute for primates, because so many of the approximately 200 primate species [Fleagle, 1988] are rare or endangered, including such important groups as the lemurs of Madagascar and the tarsiers. Of course, brain tissue obtained post mortem can be stained for Nissl and myelin, but these techniques...
provide limited information about brain structure, and inferences about brain evolution derived from the study of cyto- and myeloarchitecture alone have proven highly controversial [see, for example, Lashley and Clark, 1946]. Therefore, it is important to expand the array of non-invasive techniques for studying brain organization, techniques that can be used to study brains of rare or protected species that have died of natural causes in captivity or that have been euthanized.

In recent years, neuroscientists have developed a variety of new histochemical and immunocytochemical techniques for localizing specific brain molecules, ranging from metabolic enzymes such as cytochrome oxidase (CO), neurotransmitters and related enzymes, such as acetylcholinesterase (AChE), and structural proteins, such as the extracellular matrix constituent recognized by the monoclonal antibody Cat-301. These molecules can serve as markers for particular structures and systems within the brain [Horton, 1984; Tootell et al., 1985; Wong-Riley, 1989; Krubitzer and Kaas, 1990; Morel and Kaas, 1992]. For example, Cat-301 preferentially labels the magnocellular layers of the lateral geniculate nucleus as well as portions of visual cortex that receive magnocellular inputs [see Jain et al., 1994, for review]. Moreover, because many of these techniques do not require perfusion fixation, in principle they can be used to study a very wide range of taxa.

One feature of particular phylogenetic interest that can be studied using non-invasive techniques is the distribution of cytochrome oxidase in primary visual cortex (V1). In most primate species examined to date, histological sections through V1 exhibit a periodic array of circumscribed territories with high levels of CO activity. These territories have been referred to as CO ‘patches’ [Horton, 1984], ‘puffs’ [Wong-Riley, 1989], and most commonly, ‘blobs’ [Livingstone and Hubel, 1984]. The spatial arrangement of blobs is best appreciated in sections that have been flattened and sectioned parallel to the cortical surface. In sections that pass through the thickness of the cortex (such as standard transverse sections), blobs appear as localized regions of increased CO staining density both superficial to and deep to layer IV, which itself is very densely stained in primary visual cortex [Casagrande and Kaas, 1994]. Blobs are surrounded by ‘interblob’ regions of lower CO density. In addition to their different levels of metabolic activity, blobs and interblobs have different afferent and efferent connections. Thus, blobs and interblobs are regarded as distinct modular subdivisions of V1 [Livingstone and Hubel, 1988; Casagrande, 1994; Casagrande and Kaas, 1994].

Blobs have been demonstrated in a wide variety of different primate species (fig. 1), including all New World and Old World anthropoids that have been examined so far, as well as several strepsirhine (prosimian) species (reviewed by Horton [1984] and Preuss et al. [1993]). Most other mammalian groups that have been examined appear to lack blobs. Among the mammals thought to be most closely related to primates, CO blobs have not been observed in tree shrews [Wong-Riley and Horton, 1988] or megachiropteran bats [see fig. 9 of Rosa et al., 1993]. Carnivores show a periodic pattern of CO staining in V1 [Murphy et al., 1991; Cresho et al., 1992; Dyck and Cynader, 1993], but it is unlikely these patches are homologous to primates blobs, given the apparent absence of blobs in taxa more closely related to primates.

In view of the phylogenetic distribution of blobs, it has been suggested that they are an evolutionarily derived feature (autapomorphy) of primate V1, that is, a feature of organization that evolved in early primates, prior to the radiation of modern forms [Horton and Hubel, 1981; Horton, 1984; Allman and McGuiness, 1988; Kaas and Preuss, 1993; Preuss et al., 1993; Casagrande and Kaas, 1994]. However, blobs are reported to be absent in some primates. McGuiness et al. [1986] were able to obtain the brains of two Madagascar primates, the gentle lemur, Hapalemur

<table>
<thead>
<tr>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE</td>
</tr>
<tr>
<td>Aud</td>
</tr>
<tr>
<td>CaS</td>
</tr>
<tr>
<td>CC</td>
</tr>
<tr>
<td>CO</td>
</tr>
<tr>
<td>EB</td>
</tr>
<tr>
<td>ER</td>
</tr>
<tr>
<td>HC</td>
</tr>
<tr>
<td>IB</td>
</tr>
<tr>
<td>IT</td>
</tr>
<tr>
<td>LGN</td>
</tr>
<tr>
<td>LMZ</td>
</tr>
<tr>
<td>LS</td>
</tr>
<tr>
<td>MT</td>
</tr>
<tr>
<td>OB</td>
</tr>
<tr>
<td>OT</td>
</tr>
<tr>
<td>Pcl</td>
</tr>
<tr>
<td>Pir</td>
</tr>
<tr>
<td>PSub</td>
</tr>
<tr>
<td>RS</td>
</tr>
<tr>
<td>S1</td>
</tr>
<tr>
<td>SL</td>
</tr>
<tr>
<td>V1</td>
</tr>
<tr>
<td>V2</td>
</tr>
<tr>
<td>WM</td>
</tr>
</tbody>
</table>

Preuss/Kaas
Fig. 1. Two views of the phylectic distribution of cytochrome oxidase-rich blobs in the primary visual cortex of primates and related mammals. On current evidence, recently reviewed by Simmons [1993], the closest relatives of primates are bats (Order Chiroptera; Suborders Megachiroptera and Microchiroptera), flying lemurs (Order Dermoptera), and tree shrews (Order Scandentia). Primates comprise two suborders, Strepsirhini and Haplorhini. The entire clade is referred to as Superorder Archonta. Among archontans, the existence of blobs has been clearly demonstrated in New World anthropoid primates and the lorisoids (galagos and lorisises), as indicated by the filled boxes and branches beneath the taxon names. Tree shrews and megachiropteran bats lack blobs, as indicated by the open boxes and branches. The states for microchiropteran bats and flying lemurs are unknown and are represented with gray branches. At issue here are the character states of lemuroid primates and tarsiers. A McGuiness et al. [1986] reported that blobs are absent in the lemuroid Hapalemur griseus, in the cheirogaleid Cheirogaleus medius, and in two species of tarsiers (Tarsiidae). (The taxa they studied are indicated with asterisks.) The most parsimonious interpretation of this character distribution (requiring two changes) is that blobs evolved twice in primate history, once in lorisoids and once in anthropoids. An alternative account (requiring three changes), would have blobs evolve once in primate ancestry, with independent loss in the lemuroid and tarsiid branches. B An alternative interpretation of the phylogeny of blobs. The present study documents the existence of blobs in a Cheirogaleus medius individual, contrary to McGuiness et al. [1986]. Our positive results, obtained with different techniques, suggest that previously reported negative results for Cheirogaleus, Hapalemur, and Tarsiidae, may be erroneous. If cheirogaleids are coded as having blobs, and the condition for lemuroids and tarsiers coded as unknown, the most parsimonious interpretation of character distribution is that blobs evolved only once, early in primate evolution.

It should be noted that some morphologists believe that cheirogaleids belong to the loris-galago group, rather than to the lemuroids. If that is the case, then the present study provides no information about the lemuroid condition. However, recent reviews of molecular, morphological, and genetic data make a strong case that cheirogaleids are indeed lemuroids [Martin, 1990; Yoder, 1994].

Note Added in Proof

Paolini et al. [1994] report the presence of CO-dense blobs in area V1 of a brown lemur (Lemur fulvus). This result further strengthens the case for the monophyletic origin of primate blobs.
griseus (n = 2 individuals), and the fat-tailed dwarf lemur, *Cheirogaleus medius* (n = 1), as well as brains from two tarsier species, *Tarsius syrichta* (n = 2) and *T. bancanus* (n = 1). These animals were acquired from the Duke University Primate Center, where their brains were removed and frozen at −70°C within minutes after death before being shipped to McGuiness et al. The brains were subsequently fixed by immersion in cold glutaraldehyde, sectioned in conventional planes, and stained for CO. As a control, brains of the strepsirhine Galago crassicaudatus (n = 3), which is known to possess blobs, were prepared in the same fashion. McGuiness et al. reported that blobs were absent from the brains of *Hapalemur*, *Cheirogaleus*, and *Tarsius* so prepared, despite evidence that CO activity was preserved in the tissue. Furthermore, blobs were present in the *Galago* brains. The results of McGuiness et al. have important implications for our understanding of visual cortical evolution in primates. The most parsimonious explanation of the phylogenetic distribution of blobs suggested by McGuiness et al. is that blobs evolved independently in anthropoids and lorises and are therefore not homologous in these groups (fig. 1A). Alternatively, but less parsimoniously, blobs may have evolved once, early in primate ancestry, but were lost independently in lemuroids and tarsiers.

Since McGuiness et al. are the only workers so far to report primates without blobs, we considered it important to attempt to validate their results. In particular, we wanted to look for blobs in flat-mounted cortical sections, the preparation in which they are most easily demonstrated. We obtained the brain of a single dwarf lemur (*Cheirogaleus medius*), shipped to us by the Duke University Primate Center shortly after its death. One of the smallest primate species [Fleagle, 1988, pp. 72–75], *C. medius* has small, unfissured hemispheres ideal for flattening. We did not freeze the brain but rather fixed it by immersion in paraformaldehyde. The cortex of one hemisphere was flattened before sectioning parallel to the cortical surface. The other hemisphere was sectioned in the coronal plane. Blobs dense with CO were clearly visible in both flattened and transverse sections through the primary visual cortex of this individual, as were other features of cortical organization.

tain itself, they euthanized it. Within two hours of death, the head was removed, immersed in a solution of 2% paraformaldehyde and 30% sucrose, and shipped on ice to our laboratory. Upon arrival the following morning, the foramen magnum was enlarged and the head immersed in a cold solution of 4% paraformaldehyde and 30% sucrose on a rocker platform. After two hours, the left cerebral cortex was dissected away from the underlying white matter and manually unfolded and flattened, in the manner described by Preuss et al. [1993]. The flattened hemisphere was then frozen and sections cut at 40 μm, parallel to the cortical surface. The right hemisphere and attached brainstem were immersed in a cold solution of 4% paraformaldehyde and 30% sucrose overnight on a rocker prior to being frozen sectioned in the transverse plane at 50 μm.

From the flattened left cortex, alternate series of sections were stained for Nissl, myelin, and cytochrome oxidase. From the right hemisphere and brainstem, alternate series of sections were stained for Nissl, Cat-301, myelin, CO, and acetylcholinesterase (AChE). Myelinated fibers were stained using a modification of the technique of Gallay [1979]. Cytochrome oxidase was revealed histochemically by immersing the sections in 0.1% cobalt chloride for 20 minutes and then reacting them according to the protocol of Wong-Riley [1979]. Acetylcholinesterase activity was revealed with the histochemical procedure of Geneser-Jensen and Blackstad [1971]. For Cat-301 immunostaining, sections were incubated in chondroitinase ABC, rinsed, and incubated in a 1:20 dilution of the Cat-301 antibody for four days at 4°C. Bound antibody was revealed by immersing the sections in biotinylated secondary antibody, then in streptavidin-peroxidase conjugate, and then in a solution of diaminobenzidine, nickel, and hydrogen peroxide. For more details of our immunostaining procedure, see Jain et al. [1994]. The Cat-301 antibody was kindly supplied by Dr. Susan Hockfield. Sections were examined, drawn, and photographed at a variety of magnifications. The dimensions of isocortical surface area, of area V1, and of blobs, were measured from drawings of flattened sections. Sections processed for Nissl, CO, and myelin shrank to varying degrees; because the Nissl sections evinced the least shrinkage, all measurements were adjusted to a Nissl standard. In estimating the surface area of isocortex in this individual, we excluded olfactory structures and the tissue lateral to the rhinal sulcus, which consists of allocortex (see fig. 2). Alternative interpretations of character evolution were evaluated, and the trees in figure 1 generated, using MacClade software [Maddison and Maddison, 1992].

**Results**

Using the histological material described above, we were able to identify the primary visual area (V1) and several extrastriate visual territories in this dwarf lemur individual.

**Characteristics of V1**

The V1 was readily identifiable along the occipital pole of the hemisphere in both flattened and transverse sections (fig. 2, 3). Its location and extent were best appreciated in flattened sections stained for CO or Nissl, in which V1 stained more darkly than surrounding areas in sections taken through middle levels of the cortex. Also, the border...
Fig. 2. The location of cortical areas in a *Cheirogaleus medius* individual, based on examination of sections stained for cytochrome oxidase, myelin, and Nissl. **Above:** A composite reconstruction of the flattened left hemisphere. Rostral is to the left, dorsal and medial to the top. V1 and V2 are the primary and secondary visual areas, respectively. MT is the middle temporal visual area. A heavily myelinated region denoted here as 'Aud' resembles the cortical region which in other primates includes the primary auditory area and the rostral auditory area [Morel and Kaas, 1992]. In addition to visual and auditory areas, we observed a region within the frontoparietal cortex characterized by dense CO and Nissl staining. This region resembles in location and architectonic appearance the primary somatosensory area (S1, also known as area 3b) identified in other strepsirhine and anthropoid primates [e.g., Tootell et al., 1985; Preuss and Goldman-Rakic, 1991]. The calcarine sulcus (CaS) and lateral sulcus (LS) were cut as part of the process of flattening the cortex. Rostral is to the left. **Below:** The location of cortical areas depicted on a dorsolateral view of the brain.

Fig. 3. The appearance of area V1 in sections stained for CO. **A** A flattened section through the superficial layers of the left cortex. Rostral is to the left, dorsal and medial to the top. Dark CO blobs are clearly visible in V1, although they appear blurred or merged along the paracalcarine sulcus (PcS). Several blobs located along the rostral border of V1 are marked by arrows. At this superficial level of the cortex, the imprint of blood vessels running along the cerebral surface can be seen rostral to V1. Scale bar = 2 mm. **B** A coronal section through the occipital lobe of the right hemisphere. Although less obvious in such sections, blobs are nonetheless clearly visible (as indicated by the arrows). Observe the apparent merging of CO blobs in the superficial cortical layers within the depth of the CaS. The very dense band of CO staining in V1 corresponds approximately to layer IV. Medial is to the left, dorsal to the top. Scale bar = 2 mm.
between V1 and the second visual area, V2, was marked by a fiber-sparse gap in myelin-stained sections taken through superficial layers. In the left hemisphere, the surface area of V1 measured 66 mm², accounting for approximately 12% of the cortical mantle (i.e., isocortex plus prosocortex) in this hemisphere.

The most striking histological characteristic of V1 was the pattern of CO-rich blobs visible in flattened sections taken from levels superficial to layer IV (fig. 3A). Blobs were evident over virtually the entire extent of V1, an exception being the extreme dorso medial region, where CO-dark regions appeared more stripe-like than blob-like. This may reflect a tendency for blobs to be aligned or to merge in certain parts of V1. Blobs were rather irregular in form, and neighboring blobs were sometimes poorly demarcated. Individual blobs were typically somewhat elliptical in appearance. For a set of 35 relatively discrete blobs in the left hemisphere, we calculated a median width of 0.27 mm and a median length of 0.35 mm. The median distance between blobs, measured center-to-center for 28 pairs of blobs, was 0.51 mm.

Although V1 blobs were most apparent in flattened sections, they could also be observed in sections cut transversely and stained for CO (fig. 3B). In such sections, it could be seen that elevated levels of CO staining were not restricted to superficial layers (II–III) of cortex but were present in the deep layers (V–VI) as well. Staining of CO in layer IV was extremely dense in all parts of area V1, but even in this layer there was some suggestion of increased staining in register with the dark CO zones of the superficial and deep layers. It is noteworthy that within the calcarine sulcus, where the dorsal and ventral banks of the sulcus were closely apposed, neighboring blobs appeared to merge.

In Nissl-stained sections, area V1 was distinguished by a specialization of layer IV (fig. 4A). As in other primates, and most other mammals, layer IV was very densely
packed with cells and was coincident with a band of dense CO staining (fig. 4B). Furthermore, as in other primates, layer IV was comprised of several sublayers, although the stratification of layer IV in this individual was noteworthy in certain respects. Specifically, within layer IV there was a conspicuous light band composed of very small cells, which separated the broad, dark upper band from a thinner, dark lower band. The light intermediate band, here dubbed layer IVL, was prominent over most of V1, but absent in the portion of V1 lying in the depth of the calcarine sulcus. The upper band, which consisted of a mixture of small cells and larger (apparently pyramidal) cells, resembled layer IVα of other primates, which is the portion of layer IV that receives projections from the magnocellular layers of the LGN. The lower stratum, comprising predominantly small cells, resembled layer IVβ of other primates, which receives inputs from the parvocellular layers of LGN [Casanegra and Kaas, 1994].

In addition to its striking appearance in CO- and Nissl-stained sections, area V1 evinced distinctive staining for AChE and for myelin (fig. 4C, D). There was a very high level of AChE activity in area V1, with bands of densely stained neuropil in layer I (and possibly layer II), in layer IV, and in layer VI. Myelinated fibers were concentrated in three bands, located in layer I, in the upper part of layer IV and deep layer III (corresponding to the stria of Gennari, or the external band of Baillarger), and in layers V and VI. In general, however, horizontal fiber bands were not well developed: the external band was rather indistinct and the internal band was not distinguishable from the substrate lamina. (The terminology of cortical fibers band used here is modified from Braak [1980].)

Cortical sections reacted with the Cat-301 antibody showed no evidence of specific staining in V1 or in other cortical areas. The absence of specific staining in the cortex probably does not reflect a failure of the immunocytochemical procedures, because we observed strong staining in certain brainstem regions, including the red nucleus, deep cerebellar nuclei, reticular formation, and pontine gray. Furthermore, we observed somewhat higher levels of reaction product in the magnocellular laminae of the lateral geniculate nucleus compared to the parvocellular layers, although the difference was marginal.

Other Visual Areas

In myelin-stained flattened sections through the upper layers of cortex, we identified an oval-shaped region of dense staining situated between V1 and the auditory region (fig. 5). The same territory was densely stained in CO sections. Based on its location, and its dark appearance in myelin- and CO-stained sections, this region is probably homologous to the middle temporal area (MT) identified in other primates [Allman and Kaas, 1971; Krubitzer and Kaas, 1990; Preuss et al., 1993]. On the inferior temporal gyrus, lateral and somewhat posterior to MT, there was an additional region of moderately well-myelinated tissue that may correspond to a portion of the inferotemporal (IT) visual cortex identified in other primates [Preuss and Goldman-Rakic, 1991]. Immediately lateral to MT, intercalated between the heavily myelinated auditory region and the moderately myelinated cortex of the inferior temporal gyrus, we noted an elongated, lightly myelinated zone of cortex (LMZ), similar to that observed in Galago [Preuss and Goldman-Rakic, 1991]. The LMZ resembles a portion of the polysensory cortex buried within the superior temporal sulcus of anthropoid primates [Preuss and Goldman-Rakic,
we were not able to identify the rostral border of V2 in our dwarf lemur material. In anthropoid primates, V2 can be identified in flattened sections by its alternating dark and light bands of CO staining [Tootell et al., 1983; Horton, 1984; Krubitzer and Kaas, 1990]. By contrast, banding of V2 is weak in the strepsirhines Galago crassicaudatus and Nycticebus coucang, and virtually undetectable in Galago senegalensis [Condo and Casagrande, 1990; Krubitzer and Kaas, 1990; Preuss et al., 1993]. In the dwarf lemur, we could detect no definite bands of CO staining in V2.

Discussion

Our results suggest that brains fixed by immersion in 2–4% paraformaldehyde for 24–48 hours are potentially very useful for comparative anatomical studies. We were able to demonstrate high levels of enzymatic activity in the brain of a dwarf lemur so prepared, using routine histochemical stains to reveal CO and AChE, and good results were obtained with conventional Nissl and myelin stains. Moreover, we were successful in flattening a cerebral hemisphere in this animal, a procedure that is especially valuable for investigating the areal and modular organization of the cortex.

The most important result of this study is the clear demonstration of CO-dense blobs in V1 of both the flattened and transversely sectioned hemispheres in this animal. The blobs of this Cheirogaleus appeared rather irregular and indistinct in form and thus similar to those of other strepsirhine primates in which blobs have been described (Galago, Nycticebus), in contrast to the very well-defined, punctate blobs that have been observed in anthropoid primates (see especially Condo and Casagrande [1990], Krubitzer and Kaas [1990], and Preuss et al. [1993]). The blobs of this dwarf lemur were slightly larger than those of the strepsirhine primates Galago and Nycticebus, despite the smaller size of the dwarf lemur brain and V1 [cf. Condo and Casagrande, 1990; Krubitzer, 1989; Preuss et al., 1993]. This is an exception to the generalization that blob size increases as V1 increases in size [Horton, 1984].

Given the similar patterns of cytochrome oxidase staining in the left and right hemispheres of this individual, and the qualitative and quantitative similarities to staining patterns demonstrated in Galago and Nycticebus, we feel confident in concluding that the blob-like distribution of CO staining in area V1 of this individual Cheirogaleus medius was not artifactual. Our demonstration of V1 blobs in a Cheirogaleus medius individual stands in contrast to the results of McGuiness et al. [1986], who reported that blobs were absent in the one individual of this species they examined. There are at least two ways to interpret this discrepancy. One possibility is that dwarf lemurs are polymorphic in their expression of blobs; that is, some individuals have them and some do not. A second possibility, and more likely in our view, is that the techniques used by McGuiness et al., which involved freezing the unfixed brain, followed by thawing and postfixation in glutaraldehyde, were not as conducive to the preservation and demonstration of cytochrome oxidase as the procedures we employed. Moreover, blobs are most readily demonstrated in flattened sections, and the techniques used by McGuiness et al. did not include flattening the cortex. This interpretation of the negative results reported by McGuiness et al. in Cheirogaleus also calls into question their negative results in the lemuroid Hapalemur and the tarsiod Tarsius.

Based on presently available evidence, we believe the most parsimonious — and thus most defensible — interpretation of the phylogeny of primate blobs is that they evolved just once early in primate history, prior to the diversification of modern primate groups, and have been retained in these modern groups (fig. 1B). We stress that we regard this conclusion as tentative. The available evidence is extremely limited, both with regard to the range of primate and non-primate taxa that have been investigated and the number of individual animals studied. Certainly, we would not rule out the possibility that blobs have evolved (or been lost) on multiple occasions in mammalian evolution. Indeed, as discussed above, we consider it likely that carnivores evolved a blob-like CO pattern in area V1, independently of primates.

In addition to the presence of blobs, area V1 of the dwarf lemur examined here showed a number of similarities to other strepsirhine primates that have been studied. In particular, layer IV consisted of two main cellular strata, an upper band composed of small and larger cells and a lower band of mainly small cells. We designated these strata as IVα and IVβ, using the laminar typing of V1 developed by Hässler [1967]. In Galago, in which the organization of layer IV is very similar, the upper band is the target of projections from the magnocellular layers of the lateral geniculate, while the lower band receives projections from the parvocellular LGN layers [Diamond et al., 1985; Florence and Casagrande, 1987]. In area V1 of most anthropoids, in Tarsius, and in some strepsirhines, there is an additional, dense stratum of small cells superficial to layer IV proper. This stratum, which is termed layer IIIb in the terminology of Hässler [1967], or layer IVA according to Brodmann [1909], was not apparent in our dwarf lemur material, nor has it been observed in Galago.
One notable feature of our dwarf lemur material was the presence of a well-defined light band separating IVα from IVβ. This does not appear to be an individual anomaly, for a very similar band has been described in the closely related cheirogaleid primate, *Microcebus murinus* [Le Gros Clark, 1931; Cooper et al., 1976]. A pale band within layer IV is also present in the lorisid genus *Galago*, having been described in *G. demidovii* by Solntzky and Harman [1946]. It should be noted that much of the cortex we would include within sublayer IVα corresponds to cortex included within layer III by Solntzky and Harman [1946] and Cooper et al. [1976]. A pale intermediate band can be seen in some photomicrographs of *Galago senegalensis* (for example, fig. 4b of Diamond et al. [1985]), and we have observed it in *G. garnettii* [unpubl. observ.]. It appears to be present in the haplorhine *Tarsius* also, based on the descriptions and photomicrographs of Woolard [1925] and Hässler [1967]. To our knowledge, a pale band between Hässler’s layers IVα and IVβ (corresponding to layers IVCα and IVCβ in Brodmann’s terminology) has not been described in the adult cortex of any anthropoid primate.

On present evidence, it is unclear whether the pale intermediate band of layer IV in *Galago*, and by inference that in *Cheirogaleus*, receives projections from the magnocellular or parvocellular layers of the LGN. However, in *Galago* the cells in layer IVβ, which receive parvocellular LGN projections, are generally smaller than those in layer IVα [Diamond et al., 1985], the target of magnocellular LGN projections. This suggests that the intermediate band, composed exclusively of very small cells, may also receive projections from the parvocellular LGN.

The laminar distribution of CO and AChE in the VI of this *Cheirogaleus* was generally very similar to that described previously for *Galago*, with dense concentrations of both enzymes in layers IV and VI [Fitzpatrick and Diamond, 1980; Condo and Casagrande, 1990]. One possible difference is that the band of AChE staining in layer VI appeared to be much denser in the *Cheirogaleus* individual than in *Galago*, based on the published photographs of Fitzpatrick and Diamond [1980] and on examination of our own unpublished *Galago* material. By contrast to the simple, trilaminar patterns of CO and AChE staining of VI exhibited by *Cheirogaleus* and *Galago*, anthropoid primates show more complex patterns, with stratification of layers III and IV [Fitzpatrick and Diamond, 1980; Hedreen et al., 1984].

The monoclonal antibody Cat-301 has been used to explore the organization of the visual thalamus and cortex, where it appears to preferentially bind to elements within the Y-cell (or magnocellular) systems of the cortex or thalamus [Hendry et al., 1988; DeYoe et al., 1990; Hockfield and Sur, 1990]. However, Cat-301 is not a completely reliable marker for magnocellular systems, as it fails to bind preferentially to magnocellular-recipient layer IVα in macaques [as discussed by Jain et al., 1994]. Moreover, whereas workers have reported good immunostaining with Cat-301 in anthropoid primates, carnivores, and tree shrews, studies have revealed little if any specific staining in the lateral geniculate or visual cortex of the strepsirhine *Galago* [Hendry et al., 1988; Jain et al., 1994]. We obtained similar, virtually negative, results with our *Cheirogaleus* material, despite staining of brainstem structures. This suggests that proteoglycan recognized by Cat-301 is expressed at very low levels in the thalamus and cortex of strepsirhines, or alternatively, that a variant form of the proteoglycan is expressed in the thalamus and cortex of these animals, which lacks the epitope recognized by Cat-301 [Jain et al., 1994].

**Acknowledgments**

Supported by NIH EY02686 and the McDonnell-Pew Program in Cognitive Neuroscience (JSMF No. 90-35). We are grateful to Dr. Kenneth Glander, Dr. Patricia Feeser, and the staff of the Duke University Primate Center for their assistance in this project. This is DUPC Publication No. 292.
References


