Retinofugal Projections in the Short-Tailed Opossum 
(*Monodelphis domestica*)

DIAANNA M. KAHN AND LEAH KRUBITZER*

Center for Neuroscience and Department of Psychology, University of California, Davis, 
Davis, California 95616

ABSTRACT

In the current investigation, retinofugal projections to midbrain and thalamic nuclei of *Monodelphis domestica* were investigated using wheat-germ agglutinin conjugated to horse-radish peroxidase (WGA-HRP). Large intraocular injections of WGA-HRP were placed into the eye, and patterns of labeled axon terminals were related to nuclear boundaries in tissue that was stained for Nissl or reacted for cytochrome oxidase (CO). Our results demonstrate that the major projection from the retina is to the contralateral dorsal lateral geniculate nucleus (LGNd) and the superior colliculus (SC). Connections were also observed with the contralateral pretectal nucleus (PRT), the lateral posterior nucleus (LP), and the ventral division of the lateral geniculate nucleus (LGNv). Ipsilateral connections were with the LGNd and LGNv. These findings are consistent with reports in other marsupials as well as with studies in a number of eutherian mammals. Thus, there appears to be a common pattern of retinofugal projections that all mammals share, probably due to retention from a common ancestor. However, some features such as a lack of ipsilateral input to the SC (which are absent only in certain species like *Monodelphis*, platypus, and echidnas) may represent a primitive state retained from a common ancestor. When comparisons of retinofugal connections and LGNd organization are made across taxa, three types of organization are observed: a homogenous LGNd with a high degree of binocular overlap of projections; a partially differentiated LGNd with some segregation of eye-specific inputs; and a fully segregated structure with a large degree of segregation of eye-specific inputs. We discuss the factors that contribute to the organization observed in extant mammals and conclude that phylogeny and lifestyle appear to be the underlying factors contributing to the organization of the LGNd. J. Comp. Neurol. 447:114–127, 2002. © 2002 Wiley-Liss, Inc.

Indexing terms: lateral geniculate nucleus; evolution; marsupials; visual system; superior colliculus; WGA-HRP

One of the challenges when examining any particular neural structure is to determine the sources that contribute to its present organization and whether some subset of its morphological features is requisite for its function. Is a specific type of organization necessary to perform a particular task, or are some features of organization epiphenomenal and the result of underlying, highly constrained, developmental regimes? For example, are ocular dominance columns in the primary visual area of primates necessary for binocular vision? Are the barrels in the primary somatosensory cortex of some rodents necessary for a specialized type of tactile discrimination? In the case of the dorsal lateral geniculate nucleus (LGNd), is the striking laminar organization observed in a number of species requisite for some fundamental function?

When examining the LGNd, one is struck by the differences in morphology that different species possess, as well as the differences in the segregation of inputs from the two eyes (Kaas et al., 1978; Sanderson et al., 1984, 1987). However, within the realm of possible organizations, the
LGNd encompasses only a few types. For instance, although some animals have highly segregated inputs from each eye and others have a large amount of binocular overlap, the way in which inputs are segregated is restricted to sets of interleaving laminae. Such observations argue that only a few sources or factors have a significant impact on the organization of the LGNd in mammals. One of the factors thought to contribute to the variation in visual system organization is the ecological niche of the animal, or the degree to which an organism depends on its visual system for survival behaviors such as prey capture and resource acquisition (Sanderson et al., 1984; Barton and Purvis, 1995; Chiel and Beer, 1997). Another factor, at least for eutherian mammals, is whether an animal is diurnal or nocturnal. This is less of an issue for prototherians, because they are exclusively nocturnal, and metatherians, which are almost exclusively nocturnal and/or crepuscular.

In eutherian mammals, cytoarchitectural lamination of the LGNd and segregation of retinal afferents into eye-specific layers is generally associated with animals with more frontally placed eyes, such as carnivores and primates. Both groups are highly dependent on visual processing for survival and their visual systems are organized in a complex fashion. For example, the LGNd of carnivores and primates consists of precisely organized eye-specific laminae, each receiving information from functionally distinct retinal cell types (Stone and Hansen, 1966; Kaas et al., 1989; Nieuwenhuys, 1994). During development, the structure in question (Brauer et al., 1978; Pettigrew et al., 1989, 1994). During development, the presence of homologous genes and the similarities in their patterns of spatial and temporal expression presumably result in similar phenotypes. Conversely, changes in these patterns, or mutations, can cause large phenotypic differences. However, because genetic change in the form of deletions that produce viable offspring are rare, the types of observed change are rather limited. Thus, the course of future evolution in any species is restricted by the cascade of developmental events that generate the nervous system of a particular group, and presumably more closely related species would share more ontogenetic features than distantly related ones. One way to tease out the various contributions to the existing phenotype, in particular to the organization of the visual system, is to examine a number of species from a given lineage that occupy a variety of niches.

In the present investigation we examined the retinofugal connections of the South American short-tailed opossum, Monodelphis domestica, by injecting anatomical tracers into the eyes and examining the patterns of labeling in thalamic and midbrain structures. We compared our results with those of other marsupials and mammals in an effort to determine common features of visual system organization across groups, and the factors that may contribute to phenotypic variability in the organization of the lateral geniculate nucleus.

**MATERIALS AND METHODS**

**Intraocular injections**

Six short-tailed opossums (M. domestica) were used to examine retinal projections (Table 1). Each animal was anesthetized with a cocktail of ketamine (40 mg/kg) and...
xylazine (5 mg/kg) administered intramuscularly. Lidocaine was applied topically onto the eye and subcutaneously into the tissue surrounding the eye. Body temperature was maintained by placing the animal on a heating pad during the surgery and was monitored throughout the experiment. Once a surgical level of anesthesia was reached, a monocular intravitreal injection of a 4% solution of wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP; Sigma, St. Louis, MO) in 0.9% sterile saline was made in one eye. Injections were made by inserting the needle into the eye so that the tip of the needle could be visualized at the edge of the vitreous humor. In two cases (98-2, and 98-27) 5 μl of WGA-HRP was injected using a 10-μl Hamilton syringe with a 25-gauge needle. In four cases (98-14, 98-16, 98-23, and 99-44), a catheter needle was inserted into the opposite pole of the eye to relieve intraocular pressure while a large volume of tracer (50 μl) was injected. Immediately following the injections, an antibacterial ointment was topically applied to the eye and the animal was allowed to recover.

To study transport of WGA-HRP in this species, the first goal was to produce consistent labeling in the LGN. Initial experiments, using injections that had proved successful in other small animals, produced variable labeling of terminals in this species. To clarify these results, different transport times were adjusted to maximize intensity and density of labeling in the thalamus. We used transport times of 2, 7, or 14 days (Table 1). We found that a transport time of 2 days produced optimal staining of labeled axons in the LGN and that at 7 and 14, days the density and intensity of labeled terminals decreased.

At either 2, 7, or 14 days after injection, animals were euthanized with an intrapitoneal injection of sodium pentobarbital (250 mg/kg) and perfused transcardially with 0.9% saline and 0.1% heparin, followed by a fixative of 3% paraformaldehyde and then 3% paraformaldehyde with 10% sucrose. Following fixation, the brain was removed from the skull, and the thalamus and brainstem were dissected free from the rest of the brain and immersed in 30% sucrose overnight. The fixed, cryoprotected brains were sectioned on a freezing sliding microtome in the coronal plane at 40 μm thickness. Sections were collected in 0.1 M phosphate buffer (PB; pH 7.4). All experimental protocols were approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, and conformed to NIH guidelines.

**Tissue processing**

To visualize the WGA-HRP labeling, free-floating sections were processed with tetramethylbenzidine (TMB) using the method of Mesulam et al. (1980), as modified by Gibson et al. (1984). Alternate sections were processed for cytochrome oxidase (CO; Caroll and Wong-Riley, 1984), myelin (Gallyas, 1979) and Nissl substance.

Additionally, the extent of the injection was examined in flat-mounted retinas processed for TMB. Retinas of the un.injected eyes were also processed for TMB, as a negative control. The low levels of background staining in the control eyes were clearly distinguishable from the labeling in the injected retinas. Following perfusion, the eyes were removed from the skull, and the lens and vitreous were dissected from the retinas. The sclera and pigment epithelium were dissected away, and the retinas were reacted for TMB and then flattened using four radial cuts. Following the TMB reaction, all tissue was mounted onto gelatin-coated slides, TMB sections were dehydrated in methyl salicylate, and other sections were dehydrated in alcohol, then all slides were cleared in xylene and coverslipped using DPX (Sigma-Aldrich) mounting media.

**Data analysis**

TMB-reacted tissue was analyzed using polarized lenses attached to a stereomicroscope. With the aid of a camera lucida drawing tube, outlines of individual sections were drawn, along with labeled cell bodies and axon terminals. For the thalamus, each drawing included blood vessels and tissue artifacts to aid with reconstruction of the tissue. Adjacent myelin-, Nissl-, and CO-stained sections were drawn and included the outline of each section, blood vessels, tissue artifacts, and architectonic boundaries. All drawn sections were then aligned using the landmarks indicated above and were compiled into one comprehensive reconstruction. In this way, the location of WGA-HRP-labeled retinal axon terminals could be directly related to individual thalamic nuclei.

**Production details**

Digital photomicrographs of tissue sections were taken using a Spot RT camera and software (Diagnostic Instruments, Sterling Heights, MI). Adobe Photoshop 6.0 software was used to make linear adjustments of brightness and contrast so that the electronic version most closely matched the tissue sections.

**RESULTS**

**Architectonic subdivisions of the dorsal thalamus and midbrain structures**

In coronally cut Nissl sections, the dorsal and ventral divisions of the lateral geniculate nucleus (LGNd and LGNv, respectively) were easily identified (Fig. 1). The darkly stained LGNd was not overtly striated; however, it appeared to have at least two major subdivisions (Fig. 1b), which have been referred to as α and β subregions in most marsupials studied (Sanderson et al., 1979; Haight and Neylon, 1981; Haight and Sanderson, 1988, 1990). Along the lateral border of the LGNd, adjacent to the optic tract, there was a region extending from the dorsal to ventral poles, comprised mainly of large cells that were moderately to darkly stained. This subregion corresponded to the LGNα region described in other marsupials. More medially, cells were smaller, lightly stained, more loosely packed, and architecturally similar to the LGNβ region described in some marsupials. We have denoted the boundary between the subregions of the LGNd with a dashed line. Unlike the situation in other marsupials (Sanderson et al., 1987), the LGNα did not appear to be further subdivided. In CO-reacted tissue, the entire LGNd was darkly stained across the nucleus (Fig. 1c).

The divisions we describe below are like those described by Jones (1985) for a variety of mammals. The wedge-shaped LGNv could be clearly distinguished immediately ventral and slightly caudal to LGNd. In Nissl-stained tissue, the LGNv was identified as a nucleus that contained small, tightly packed, darkly stained neurons (Fig. 1b). In tissue reacted for CO, the LGNv was darkly stained (Fig. 1c).

Dorsal to the LGNv and ventral to the LGNd was a small region that was cell-sparse in Nissl-stained tissue.
In CO-reacted tissue, this area was lightly stained. This region corresponds to the intergeniculate leaflet (IGL; see arrows in Fig. 1), described in some marsupials and placental mammals. In the rat, the IGL has been characterized as a subnucleus of the LGNv (Brauer et al., 1984).

The lateral posterior nucleus (LP) is an elongated nucleus whose lateral border extends approximately to the midlevel of the LGNd. In Nissl-stained sections, cells in the mid-LP were more darkly stained and slightly more densely packed than in anterior regions. The LP has been subdivided into lateral (LPL), intermediate (LPi), and medial (LPM) subnuclei in the opossum (Benevento and Ebner, 1970). However, in the present study no distinction has been made between the subdivisions of the LP.

Layers of the superior colliculus (SC) were easily identified in Nissl-stained sections (Fig. 2), in CO-reacted tissue, and in myelin-stained sections (not shown). In all staining preparations, a distinguishing feature of the SC was the appearance of alternating mediolateral stripes of light and dark layers. The most superficial layer (I), was a cell-sparse thin stripe, identified in Nissl sections by a lack of staining. Layer I reacted very darkly for CO and also appeared very dark in myelin-stained tissue. Immediately underlying layer I was the wide, cell-dense layer II. In Nissl-stained sections, cells were darkly labeled throughout the entire layer II and were relatively larger in the medial region than in the more lateral region. Layer III was narrow, and in Nissl-stained sections, cells were small and darkly labeled. The intermediate layer IV appeared similar, but cells were darker in Nissl-stained sections. The deeper layers V, VI, and VII appeared lighter, and cells were lightly stained and less densely packed. Layer III reacted very lightly for CO, as did the deeper V layer. The intermediate layer IV reacted darkly for CO.

Retinal projections

Examination of the retinal projections revealed the highest density of retinal ganglion cell axon terminals in the contralateral dorsal lateral geniculate nucleus (LGNd) and contralateral superior colliculus (SC) in all cases (Figs. 1–6). Labeled axon terminals were observed in the LGNd ipsilateral to the injected eye in 2 cases (Figs. 3, 4). In contrast, no labeled terminals were observed in the ipsilateral SC in any case. In five cases labeled terminals were observed in the contralateral pretectum (PRT), and in four of these cases, also in the nucleus of the optic tract (NOT; Figs. 3, 4; Table 1). In one case (98-2), labeled terminals were observed in the contralateral medial terminal nucleus (MTN; not shown). Surprisingly, in two cases (98-14, not shown, and 98-44; see Fig. 6), retrogradely labeled cell bodies were found in the trochlear nucleus (NT), an oculomotor nucleus. The labeled cells observed in the NT are most likely a result of retrograde
transport due to leakage of the tracer onto the axon terminals innervating the intraocular muscles.

Although overall labeling patterns were similar in all cases, a 2–7-day transport time appeared to be optimal in terms of density of labeled terminals. In one 2-day case (98-2) and one 7-day case (99-44), labeled terminals were observed in the ipsilateral and contralateral LGNd and LGNv (Figs. 3, 4). In the other 2-day (98-14) and 7-day (98-27, not shown) cases, labeled terminals were observed in the contralateral LGNd and LGNv only (Table 1). In all 14-day cases, labeling was considerably more sparse and was observed solely in the contralateral LGN.

Examination of the TMB-reacted retinas revealed that in four cases the WGA-HRP extended over most of the entire retina (98-2, 99-44, 98-16, and 98-14). In two retinas, spread of the dye covered approximately two-thirds of the retinal surface (98-27; however, see Discussion).

**Lateral geniculate nucleus**

In the six cases in which labeled terminals were observed, terminals were consistently located in the contralateral LGNd and LGNv (Figs. 3, 4; Table 1). Labeled terminals were dense across the mediolateral and rostrocaudal extent of the nucleus, and portions of labeled axons were dense throughout the optic tract in most cases. The labeled terminals were not strictly organized into sublaminae, although their distribution within the nucleus was not homogeneous, rather, dense patches of labeled terminals were adjacent to sparsely labeled or unlabeled patches of the nucleus. In all cases, at caudal levels of the LGNd, patches of labeled axon terminals tended to be concentrated in the ventrolateral region (Figs. 3e, 4e). In two cases (98-2 and 99-44), labeled terminals were observed in the LGNd ipsilateral to the eye injection, and this labeling was patchy and considerably less dense and tended to avoid more lateral portions of the nucleus along the optic tract.

The contralateral LGNv was labeled in five of six cases, although the density of labeled axon terminals in these cases was less dense than in the LGNd (Figs. 1, 3, 4). In these cases, labeled terminals were largely confined to the dorsolateral region of the nucleus. Dense labeling was also observed in the intergeniculate leaflet (IGL).

In two cases (98-2 and 99-44), a few labeled retinal axon terminals were observed in the ipsilateral LGNv. Label in the ipsilateral LGNv was more sparse than in the contralateral hemisphere (Figs. 3, 4). In these cases, labeled axons were clearly present at midlevels of the ipsilateral LGNv, and labeled terminals were confined to the posterior portion of the nucleus.

**Superior colliculus**

In all cases, WGA-HRP labeled terminals were observed in the superior colliculus contralateral to the eye injection (Figs. 2, 5, 6). No labeled terminals were observed in the ipsilateral SC. In all cases patches of alternating high and low density of labeled terminals were located throughout the entire rostrocaudal extent of the SC. Terminals were predominantly confined to the superficial layers, I and II, and sparse labeling was observed in layer III.

**Other retinorecipient nuclei**

In all cases (Table 1), the contralateral pretectum contained densely packed terminals located mostly posterior and medial to LP and extending to the rostral level of the
Fig. 3. Reconstructions of labeled retinal axon terminals in a coronally sectioned thalamus following an intraocular injection of WGA-HRP in case 98-2. In this case, the time allowed for transport of the tracer was 2 days. Labeling was most dense in the contralateral LGNd (a–e). In the rostral sections (a–c), labeling appears to fill the entire LGNd, whereas at more caudal levels, labeled terminals were sparse and did not occupy the entire nucleus. Ipsilateral projections to LGNd and LGNv (f–j) were distributed throughout the LGNd, with the zone immediately adjacent to the optic tract devoid of labeled terminals. Labeled retinal terminals were also observed in the contralateral LP and PRT. The inset, above right, is an illustration of the extent of uptake of the tracer in the retina (stippled). The injection covered virtually the entire retina. The thalamic sections are arranged from rostral (a,f) to caudal (e,j). Dots represent labeled axon terminals, and dashes represent portions of axons. Thick lines represent the border of the thalamus, and thin lines represent borders of individual thalamic nuclei. For abbreviations, see list. Dorsal is up.
Fig. 4. a–j: Reconstructions of WGA-HRP-labeled axon terminals in a coronally sectioned thalamus following intraocular injection in case 99-44. In this case, 7 days were allowed for transport of tracer. Although the amount of labeled terminals was less dense than in case 98-2, labeling patterns were similar to other cases. Labeled axon terminals were observed in the contralateral LGNd, LGNv, LP, and PRT and in the ipsilateral LGNd and LGNv. For abbreviations, see list. Dorsal is up.
Fig. 5. Reconstruction of labeled retinal axon terminals in a coronally sectioned SC following intraocular injections of WGA-HRP in case 98-2. In this case, transport time allowed for the tracer was 2 days. Alternating patches of high and low density of labeled terminals were visible throughout the entire rostrocaudal extent in the superficial layers of the SC. Labeled terminals were also seen in the PRT. Labeled terminals were observed solely in the SC contralateral to the eye injection. Sections are arranged rostral (R, top section) to caudal (C, bottom). For abbreviations, see list. Dorsal is up.

Fig. 6. Reconstruction of labeled retinal axon terminals in the coronally sectioned SC in case 99-44. Transport time of WGA-HRP was 7 days. Labeling patterns were very similar to those of the other cases, with alternating dense and light patches of labeled terminals in the superficial layers of the SC in the medial to lateral direction. As in case 98-14, labeled cells were observed in the NT. For abbreviations, see list. Dorsal is up.
SC (Figs. 3, 6). A population of labeled terminals of moderate density was also observed in the contralateral nucleus of the optic tract in three cases (Figs. 3e, 4e). In five of the six cases, labeled axon terminals were observed in the contralateral lateral posterior nucleus (LP; Figs. 3, 4). In four cases, the amount of staining was relatively sparse and located along its dorsal border. In one case (98-2), labeling was also observed in the contralateral medial terminal nucleus (MTN) of the accessory optic system (not shown). In this case, labeled retinal axon terminals were very dense and were located in the posterior region of the MTN, just bordering the cerebral peduncle. There were no labeled terminals observed in the ipsilateral MTN. Labeled cell bodies were observed bilaterally in the trochlear nucleus (NT) in two cases (Fig. 6). Unlike label observed in other subcortical nuclei, intensity of the label was not diminished in the 7-day group.

DISCUSSION

In the present investigation, intraocular injections in the marsupial Monodelphis domestica revealed that the dominant projections from the eye are to the contralateral dorsal lateral geniculate nucleus (LGNd) and superior colliculus (SC). Retinorecipient nuclei also include the contralateral LGNv, LP, and PRT. In a few cases, the ipsilateral LGNd and LGNv were also labeled. The lack of ipsilateral label in all cases is probably due to the differences in survival times and differences in the effective uptake of the tracer. In the following discussion, we compare patterns of retinal projections and thalamic architecture across marsupials and across mammals, and we suggest possible sources that contribute to the different types of neuroanatomical and structural organization of the LGNd in mammals.

Retinal projections in mammals

Superior colliculus. Dense projections from the retina to the contralateral SC have been observed in all marsupials investigated (Cavalcante et al., 1975; Pearson et al., 1976; Royce et al., 1976; Sanderson and Pearson, 1977; Sanderson et al., 1979), as well as all eutherians that have been studied (Campbell et al., 1967; Laemle and Noback, 1970; Tigges and Tigges, 1970, 1972). In birds, reptiles, turtles, and amniotes, the retinotectal projection is completely crossed as well (Bass and Northcutt, 1981a, b; see Butler and Hodos, 1996, for review). Thus, one interpretation of our results is that a strict contralateral retinocollicular projection is a primitive feature that has been retained in extant monotremes and lost in all but a few species of marsupials, such as Monodelphis. An alternative interpretation is that the ipsilateral SC connections arose with the emergence of marsupials and was subsequently lost in some marsupials such as Monodelphis.

Lateral geniculate nucleus. Although results from the present investigation did not demonstrate clear regions of segregated contralateral and ipsilateral retinal projections within the LGNd, label was very patchy, and the contralateral projection was extremely dense. In other marsupials investigated, the degree to which inputs from the two eyes is segregated varies dramatically. For instance, there is a high degree of binocular overlap of inputs in the Virginia opossum (Benevento and Ebner, 1970), and the Tasmanian devil (Sanderson et al., 1979), much like the pattern observed in the present investigation. In other groups of marsupials including brown bandicoots (Haight and Sanderson, 1986), the grey kangaroo (Sanderson et al., 1984), and the Tasmanian potoroo (Sanderson et al., 1984; Wilson and Astheimer, 1989), retinal ganglion cell axons projected to eye-specific regions in the LGNd, although the degree of segregation of retinal inputs varied among different species of marsupials (Table 2).

Cytoarchitectural differences have also been observed in the LGNd of different marsupials. For instance in Virginia opossums (Royce et al., 1976; Wilson and Astheimer,
In marsupials, there are at least three types of cytoarchitectonic segments: a completely homogenous LGNd, an LGNd divided into α and β segments only, and an LGNd in which the α segment contains multiple laminae. Not surprisingly, the presence of multiple cytoarchitectonic layers in the LGNd is often coincident with the presence of multiple retinal terminal regions (RTRs), whereas a relatively homogeneous LGNd is coincident with fewer RTRs (Table 2). For example, among the polyprotodont marsupials, the cytoarchitecture of the LGNd of the Virginia opossum (Royce et al., 1976) and the Tasmanian devil (Sanderson et al., 1979) is relatively simple, with no clear α and β subregions, and these animals have substantial overlap of retinal inputs. Other polyprotodont marsupials such as the kowari and ffaith-tailed dunnart, from Haight and Sanderson, 1988; brush-tailed possum, from Hayhow, 1967; Sanderson et al., 1978; Tasmanian devil, from Sanderson et al., 1979; gambal, from Lent et al., 1976; ring-tailed possum, from Pearson et al., 1976; Virginia opossum, South American opossum, from Royce et al., 1976; Eastern quoll, from Sanderson and Pearson, 1977; honey possum, from Harman et al., 1990; dunnart, from Dunlop et al., 1997.

In a variety of eutherian mammals, a similar correspondence exists between cytoarchitecture and segregation of retinal inputs in the LGNd. For instance, the LGNd of hedgehogs (Campbell et al., 1967; Dinopoulos et al., 1987), rabbits (Takahashi et al., 1977), microchiropteran bats (Pettigrew et al., 1989), and one species of tree shrew have a more complex LGNd cytoarchitecture, with four to seven cytoarchitectonic laminae and three to ten retinal terminal regions (RTRs) with little or no binocular retinal terminal regions.
(Simmons, 1979) consists of a fairly homogenous cell population with no obvious laminar arrangement. Examination of retinal projections to the LGNd in these animals indicates a large amount of overlap of projections from the ipsilateral and contralateral eye, with very few eye-specific retinal terminal bands or regions. In the rat LGNd, the α and β segments are relatively undifferentiated, but the retinal projections are segregated into separate, patchy regions (Cunningham and Lund, 1971; Lund et al., 1974; Reese, 1988). This concealed lamination described in rats is similar to the LGNd organization in the ring-tailed possum (Pearson et al., 1976) and the brown bandicoot (Haight and Sanderson, 1990), in which no clear cytoarchitectonic distinctions are apparent, yet projection patterns indicate at least some segregation of retinal inputs. Finally, in cats (Stone and Hansen, 1966; Peters and Paley, 1966) and primates (Kaas et al., 1978; Florence et al., 1986; see Kaas and Huerta, 1988, for review), the LGNd is differentiated into a number of cytoarchitectonic and eye-specific layers, although the number of laminae varies across these species. Thus, the three types of organization described above for marsupials are present in eutherian mammals as well.

Factors that contribute to lamination and eye-specific domains in the lateral geniculate nucleus of mammals

Comparative studies indicate that three general types of organization of the LGNd have evolved in the mammalian visual system, suggesting that there are a restricted number of factors that contribute to its organization. One source of current organization is genetic. If genes are responsible for the present organization of the LGNd, then one would predict that closely related species would have a similarly appearing LGNd organization. Marsupials are an order of mammals composed of two suborders, polyprotodonts and diprotodonts (Fig. 7; Table 2). Comparisons of closely related marsupials reveal that in polyprotodont marsupials the LGNd is organized in a less complex fashion than in diprotodonts. For example, polyprotodonts such as the Virginia opossum, Tasmanian devil, and brown bandicoot possess a cytoarchitectonically homogenous LGNd, and most other polyprotodont species have two or three laminae. Only the fat-tailed dunnart and kowari have more cytoarchitectonic laminae. Additionally, most polyprotodont species have a very high degree of binocular overlap of retinal projections (30–50% of RTR receive input from both eyes). There is evidence that polyprotodonts represent an evolutionarily older lineage than diprotodonts (Richardson, 1988), suggesting that this reduced cytoarchitectonic and connectional segregation reflects a primitive feature of LGNd organization. This supposition is supported by studies in monotremes demonstrating that the LGNd is small and cytoarchitectonically homogeneous (Campbell and Hayhow, 1971, 1972).

Diprotodonts, on the other hand, have an LGNd that is subdivided cytoarchitectonically into four to seven laminae, as well as a large number of RTRs, which receive input almost exclusively from one eye (Table 2). Thus, a comparative analysis reveals that in marsupials a predictor of LGNd organization is phylogeny. This observation is likely to be true for other mammals as well. For instance, although the organization and complexity of the LGNd varies across primates, as an order, the LGNd of primates is more complex than the LGNd in the order Rodentia. Thus, the complexity of the LGNd organization in terms of the number of laminae, RTRs, and segregation of inputs is, at least in part, genetically determined. However, within the two suborders of marsupials (and within other orders such as primates) variability still exists, suggesting that other factors contribute to the cytoarchitectonic and neuroanatomical organization of the structure in question.

Another factor believed to contribute to current organization is the ecological niche of different mammals, because mammals with similar environmental demands often adapt similar underlying neural organizations (Sanderson et al., 1984; Barton and Purvis, 1995). With respect to the visual system, one would predict that animals such as predators would have evolved morphological features such as eye placement and receptor type, and corresponding sensory systems that are more like each other than they are to mammals that have different lifestyles, such as herbivores.

A comparative analysis of lifestyle and LGNd organization in marsupials that have been examined does suggest that within a suborder, lifestyle is likely to contribute to some of the variability observed in LGNd organization in different species. For example, the predatory polyprotodont marsupials that have been studied range in size from approximately 15 g to 5 kg; they are all terrestrial and nocturnal. In all of these species, the LGNd contains three to five cytoarchitectonic laminae, with the exception of the Tasmanian devil (Sanderson et al., 1979), compared with the lack of cytoarchitectonic distinctions (one to two laminae) of the LGNd of nonpredatory polyprotodonts (Table 2). Furthermore, examination of retinal terminal projection patterns in predatory and nonpredatory polyprotodont marsupials indicates that the LGNd of the predatory species has five or six RTRs, whereas nonpredatory polyprotodonts have two or three RTRs (Table 2; Sanderson and Pearson, 1977; Sanderson et al., 1979; Haight and Sanderson, 1988; Dunlop et al., 1997).

Although the LGNd organization of predatory polyprotodonts is more complex than that of nonpredatory polyprotodonts, the idea of a predatory lifestyle as a predictor of complex LGNd organization in mammals in general is weakened by observations of the diprotodont marsupials. The diprotodonts that have been studied are mostly herbivores, yet in almost all species, their LGNd is organized in a complex fashion, with four to seven cytoarchitectonic laminae and four to ten eye-specific retinal terminal regions. In only one of the diprotodont herbivores that have been studied (honey possum; Harman et al., 1990) does the input from both eyes converge in the same RTR.

Although lifestyle, because it determines peripheral morphology, does appear to contribute to current organization, the spontaneous and patterned activity that arises from particular receptor types, distributions, and use plays an important role in the establishment and refinement of connections, and in the initial wiring of primary afferents. There is a wealth of data on the role activity plays in establishing normal patterns of ocular dominance columns and orientation columns in primary visual cortex (Shatz and Stryker, 1978; Antonini and Stryker, 1993; Chapman et al., 1996; Katz and Shatz, 1996; see Sur et al., 1999, for review), as well as barrel fields in the primary somatosensory cortex of mice (Van der Loos and Woolsey, 1973; Welker and Van der Loos, 1986). At the level of the
Fig. 7. A simplified cladogram depicting the relationship between the suborders of marsupials and other mammals (modified from Eisenberg, 1981). Prototherian mammals compose the oldest lineage, and only three species exist today. Metatherian mammals are thought to have emerged somewhat later (However, see Penny and Hasegawa, 1997, for an alternate interpretation) and Eutherian mammals later still. Xs denote lineages that are extinct.
dorsal thalamus, it is well established that activity-dependent competitive mechanisms are necessary for the formation of eye-specific laminae in the LGNd (see Shatz, 1990, for review). Both carnivores and primates have frontally placed eyes, and it is the competitive interaction of activity arising from visual stimulation from both eyes that modulates formation of laminae in the LGNd and of ocular dominance columns in V1 (Hubel and Wiesel, 1970; Hubel et al., 1977). However, although visual experience does modify ocular dominance columns, the initial formation of the columns occurs early in development, in the absence of visual stimulation (Wiesel and Hubel, 1974; Horton and Hocking, 1996).

From comparative studies, it is apparent that phenotypic variations observed in mammalian nervous system organization can be accounted for by some combination of phylogenetic relationship and lifestyle of the animal. Presumably changes in behavior and morphology associated with a particular lifestyle have a large impact on the activity patterns to which the developing nervous system is exposed. The genetic regulation of axonal development, when coupled with an activity pattern dictated by morphological features related to lifestyle (such as the location of the eyes on the head, receptor type and density, and nerve cell specialization within the retina), generates a particular type of LGNd organization observed in a species.

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