Neuroscience 142 (2006) 843–858

EARLY BLINDNESS RESULTS IN ABNORMAL CORTICOCORTICAL AND THALAMOCORTICAL CONNECTIONS

S. J. KARLEN,[a] D. M. KAHN[a] AND L. KRUBITZER[a,b]*

[a]*Center for Neuroscience, University of California, Davis, 1544 Newton Court, Davis, CA 95618, USA*

[b]*Department of Psychology, University of California, Davis, Davis, CA 95616, USA*

Abstract—Studies in congenitally blind and bilaterally enucleated individuals show that an early loss of sensory driven activity can lead to massive functional reorganization. However, the anatomical substrate for this functional reorganization is unknown. In the present study, we examined patterns of corticocortical and thalamocortical connections in adult opossums that had been bilaterally enucleated neonatally, prior to the formation of retinogeniculate and geniculocortical connections. We show that in addition to normal thalamocortical projection patterns from visual nuclei, enucleated animals also receive input from nuclei associated with the somatosensory (ventral posterior nucleus, VP), auditory (medial geniculate nucleus, MGN), motor (ventrolateral nucleus, VL), and limbic/hippocampal systems (anterior dorsal nucleus, AD; and anterior ventral nucleus, AV). Likewise, in addition to normal corticocortical projections to area 17, bilaterally enucleated opossums also receive input from auditory, somatosensory, and multimodal cortex. These aberrant patterns of thalamocortical and corticocortical connections can account for alterations in functional organization observed in the visual cortex of bilateral enucleated animals, and indicate that factors extrinsic to the cortex play a large role in cortical field development and evolution. On the other hand, the maintenance of normal patterns of connections in the absence of visual input suggests that there are formidable constraints imposed on the developing cortex that highly restrict the types of evolutionary change possible.

Key words: bilateral enucleations, marsupials, primary visual area, intracortical projections, thalamocortical projections, multiunit electrophysiology.

The neocortex has a tremendous capacity to functionally adapt to external perturbations. Such perturbations may be extrinsic to the brain, but endogenous to the individual, such as the loss of peripheral sensory receptor arrays or alterations in sensory driven activity. Other perturbations may be environmentally driven, such as alterations in the physical parameters of the sensory environment. In the former instance, the loss of receptor arrays or sensory driven activity in the auditory or visual system, as evidenced in congenitally deaf and blind individuals, leads to enormous alterations in cortical organization such that the deprived regions of cortex are activated by different modalities (Catalan-Ahumada et al., 1993; Cohen et al., 1997; Buchel et al., 1998; Levanen et al., 1998; Finney et al., 2001; see Bavelier and Neville, 2002 for review). Further, we know that in humans this re-organized cortex appears to be functionally optimized in that discriminatory and perceptual abilities of the spared sensory systems are enhanced (e.g. Roder et al., 1999, 2000).

In a previous investigation in early bilaterally enucleated *Monodelphis domestica*, we demonstrated that all of visual cortex was functionally re-specified and devoted to auditory and somatosensory processing (Kahn and Krubitzer, 2002). Further, architectonically defined area 17 was smaller, and a new area, area X, was observed just lateral to area 17. These results are similar to studies in cats which found that early visual deprivation resulted in auditory activation of neurons in "visual" cortex (Yaka et al., 1999), and studies in monocularly enucleated rats which found that somatosensory stimulation evoked activity in visual cortex (Toldi et al., 1994; see Toldi et al., 1996 for review). The question we address here is: What is the anatomical substrate for this re-organization?

While alterations in connections of both deprived and non-deprived neural structures must account for the functional and behavioral plasticity observed with congenital loss of peripheral receptors or sensory driven activity, the anatomical substrate for this observed plasticity is not known. One possibility is that connections of primary sensory afferents have been altered, such that projections from the retina, or projections from the cochlea, invade subcortical structures normally occupied by the lost system. This appears to be the case for congenitally deaf mice where the eyes not only project to their normal targets, but also to abnormal targets such as the medial geniculate nucleus (MGN) (Hunt et al., 2005).

Other types of nervous system alterations that may account for cortical re-organization include changes in thalamocortical or corticocortical connections. To investigate the possibility that connections are altered at both cortical and subcortical levels, we examined the corticocortical and thalamocortical connections of bilaterally enucleated short-tailed opossums (*M. domestica*). These animals make ideal models for examining the role of sensory

*Correspondence to: L. Krubitzer, Center for Neuroscience, 1544 Newton Court, Davis, CA 95618, USA. Tel: +1-530-757-8868; fax: +1-530-757-8827.
E-mail address: lakrubitzer@ucdavis.edu (L. Krubitzer).

Abbreviations: A, auditory area; AD, anterior dorsal nucleus; AV, anterior ventral nucleus; CO, cytochrome oxidase; CT, caudal temporal area; ER, entorhinal cortex; FE, fluoroemerald; FR, fluororuby; LD, laterodorsal nucleus; LG, lateral geniculate nucleus; LGD, dorsal lateral geniculate nucleus; LGV, ventral lateral geniculate nucleus; LP, lateral posterior nucleus; MGN, medial geniculate nucleus; MM, multimodal cortex; P, postnatal day; PB, phosphate buffer; S1, primary somatosensory area; VL, ventrolateral nucleus; VP, ventral posterior nucleus; V1, primary visual area; V2, secondary visual area.

0306-4522/06$30.00+0.00 © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2006.06.055

Table 1. Neuroanatomical abbreviations

Abbreviation	Area
17	Area 17
A	Auditory area
AD	Anterior dorsal nucleus
AV	Anterior venral nucleus
CO	Cytochrome oxidase
CT	Caudal temporal area
ER	Entorhinal cortex
FE	Fluoroemerald
FM	Frontal myelinated area
FR	Fluororuby
LD	Laterodorsal nucleus
LG	Lateral geniculate nucleus
LGD	Dorsal lateral geniculate nucleus
LGV	Ventral lateral geniculate nucleus
LP	Lateral posterior nucleus
M	Medial
MGN	Medial geniculate nucleus
MM	Multimodal cortex
OB	Olfactory bulb
P	Postnatal day
PYR	Pyriform cortex
R	Rostral
S1	Primary somatosensory area
SC	Superior colliculus
V1	Primary visual area
V2	Secondary visual area
VL	Ventrolateral nucleus
VP	Ventral posterior nucleus
X	Area X

input in determining the functional organization and connectivity in the developing cortex, since most of their neural development occurs postnatally and the patterns of retinofugal projections in the marsupial are similar to that of placental mammals (see Kahn and Krubitzer, 2002, for review). In these experiments, injections of anatomical tracers were made into area 17 (primary visual area, V1; see Table 1 for abbreviations) and patterns of connections were compared in bilaterally enucleated and normal animals.

These studies are essential for understanding the role of factors extrinsic to the neocortex, such as retinal input, in organizing features of the developing neocortex, such as corticocortical and thalamocortical connectivity. Further, these studies could provide important insight into the extent to which sensory inputs specify functional modality of cortical areas, and ultimately sculpt perceptual behavior.

EXPERIMENTAL PROCEDURES

All procedures were approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, and conform to National Institutes of Health guidelines. All efforts were made to minimize stress to the animals, and multiple techniques were used in each case to maximize the amount of data obtained from each animal and to minimize the total number of animals required. Eight adult bilaterally enucleated *Monodelphis domestica* were examined in these studies (Table 2). Cortical connections were examined in a total of seven animals, four of which were also mapped electrophysiologically. An additional animal was used for electrophysiological recordings only.

Enucleation surgery

All animals were enucleated on postnatal day 4 (P4) to ensure survival of the pups. At this stage, the ganglion cell axons have just entered the optic tract (Dunn et al., 2001), and thalamocortical afferents have not yet arrived at the cortex (which occurs between P5–P15; Molnar et al., 1998b). For these experiments, adult females were anesthetized with alphaxalone (45 mg/kg) and alphadolone (15 mg/kg) administered intramuscularly to keep them immobilized, and the individual young were anesthetized by hypothermia. Body temperature, heart rate, and respiration were continuously monitored throughout the surgery. Once the pups were anesthetized, their eyes were manually excised under microscopic guidance. The skin surrounding the eyes was replaced over the exposed eye socket and secured with surgical glue. Pups remained with the mother for four weeks, and then were hand-reared until postnatal week seven. The animals were then housed individually for 8–12 months. Although the eyelids did form, they did not open, and postmortem dissection revealed a membrane covering the eye sockets.

Injections of anatomical tracers

Adult animals were placed in an anesthesia chamber with 2–3% isoflurane. Once anesthetized, the animals were placed in a stereotaxic apparatus, and anesthesia (1–2% isoflurane) was delivered through a mask fitted over the snout. An incision in the skin over the midline of the skull was made, the temporal muscle was retracted, and holes were drilled in the caudomedial region of the skull overlying the occipital region of the right cortical hemisphere. The location of the injection site was determined in part by blood vessels patterns, but the final delineation of the injection was done post hoc using myeloarchitectonic analysis (see below). A calibrated Hamilton syringe (25 gauge needle; Hamilton Syringes, Reno, NV, USA) attached to a micromanipulator was lowered through the dura into the brain

Table 2. Procedures conducted on each cortical hemisphere by case number

Cortical hemisphere			
Case no.	Area 17 injections		Electrophysiological recordings
	FE	FR	
Normal opossums			
00-32			Left
96-18		Left	
97-5	Left	Left	
97-7	Left	Left	
99-53		Right	
Bilateral enucleates			
01-03	Right		Left
01-09	Right		
01-20			Left
03-92	Right		Left
04-03	Right		
04-34		Right	Left
04-56		Left	Left
05-103	Right		

to a depth of 250–300 μm below the pial surface, approximately into the middle cortical layers. One injection of either the dextran amine tracer fluororuby (FR; Molecular Probes, Eugene, OR, USA) or fluoroemerald (FE; Molecular Probes) was injected into the cortex. In each case, 0.3 μl of a 7% solution of the tracer was injected with the objective of incorporating all of the cortical layers in the injection site. Bone wax was used to replace the section of skull overlying the injection site, and the temporal muscle and skin were sutured. Animals had survival times of 4–7 days to allow for the transport of the tracer.

Electrophysiological recordings

Electrophysiological recordings of the neocortex in the hemisphere opposite to that injected (except case 04–56) were obtained in five animals; one of these cases (01-20) has been previously reported (Kahn and Krubitzer, 2002). The same methods were used for electrophysiological recordings as described in Kahn and Krubitzer (2002). Briefly, the animals were anesthetized with isoflurane (1–2%) delivered via an endotracheal tube. The animals were placed in a stereotaxic apparatus, the skin over the skull was cut, the temporal muscle was retracted, and the skull and dura were removed to expose the entire hemisphere. Body temperature, heart rate, and respiration were monitored continuously throughout the experiment. Subcutaneous injections of lactated Ringer's solution were administered every 3–4 h to maintain hydration. The exposed cortex was digitally imaged with a Pixera PVC100C camera (Pixera Corporation, Los Gatos, CA, USA). This image was used as a reference map to relate the electrode penetrations to cortical vasculature. A tungsten microelectrode (5 MΩ; 0.02 inch) was used to make recordings 200–400 μm below the pial surface, approximately in layer IV. Neural activity was amplified, filtered (250 Hz to 4 kHz), heard through a speaker, and monitored on an oscilloscope. Auditory stimuli consisted of broadband clicks presented in a free field. Somatic stimuli consisted of light taps, displacement of hairs with brushes, light brushing of skin, hard taps, and manipulation of muscles and joints. Receptive fields obtained for neurons at each recording location were drawn onto illustrations of the body. For each experiment, three injections of Fast Blue (20% concentration; Sigma Aldrich, St. Louis, MO, USA) were made in the cortex and marked on the photograph so that electrophysiological recording results could be related to histologically processed tissue.

Histology and data analysis

At the completion of the experiments, animals were given a lethal dose of sodium pentobarbital (250 mg/kg), administered intraperitoneally, perfused transcardially with 0.9% saline plus 0.1% heparin in 0.1 M phosphate buffer (PB; pH 7.4), then with 4% paraformaldehyde in PB, and followed by 4% paraformaldehyde with 10% sucrose in PB. Following perfusion, the brain was removed from the skull, and the cortical hemispheres were separated from the diencephalon and flattened between two glass slides. The entire brain was cryoprotected by immersion in 30% sucrose overnight. The thalamus and midbrain were frozen and sectioned on a sliding microtome in the coronal plane at a thickness of 40 μm. The flattened cortex was frozen and sectioned in a plane tangential to the pial surface at 30 μm. Sections were collected in PB. For the thalamus, alternate sections were reacted for cytochrome oxidase (CO) (Carroll and Wong-Riley, 1984), stained for Nissl, or mounted directly onto glass slides for fluorescent microscopy of anatomical tracers. Cortical sections were also collected in PB, and alternate sections were stained for myelin (Gallyas, 1979) or mounted for analysis of fluorescent tracers.

Table 3. Percentage of retrogradely labeled cells by area

Location	Normal opossums		Bilateral enucleates	
	Mean	SD	Mean	SD
Cortical areas	(*n*=5)		(*n*=6)	
V1 (area 17)	59.40	27.85	32.39	15.77
V2	5.30	1.16	n/a	n/a
Area X	n/a	n/a	7.29	5.84
Area A	1.20	1.04	7.26	5.64
CT	20.79	19.92	20.64	20.44
ER	8.39	10.66	6.36	8.73
FM	0.00	0.00	0.70	1.41
MM	4.93	3.74	14.05	9.25
S1	0.00	0.00	5.50	5.97
Other	0.00	0.00	5.81	7.12
Thalamic nuclei	(*n*=4)		(*n*=5)	
AD	0.00	0.00	2.82	4.29
AV	0.30	0.61	5.07	4.95
LD	16.19	10.36	7.96	10.17
LGD	57.76	21.09	36.99	16.08
LGV	0.00	0.00	0.27	0.60
LP	19.91	8.62	16.76	7.36
MGN	0.00	0.00	0.27	0.60
VL	1.74	2.84	8.44	6.82
VP	0.34	0.49	8.95	3.70
Other	3.76	2.08	12.47	8.14

Analysis of fluorescent tracers

The injection sites and retrogradely labeled cell bodies were plotted using a computerized X/Y stage encoding system (Accustage Inc., Shoreview, MN, USA) that was mounted to a fluorescent microscope. The entire series of sections for both the cortex and the thalamus was plotted. In addition to labeled cells, the outlines of the tissue and blood vessels were also plotted. The plots of injection sites and labeled cells were directly related to architectonic boundaries. For the thalamus, these boundaries were determined from adjacent sections processed for either CO or Nissl. Cortical boundaries were determined by myelin-stained sections, and fluorescent labeling was related to myeloarchitectonic boundaries. For the cortex, the entire series of plots with myelin boundaries was collapsed into a single comprehensive reconstruction of each hemisphere.

In order to determine the percentage of retrogradely labeled cells in a particular thalamic nuclei or a cortical field, the number of cells in an architectonically defined nucleus of the thalamus, or area of the cortex, was counted and expressed as a fraction of the total number of labeled cells in the thalamus or in the cortex. For the thalamus, the number of retrogradely labeled cells in every other section in the series was counted. Although alternate sections were utilized, we examined every section in the series to ensure that labeled cells in small nuclei were not missed by this method. For the cortex, every other section in the series containing retrogradely labeled cells was counted. For both the thalamus and the cortex, only the areas that were architectonically distinct and could be confidentially and reliably determined across cases were included in this analysis. All other regions are denoted as "other." The data from this analysis are presented in Table 3.

Reconstructions of electrophysiological maps

To match electrophysiological recording data to the cortical reconstructions of architectonic boundaries, all reconstructed sections

(described above) were aligned to the digital image of the cortex that contained surface blood vessels, electrode penetrations, and the Fast Blue injections placed during electrophysiological mapping. In this way, electrode penetrations were directly related to cortical myeloarchitecture. Sensory maps of the neocortex were made by separating recording sites containing neurons that responded to different sensory stimuli, by drawing lines that were interpolated between appropriate recording sites. Within the area that responded to somatic stimuli, body part representations were delineated in the same fashion.

RESULTS

We examined the effect of early bilateral enucleation on the corticocortical and thalamocortical connections of cortical area 17 (V1) in short-tailed opossums (*M. domestica*). V1 is a functionally defined field that contains a complete representation of the contralateral visual hemifield that is coextensive with architectonically defined area 17. Because bilaterally enucleated animals do not possess a V1, but only an architectonically defined region that corresponds to area 17 in normal animals, we refer to this region as area 17 in bilaterally enucleated animals and V1 (area 17) in normal animals. Our results demonstrate that aberrant corticocortical and thalamocortical connections develop in bilaterally enucleated animals, as revealed by retrograde transport of fluorescent anatomical tracers from architectonically defined area 17. Here, we briefly describe cortical myeloarchitecture and the relation of architectonically defined fields to functional mapping results in both normal and bilaterally enucleated animals. Then, we describe the patterns of cortical connections in normal and bilaterally enucleated animals, and the relationship of each cortical field to architectonically defined regions of cortex. Finally, we describe the architectonic appearance of nuclei in the thalamus, and the thalamocortical connections resulting from injections into area 17.

Cortical myeloarchitecture and functional mapping in normal and bilaterally enucleated opossums

Both the cortical myeloarchitecture and the functional organization of the neocortex have been previously described in normal (Huffman et al., 1999; Frost et al., 2000; Kahn et al., 2000) and bilaterally enucleated *M. domestica* (Kahn and Krubitzer, 2002). Here, we expand on our previous data with additional cases, and provide a brief description of these results to better relate the patterns of connections of area 17 in bilaterally enucleated animals to functionally and architectonically defined cortical areas.

In both normal and bilaterally enucleated opossums, the primary sensory areas, including S1 (somatosensory), A (auditory), and V1 (area 17; visual; see Table 1 for abbreviations), are easily defined by their darkly myelinated appearance (Fig. 1). The major difference between normal and bilaterally enucleated opossums is that area 17 is substantially smaller in bilateral enucleates, and a darkly myelinated region just lateral to area 17, termed area X (see Rakic et al., 1991; Kahn and Krubitzer, 2002), is present. As described previously (Kahn and Krubitzer, 2002), area X appears cytoarchitectonically and myeloarchitectonically distinct from both V1 (area 17) and V2 (secondary visual area) in normal animals and is similar in location and appearance to area X described in enucleated primates (Rakic et al., 1991). Obviously area X in the present investigation is not homologous to area X in primates; the terminology is meant only to reflect the emergence of a novel field that is similar in location and appearance to area X in primates.

In normal animals, electrophysiological recording results demonstrate that the primary sensory areas are predominantly unimodal (Fig. 2A). Neurons in S1 are responsive to somatic stimulation, neurons in V1 (area 17) are responsive to visual stimulation, and neurons in A are responsive to auditory stimulation. However, at the borders

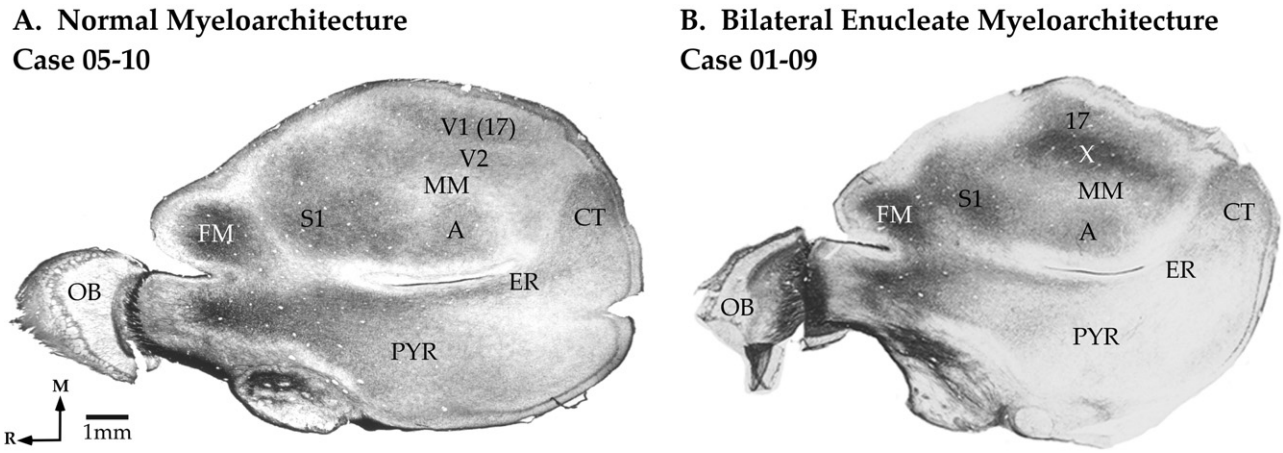

Fig. 1. Digital images showing myeloarchitecture in flattened cortical sections of a normal (A) and bilaterally enucleated (B) opossum. Because all cortical boundaries cannot be appreciated in a single section, the entire series of sections is used to determine the boundaries of cortical fields. In normal animals, primary sensory areas (V1/17, S1, and A) are all darkly myelinated. These boundaries are coextensive with functional regions of the neocortex defined using electrophysiological recording techniques (see Fig. 2). As has been reported previously (Kahn and Krubitzer, 2002), the myeloarchitectonic appearance of most cortical areas is similar in normal and bilaterally enucleated animals. However, area 17 is markedly smaller, and there is a darkly myelinated region adjacent to area 17 called area X in bilateral enucleates (B). Abbreviations are defined in Table 1.

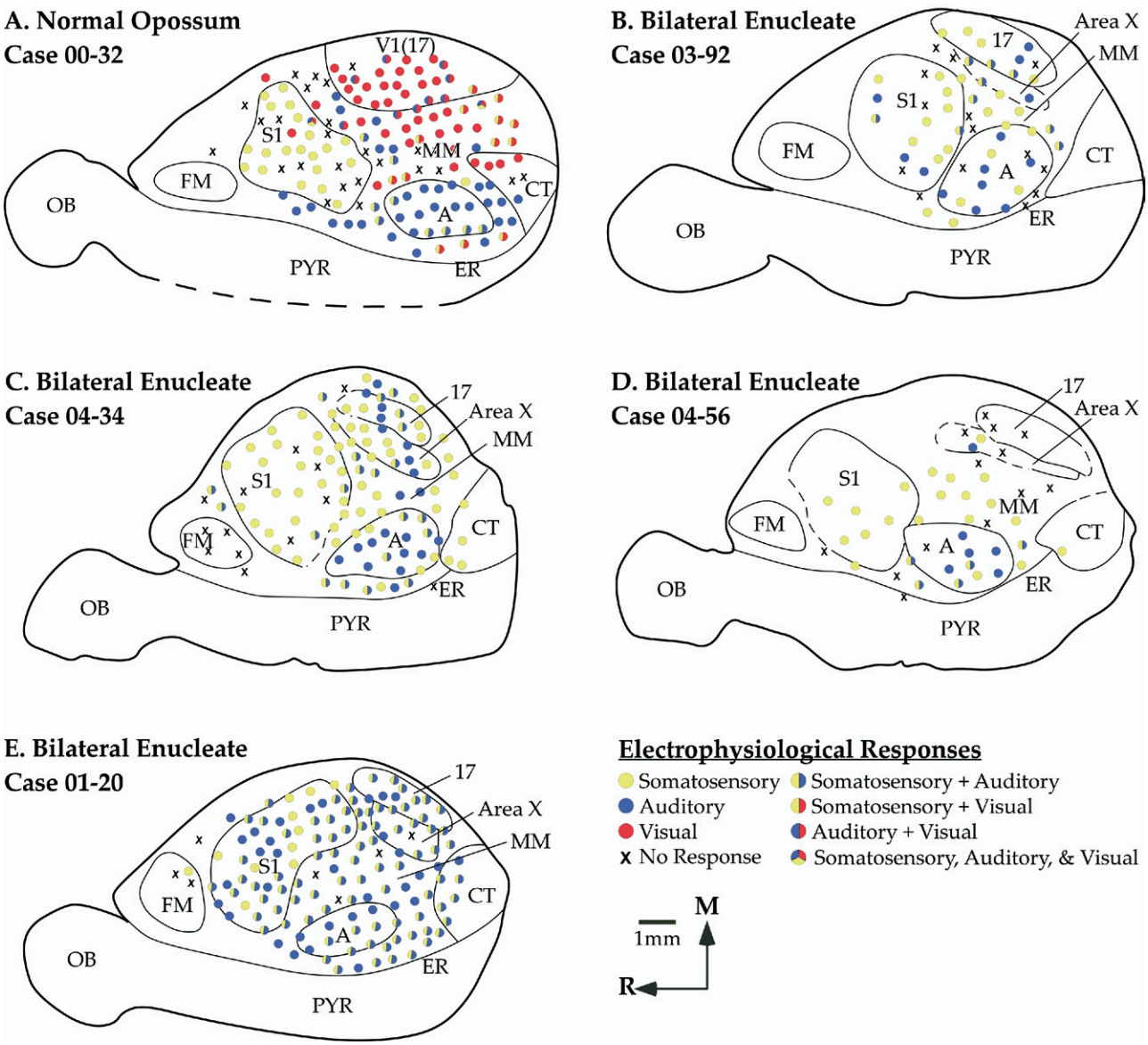

Fig. 2. Functional organization in normal (A) and bilaterally enucleated (B–E) opossums. In normal animals, primary sensory fields contain neurons that respond predominantly to a single modality of stimulation. In bilaterally enucleated animals, neurons in area 17 respond to both auditory (blue circles) and somatosensory stimuli (yellow circles), rather than visual stimulation (red circles), as in normal animals. Additionally, there is an increase in the regions of cortex in which neurons respond to more than one modality of stimulation. For example, in normal animals this cortex resides between area V2, S1, and A. In bilaterally enucleated animals, not only does area 17 contain a large number of neurons responsive to bimodal stimulation, but in some cases, areas X, MM, S1, and A have a larger number of recording sites that contain neurons which respond to an additional modality or that are bimodal. Thin lines in C and D denote myeloarchitectonic boundaries. Abbreviations defined in Table 1. Conventions as in previous figure.

of both S1 and A, a few recording sites contained neurons that were bimodal or multimodal. In normal animals, both S1 and V1 (area 17) have been shown to contain a complete, topographically organized representation of the sensory epithelium that is coextensive with myeloarchitectonic boundaries described above (Huffman et al., 1999; Frost et al., 2000; Kahn et al., 2000).

The electrophysiological recording results in bilaterally enucleated animals in the current study are consistent with those previously reported in several respects (Fig. 2B–E, see also Kahn and Krubitzer, 2002). First, cortex that would normally be devoted to processing visual inputs, including architectonically defined area 17, contained neurons responsive to auditory and somatic stimuli. Second, for neurons responsive to somatic stimuli in areas 17, X, and multimodal cortex (MM), most of the receptive fields are on the snout, face, vibrissae, and head. Third, in some cases, both S1 and A have undergone functional reorganization such that a larger number of recording sites contained neurons that were either bimodal or responded to another modality. For example, in case 01-20 (Fig. 2E), S1 contained neurons responsive to somatosensory, auditory,

or somatosensory+auditory stimuli, and auditory cortex contained neurons responsive to both modalities as well. Finally, the overall pattern of functional re-organization observed in the bilaterally enucleated opossums was not the same for different individuals. For example, in case 01-20 (Fig. 2E), area 17, MM, and even portions of somatosensory cortex were dominated by neurons that responded to bimodal auditory and somatosensory stimulation. This was different than the organization observed in case 04-34 (Fig. 2C) in which most neurons in area 17, X, and MM were unimodal and responded predominantly to somatosensory stimuli. In this case, neurons at only two sites at the border of S1 were bimodal. On the other hand, area A had several recording sites in which neurons were responsive to somatic stimuli or both somatic and auditory stimuli, in addition to pure auditory stimuli (Fig. 2C). In case 01-03 (not shown), re-organized areas, including area 17, X, and MM, were dominated by neurons that were responsive to unimodal auditory stimuli, with a few sites containing neurons responsive to bimodal stimuli. In this case, both S1 and A were unimodal with only two sites on the caudolateral border of S1 that contained bimodal neurons. Finally, the organization of MM is different for each case. In some cases, it is dominated by neurons responsive to somatosensory stimulation (Fig. 2C, D), and in other cases by neurons responsive to auditory or bimodal stimulation (Fig. 2E). It is possible that the variability observed in functional re-organization and connectivity may be the result of hand-rearing these animals. This notion is consistent with a recent study in anophthalmic mice which demonstrates differences in functional organization and connectivity of area 17 with environmental enrichment (Piche et al., 2004). However, this hypothesis needs to be experimentally verified.

Corticocortical connections in bilaterally enucleated animals

In all cases, the injection sites of our fluorescent tracers ranged in diameter from 100 to 300 μm (e.g. Fig. 3A), and spanned all cortical layers. The tracers used in this study are not taken up by fibers of passage; therefore, all of the label identified originates from the injection site in area 17. Retrogradely labeled cells in the neocortex resulting from these injections fluoresced brightly with very little non-specific background label (Fig. 3B, D–F). In some instances, the entire cell body and apical processes were filled (Fig. 3B, D, F). The corticocortical connections from V1 (area 17) of normal *M. domestica* have been reported by Kahn and colleagues (2000), and a brief description is provided here so that connections can readily be compared with the new findings in the bilateral enucleates. In normal opossums, 59% of the connections of V1 (area 17) are intrinsic, many of which are located close to the injection site (1–1.5 mm). The majority of labeled cells located outside of V1 (area 17) were in caudal temporal area (CT; 21%), V2 (5%), MM (5%), and entorhinal cortex (ER; 8%). A very small percentage of cells was observed in area A (1%; see Table 3).

In bilaterally enucleated animals, all of the injections were related to cortical myeloarchitecture. In all cases, injections were restricted to the darkly myelinated area 17 at the caudal pole of the neocortex. In five cases, the injections were placed in the caudal portion of area 17 (Figs. 4B; 5A, B, D; case 01-09, not shown), while in the

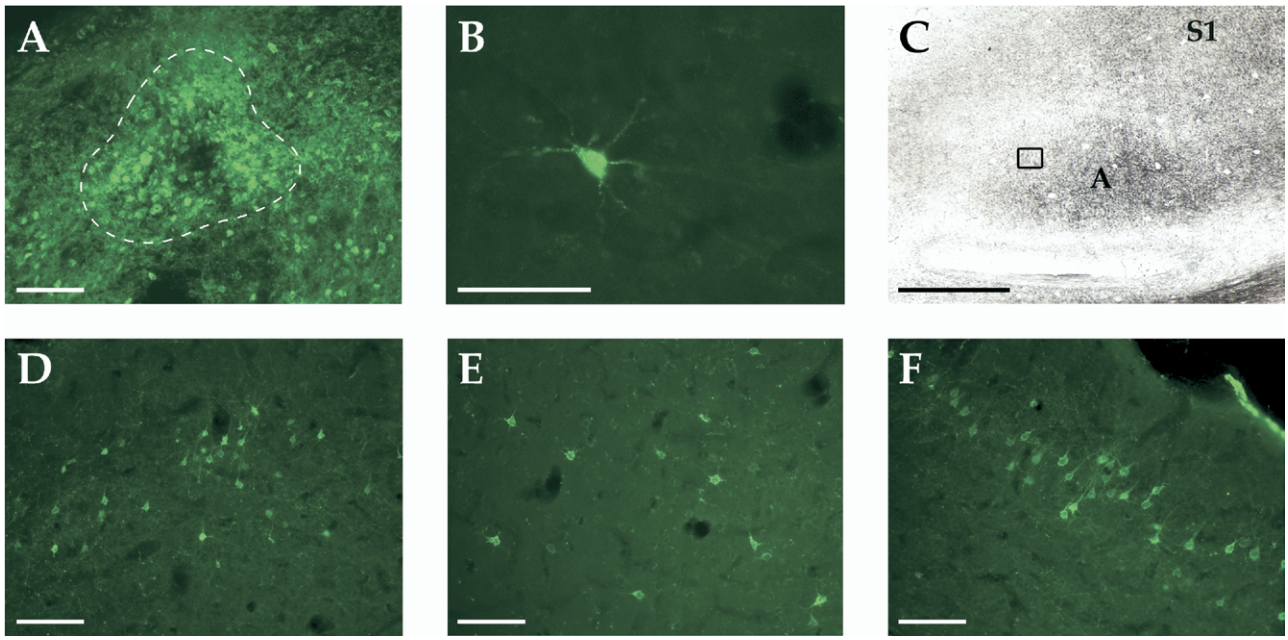

Fig. 3. Digital images depicting an injection (A) of FE into area 17 of a bilaterally enucleated animal, case 05-103. Labeled cell bodies were observed in auditory cortex (B), area 17 (D), CT (E), and S1 (F). (C) An image of cortex from an adjacent section stained for myelin. The box in area A (auditory area) depicts the location of the labeled cell shown in (B). Labeled cells were observed scattered throughout area A in this case (05-103). Scale bars=100 μm in panels A, B, D–F, and 1 mm in C.

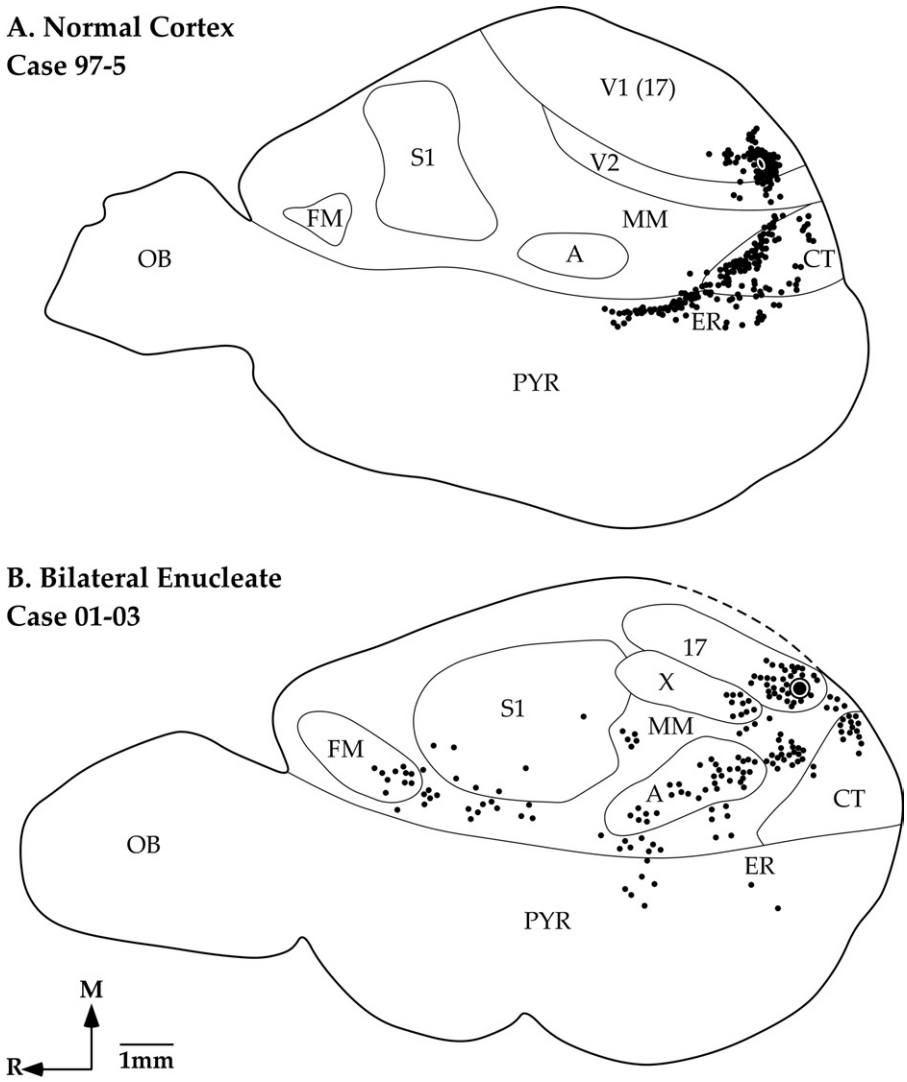

Fig. 4. Reconstructions of cortical connections of a normal (A) and a bilaterally enucleated opossum (B). The locations of cell bodies that project to area 17 are shown relative to architectonically defined cortical fields. In each case, the injection site is represented by a white circle/ellipse with a dark circle in the middle, and retrogradely labeled cells are represented as filled dots. Thin lines are myeloarchitectonic borders. In case 97-5 (A), an injection of FR was placed into area 17, and the pattern of labeled cells was relatively restricted to V1 (area 17), V2, CT, and ER. In the bilaterally enucleated animal (B; case 01-03), an injection of FE into area 17 resulted in intrinsic label in area 17, CT, and ER, as in normal animals. Aberrant patterns of retrogradely labeled cells were also observed in MM, A, S1, FM, area X, and cortex just dorsal to the rhinal sulcus. Abbreviations defined in Table 1.

remaining two cases the injections were located in the rostral region of area 17 (Fig. 5C; case 05-103, not shown). As found in normal animals, a large percentage of labeled cells (32%) was intrinsic to area 17 (Fig. 3A, D; Table 3). In all cases, a large percentage of retrogradely labeled cells was identified in the darkly myelinated area CT (21%; Figs. 3E; 4B; 5A–D), the same average percent as that observed in normal animals. In all but one case (Fig. 5C), labeled cells in this region were numerous and often densely packed. Seven percent of labeled cells were observed in cortex immediately lateral to area 17, in area X (Figs. 4B; 5A–D). In normal animals, this region of cortex normally corresponds to area 18. It is unclear whether bilaterally enucleated animals have an area 18 because it is difficult to identify this field architectonically. As in normal animals, a small to moderate percentage of labeled cell bodies (6%) was observed in ER, and in one case (01-09), the percentage of labeled cells in this region was particularly high (24%).

In addition to the normally proportionate patterns of corticocortical connectivity that we observed for area 17 in bilaterally enucleated animals, we also observed abnormal projections. For example, cortex immediately lateral to area X contained numerous labeled cell bodies. The cortex in this location in normal animals is MM, but has relatively sparse connections with V1 (area 17; 5% in normal animals; Fig. 4A). In bilaterally enucleated animals, the percentage of labeled cells in MM was high (14%) compared with that of normal animals (Table 3, Fig. 5A, D). Area 17 received a greater percentage of connections from area A

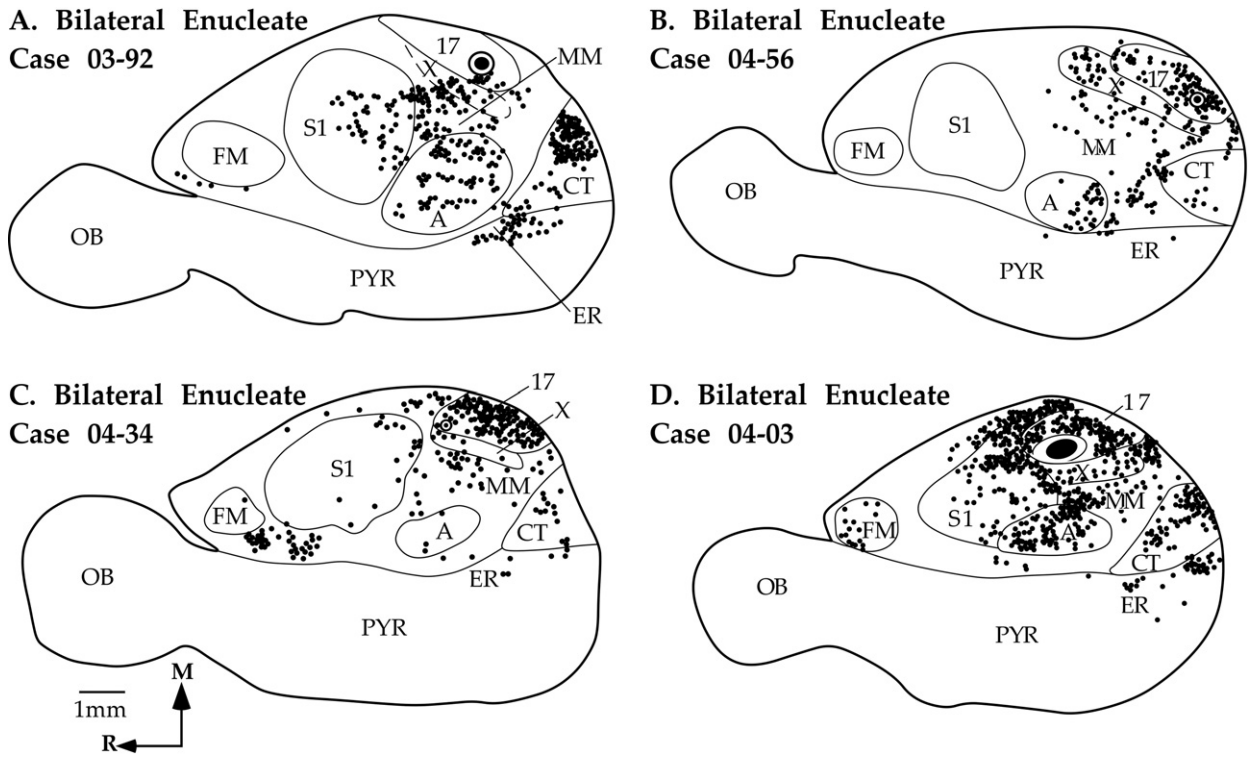

Fig. 5. Reconstructions of cortical connections of area 17 for four bilaterally enucleated animals (A–D). The locations of cell bodies that project to area 17 are shown relative to architectonically defined cortical fields. Injections are defined by a large white circle/ellipse with a dark circle in the middle, and retrogradely labeled cells are represented as filled dots. Thin lines are myeloarchitectonic borders. FE was injected in case 03-92 (A) and 04-03 (D), and FR was injected in the other two cases (B and C). In all cases, connections were observed with other portions of area 17 and CT. Additionally, aberrant connections were observed with MM, A, S1, area X, and cortex rostral to S1. Abbreviations defined in Table 1. Conventions as in previous figures.

(7% vs. 1%), and in some cases this percentage was particularly high (e.g. 10-12% in cases 04-03 and 03-92). Abnormal projections were also observed from S1 (Figs. 4B; 5A, C, D; case 05-103, not shown), although the percentage of labeled cells was relatively small (5.5%). Label in S1 did not appear to be restricted to a particular portion of the field. Finally, about 6% of labeled cells were observed in other regions of the cortex, particularly, in and around the frontal myelinated area, FM (Figs. 4B; 5A, C, D; case 05-103, not shown).

Thalamic cytoarchitecture and CO staining

The architectonic appearance of the lateral geniculate (LG) and the lateral posterior (LP) thalamic nuclei has been previously described in normal *M. domestica* using both Nissl stains and CO stains (Kahn et al., 2000). In both normal and bilaterally enucleated animals, the major sensory nuclei of the thalamus, including the dorsal lateral geniculate nucleus (LGD), the ventral lateral geniculate nucleus (LGV), the ventral posterior nucleus (VP), and the medial geniculate nucleus (MGN), are all darkly stained and densely packed with cells as determined by Nissl staining. Further, LGD, LGV, VP, and MGN stain darkly for CO compared with surrounding nuclei (Fig. 6H, I, K, L). Both LP and LGD contain small moderately packed cells and stain darkly for CO (Fig. 6E, F). In the rostral thalamus, the anterior dorsal nuclei (AD) are easily distinguished as containing darkly stained, densely packed cells as determined by Nissl and stain very darkly for CO (Fig. 6B, C). The anterior ventral nucleus (AV) also stains darkly and contains densely packed cells, but not to the same extent as AD (Fig. 6B, C). AV stains moderately for CO.

Thalamocortical projections

Labeled cell bodies resulting from injections in area 17 were related to architectonic boundaries of thalamic nuclei in both normal and bilaterally enucleated animals. In both normal and enucleated animals, the majority of retrogradely labeled cells were in the LGD (58% and 37%, respectively; Fig. 7). Moderate projections from LP were also observed in both normal and bilateral enucleates (20% and 17%, respectively; Fig. 7). In normal animals, 16% of retrogradely labeled cells were in the laterodorsal nucleus (LD), and in bilaterally enucleated animals, 8% of labeled cells were in LD. Thus, normal thalamocortical pathways appear to be maintained despite the complete lack of input from the eye. Neuroanatomical tracer injections into area 17 in bilaterally enucleated animals also revealed abnormal thalamic projections from several nuclei. As with corticocortical connections, there was some variability in the patterns of thalamocortical connections

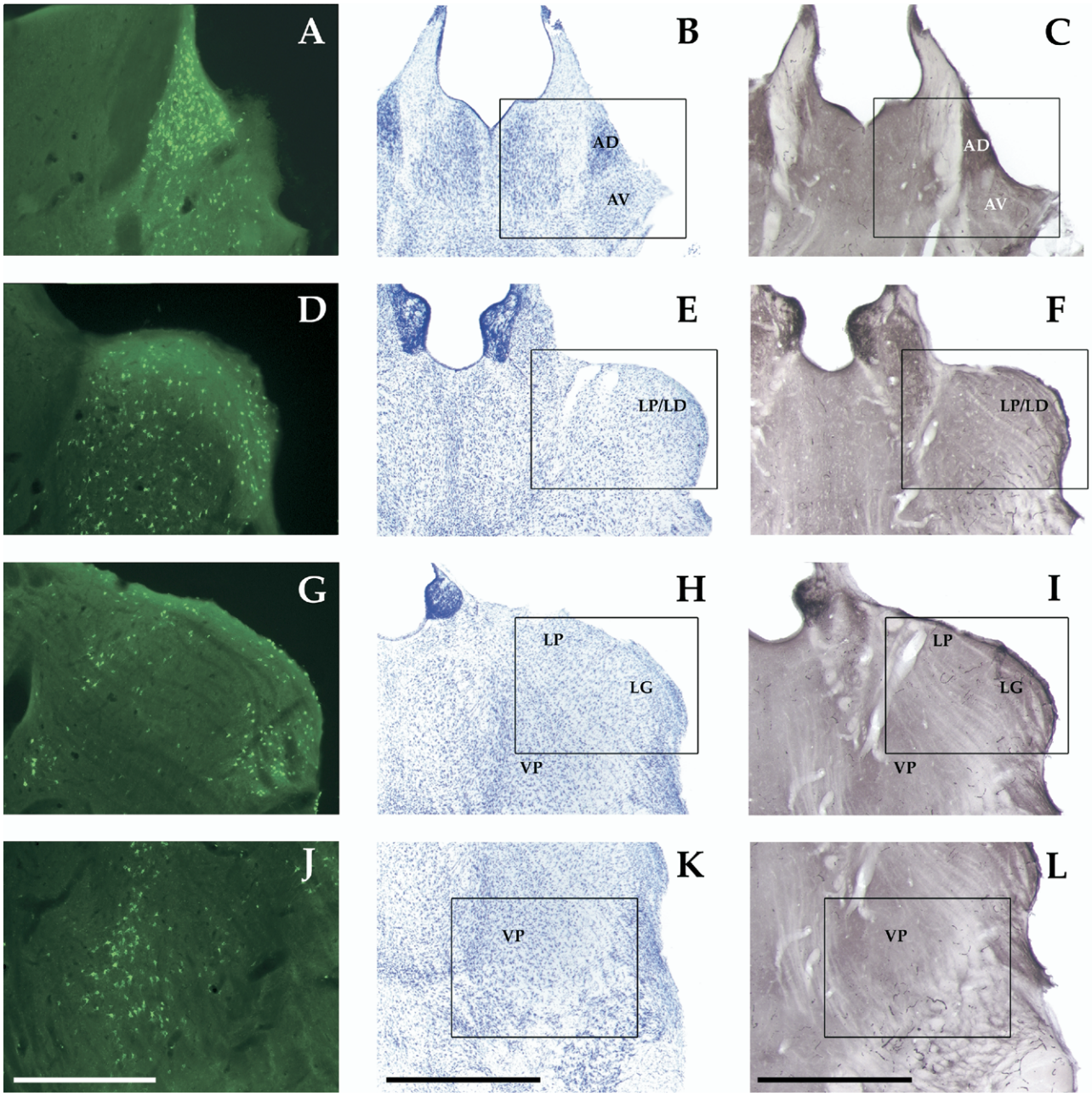

Fig. 6. Digital images of labeled cells in the dorsal thalamus (left column) resulting from injections into area 17 of a bilaterally enucleated animal, case 05-103. Adjacent sections that were stained for Nissl (middle column) or processed for CO (right column) allowed us to relate the location of labeled cells to architectonically defined nuclei of the thalamus. While normal projections from LG and LP were observed (G–I), abnormal projections were also observed from AD and AV (A–C), LD and VL (D–F), and VP (J–C). The rectangles in the middle and right columns outline the region where cells were imaged in the left column. Scale bar=500 μm in the left column and scale bar=1 mm in the middle and right columns. Abbreviations defined in Table 1. Conventions as in previous figures.

across individuals. In three cases an average of 5% of retrogradely label cells were observed in AV, and 3% of cells were in AD; both nuclei are normally associated with limbic/hippocampal cortex (Figs. 6A–C; 7B, C). Only in one normal animal were a few labeled cells observed in AV (1%), and no cells were observed in AD in normal animals in any of the cases examined. All but one injection in bilaterally enucleated animals resulted in a moderate number of labeled cells in the ventrolateral nucleus (VL; 8%), a number substantially larger than in normal animals where only two injections resulted in a few cells in VL (2%). In all bilaterally enucleated animals 4%–12.5% of retrogradely labeled cells were observed in VP (e.g. Figs. 6J–L; 7B, C; Table 3). This is in contrast with normal animals where only one or two labeled cells were observed in VP in two cases (0.3%; Fig. 7A). Finally, in one bilaterally enucleated animal a few labeled cells were observed in the MGN. The remaining labeled cells were scattered throughout portions

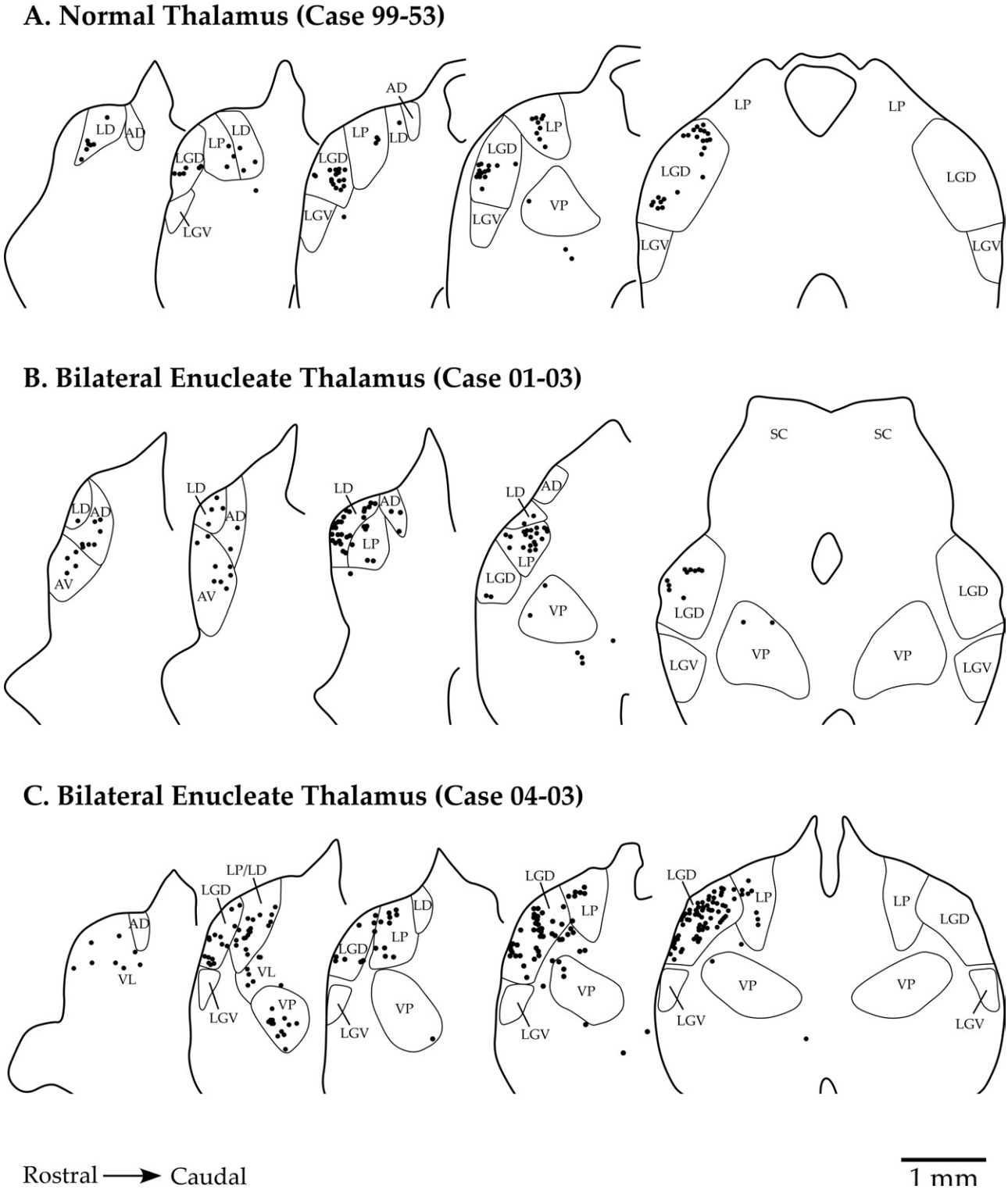

Fig. 7. A series of sections through thalami that have been sectioned in the coronal plane. Reconstructions of retrogradely labeled cells in the thalamus resulting from an anatomical tracer injection into area 17 are illustrated in a normal (A) and two bilaterally enucleated opossums (B and C). In normal animals, cells projecting to area 17 arise predominantly from LGD, LP, and LD. A few cells are observed outside of these nuclei in and around VP. In bilateral enucleated animals, the majority of cells projecting to area 17 are from LGD and LP; however, in the two cases depicted, cells projecting to area 17 are also located in AV, AD, VP, and VL. The average percentage of labeled cells across all cases is given in Table 3. Thin lines represent cytoarchitectonic borders drawn from adjacent Nissl- and CO-stained sections; retrogradely labeled cells are represented as filled dots. Sections are drawn rostral (left) to caudal (right). Abbreviations defined in Table 1.

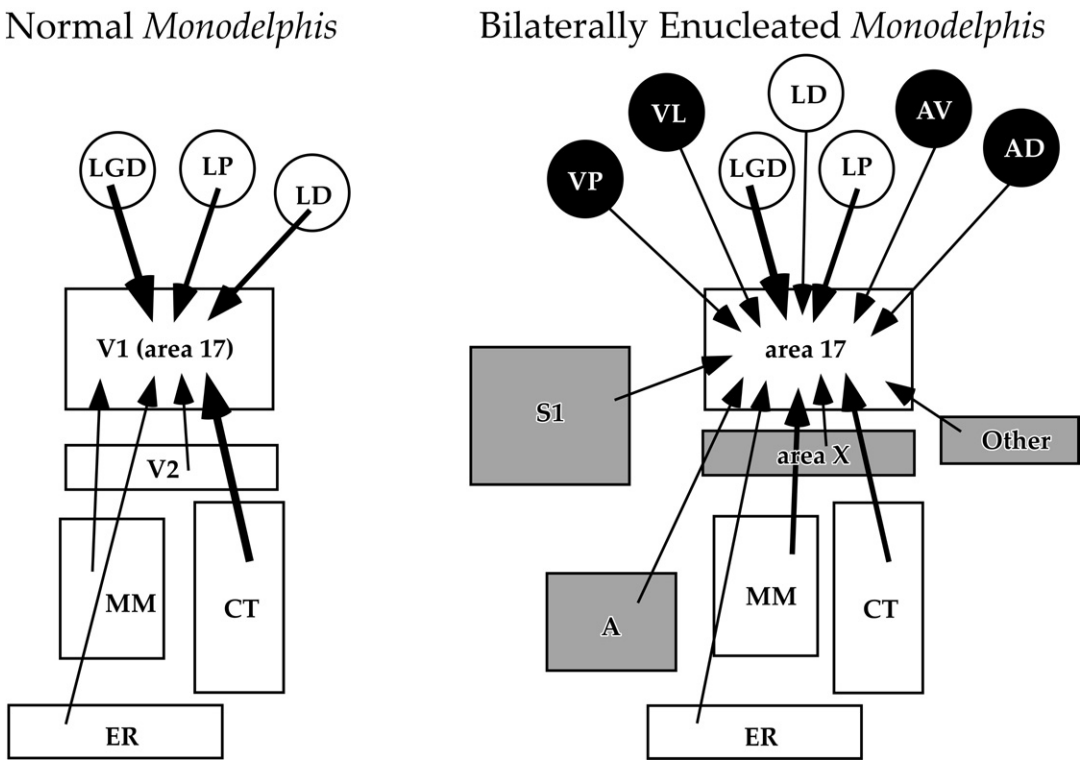

Fig. 8. A summary of the thalamic (circles) and cortical (boxes) connections of area 17 in a normal (left) and bilaterally enucleated (right) opossum. In normal animals, thalamocortical projections are restricted to LGD, LP, and LGD. Corticocortical connections of area 17 in normal animals are predominantly from visual areas V2 and CT, and from ER. In bilaterally enucleated animals, normal projections from LGD, LP, and LD of the thalamus can be identified; however, a large number of abnormal connections are also observed from VP, VL, AV, and AD. Like the thalamus, the normal pattern of corticocortical connections exist in bilateral enucleated animals; however, additional projections (shaded boxes) from S1, A, MM, area X, and other regions of the cortex have been identified as well. The thickness of arrows roughly denotes the density of connections. Abbreviations defined in Table 1.

of the thalamus in nuclei that were difficult to define architectonically, and thus are termed "other." Taken together, the data indicate that aberrant thalamocortical connections have formed in the bilaterally enucleated animal. For most injections examined, AV, AD, VP, and VL contained relatively large percentages of labeled neurons compared with normal animals (Table 3).

DISCUSSION

In the present study in bilaterally enucleated *M. domestica*, we demonstrate that in addition to its normal projections from visual nuclei of the thalamus (LGD and LP; see Table 1 for abbreviations), area 17 also receives input from nuclei associated with the somatosensory (VP), auditory (MGN), motor (VL), and limbic/hippocampal (AD, AV) systems (Fig. 8). Likewise, in addition to the highly restricted projections from cortical visual areas such as CT and ER, area 17 in bilaterally enucleated opossums receives inputs from A, S1, and MM cortex. These results are the first to provide a potential anatomical substrate for the massive functional reorganization of visual cortex observed in bilaterally enucleated opossums (Kahn and Krubitzer, 2002), hamsters (Izraeli et al., 2002), and congenitally blind humans (Sadato et al., 1996; Cohen et al., 1997; Buchel et al., 1998; Weeks et al., 2000).

Effects of early loss of visual input on subcortical pathways

In several species, including rodents, hamsters, opossums, and primates, monocular enucleation in neonatal animals causes extensive alterations in the retinogeniculate and retinocollicular pathways (see Table 4 for review). Specifically, axons from the ipsilateral eye invade areas of the ipsilateral LGD and superior colliculus (SC) that are normally occupied by projections from the contralateral eye (Lund et al., 1973; Drager, 1978; Godement et al., 1980; Lent and Mendez-Otero, 1980; Rakic, 1981; Hsiao, 1984; So et al., 1985). Similar results were demonstrated in anophthalmic and microphthalmic mice (in which one eye is reduced in size while the other is normal in size, Godement et al., 1980). In addition to alterations in connectivity, monocular or binocular enucleations also resulted in a volume reduction of the LG, due to increased cell death, retraction of axonal projections, atrophy, or less extensive arborization of retinal axon terminals (Lund et al., 1973; Heumann and Rabinowicz, 1980; Finlay et al., 1986; Berman, 1991; Trevelyan and Thompson, 1995). Thus, the lack of visual input has been shown to have a pronounced effect on retinal pathways and subcortical retinal targets.

In the present investigation, we did not quantify the size of the LGD. However, microscopic analysis of this

Table 4. Summary of previous enucleation studies

Enucleation	Outcome	Reference	Species	Age
Monocular	Alterations in retinogeniculate and retinocollicular pathways	Lund et al., 1973	Rat	P0–P100
		Godement et al., 1980	Mouse	P0
		Lent and Mendez-Otero, 1980	Opossum	P23–P38
		Rakic, 1981	Macaque	Midgestation
		Hsiao, 1984	Hamster	P0–P32
	Alteration in thalmocortical projections	Jeffery, 1984	Rat	P0
		Trevelyan and Thompson, 1992	Hamster	P0
	Alterations in corticotectal projections	Djavadian et al., 2001	Opossum	P2
	Alterations in callosal connectivity	O'Brien and Olavarria, 1995	Hamster	P0
	Decreased volume of LG	Lund et al., 1973	Rat	P1–P100
		Finlay et al., 1986	Hamster	P0
		Trevelyan and Thompson, 1995	Hamster	P0
	Visual cortical areas invaded by other sensory modalities	Toldi et al., 1994	Rat	P0
Bilateral	Normal connection patterns maintained	Negyessy et al., 2000	Rat	P0
		Izraeli et al., 2002	Hamster	P0
	Alterations in callosal connectivity	Innocenti and Frost, 1980	Cat	P1–P4
		Olavarria and van Sluyters, 1984	Mouse	P0
		Dehay et al., 1989	Macaque monkey	E77, E112
		Berman, 1991	Cat	P0–P60
		Olavarria and Li, 1995	Rat	P0
	Decreased volume of LG	Berman, 1991	Cat	P0–P60
		Dehay et al., 1996	Cynomolgus monkey	E59–E109
	LGD invaded by afferents from other sensory modalities	Asanuma and Stanfield, 1990	Mouse	P0
		Izraeli et al., 2002	Hamster	P0
	Alterations in LG lamination	Brunso-Bechtold et al., 1983	Tree shrew	P0
		Dehay et al., 1996	Cynomolgus monkey	E59–E109
	Identification of a novel cytoarchitectonic area, termed area X	Rakic et al., 1991	Macaque monkey	E81, E90
	Previously visual areas respond to other sensory modalities	Yaka et al., 1999	Cat	P3–P5
		Izraeli et al., 2002	Hamster	P0
		Kahn and Krubitzer, 2002	Opossum	P4
	Stabilization of transitory corticocortical projections	Innocenti et al., 1988	Cat	P2
Both types	Alterations in cortical lamination	Heumann and Rabinowicz, 1982	Mouse	P0
	Alterations in callosal connectivity	Olavarria et al., 1987	Rat	P0–adult
		Ankaoua and Malach, 1993	Rat	P0
	Decreased volume of LG	Heumann and Rabinowicz, 1980	Mouse	P5–P180

structure did not reveal any apparent difference in its size, as compared with normal animals. It is possible that the very early developmental stage of our enucleations allowed for more radical alterations in subcortical connectivity such that the LGD was invaded by afferents from other sensory modalities, resulting in decreased degeneration of this structure. Such major re-routing has been described in congenitally blind (Asanuma and Stanfield, 1990) and deaf mice (Hunt et al., 2005), bilaterally enucleated hamsters (Izraeli et al., 2002), and naturally blind animals (Doron and Wollberg, 1994). For example, Asanuma and Stanfield (1990) showed that in both congenitally blind mice and those bilaterally enucleated at birth, ascending somatosensory projections from the dorsal column nuclei innervate the LGD. Furthermore, in bilaterally enucleated hamsters (Izraeli et al., 2002; Piche et al., 2004) and in naturally blind animals such as the blind mole rat (Doron and Wollberg, 1994), the inferior colliculus has been shown to be the major source of input to the LGD. Finally, recent work in our laboratory has demonstrated abnormal retinal projections to the MGN in congenitally deaf mice (Hunt et al., 2005). Together, these studies demonstrate that early sensory loss, which is induced either experimentally or occurs naturally, results in alterations of primary sensory afferents within and across midbrain structures and thalamic nuclei, indicating that anatomical re-modeling begins at the earliest stages of sensory processing.

Effects of early loss of visual input on thalamocortical and corticocortical connections

In the present investigation, there were two important observations regarding the connections of area 17 in bilaterally enucleated animals. The first was that despite the lack of eyes, and therefore any type of visual input, major portions of the visual pathways remained intact. The second observation was that abnormal projections to area 17 from both the thalamus and other cortical fields had formed. Both of these observations are supported by studies in bilaterally enucleated hamsters and rats and in naturally blind animals (see Table 4).

In bilaterally enucleated hamsters, normal connections between the LGD and area 17 were maintained (Izraeli et al., 2002). Normal connections were also maintained in

bilaterally enucleated rats; however, differences in the density of projections from LGD, LP, and LD were observed (Negyessy et al., 2000). In these rats, the proportion of neurons projecting to area 17 from LP increased, while the proportion projecting from LGD decreased. In the current study, we observed a similar decrease in the average percentage of cells from LGD projecting to area 17 (Table 3). However, the average number of cells projecting to area 17 from LP was about the same in normal and bilaterally enucleated animals.

In the blind mole rat, injections into area 17 result in labeled cells in LGD (Cooper et al., 1993), although some abnormal connections to VP were noted as well (Rehkamper et al., 1994). Similar results have been reported for anopthalmic mice where normal projections from LGD to area 17 are maintained (Godement et al., 1979; Kaiserman-Abramof et al., 1980) in addition to abnormally dense projections to the posterior portions of the dorsal thalamus, including LP (and possibly VP). However, it should be noted that the extent and density of the abnormal thalamic projections in both blind mole rats and anophthalmic mice were less dramatic than in the present investigation. Finally, studies by Catalano and Shatz (1998) indicate that in addition to the loss of sensory driven activity, alterations in neural activity can result in substantially altered connections. In developing cats in which the brain was infused with the sodium channel antagonist tetrodotoxin, axons from the LGD which normally project to area 17, projected to a number of cortical regions outside of area 17, including auditory cortex.

In bilaterally enucleated animals, we observed both normal corticocortical connections to area 17, from CT, ER, and cortex lateral to area 17, and aberrant connections, from S1, A, MM, and other regions of the cortex. While a number of studies have examined the alterations in callosal connectivity that result from early bilateral enucleations (e.g. Innocenti and Frost, 1980; Olavarria and van Sluyters, 1984; Olavarria et al., 1987; Dehay et al., 1989; O'Brien and Olavarria, 1995, see Table 4), there are very few studies that examine ipsilateral corticocortical connections in bilaterally enucleated mammals (e.g. Innocenti et al., 1988; Izraeli et al., 2002). Furthermore, unlike the current study, Izraeli and colleagues (2002) found that there were no alterations in corticocortical connections of bilaterally enucleated hamsters. It is likely that the differences between the present results and those described for hamsters are a reflection of the developmental stage at which the enucleations were made. In hamsters, enucleations were made after thalamic axons had arrived at the cortex and invaded the cortical plate (Miller et al., 1993). In *M. domestica*, thalamocortical afferents do not arrive at the cortex until P5, and the process of thalamocortical development occurs through P15 (Molnar et al., 1998b). Our enucleations took place on P4.

The observation of altered corticocortical connections in the present investigation is very similar to results described previously in hamsters in which LGD, LP, and LGV were lesioned at birth (Kingsbury et al., 2002). In these hamsters, V1 not only maintained its normal pattern of connectivity with other visual areas, but also formed ectopic projections with somatosensory and auditory cortex.

While the presence of aberrant connections is extremely interesting and likely underlies the functional alterations observed for area 17 in bilaterally enucleated animals, the observation that substantial portions of the visual pathway are maintained is remarkable. This suggests that there are large constraints imposed on developing and evolving nervous systems, since even the complete loss of a sensory receptor array does not eliminate connections associated with that array. Nevertheless, neural structures associated with the lost system are often reduced in size, and alterations in connectivity can occur. The constraints on sensory systems are likely imposed by genes and the developmental contingencies that they induce. Thus, mammalian brains are saddled with a number of organizational features and connectivity that may not be the most efficient design, but that represent the best compromise for information processing.

What accounts for the variability observed in the connectivity and functional organization of bilaterally enucleated opossums?

In the present investigation, we noted that the types of alterations in connectivity observed in bilaterally enucleated animals were variable. For example, in some animals connections were relatively dense with auditory cortex (e.g. Fig. 5A, D), and in other cases such connections were sparse (Fig. 5C) or absent (case 01-09, not shown). Likewise, for the thalamus, some cases had numerous labeled cells in VL (18%; case 03-92) resulting from injections in area 17, while other cases had fewer (6%; case 04-34) or no retrogradely labeled cells in VL (case 01-03). It is likely that this variability observed in anatomical connections in bilaterally enucleated animals, underlies the functional variability observed in cortical maps of area 17 and other areas (Fig. 2). Thus, one could surmise that dense connections with auditory cortical fields and/or thalamic nuclei may be the result of tracer injections into a region of area 17 in which neurons are driven by auditory stimulation. Likewise, a preponderance of aberrant somatosensory connections may be the result of injections placed in portions of area 17 driven by somatosensory stimulation. The question is: What generates this variability in cortical connectivity?

There are several possible sources of variability observed in the current study. The first source could be due to variability in methodology. We think this is unlikely since all of the animals were enucleated at the same age, the neocortex was examined in normal and enucleated adults within the same range of ages, the anatomical tracers utilized were the same for both normal and enucleated animals, and both of the tracers that were injected used the same axonal transport mechanisms.

A second source of variability could be due to naturally occurring variation within a population. This likely accounts for at least some of the variability observed in bilaterally enucleated animals since normal animals also exhibit variability in terms of the density of projections from particular

cortical fields and thalamic nuclei (see standard deviations in Table 3), and even in the source of some of their inputs.

A final source of variability could be due to differences in sensory driven activity that each animal encounters during development and the strategies implemented by individual animals to explore their surroundings. The bilaterally enucleated animals are hand reared for several weeks after eye opening would normally occur, and all animals (including normal animals) are provided with environmental enrichment. The combination of these two events makes the sensory experiences of individual animals fairly unique. These differences may be exacerbated by different strategies used by the animals to explore their environment, including the dominant of use of one sense over another and the interaction between the dominant sensory system and the motor system. This suggests that alterations in relative activity patterns between sensory systems, either due to the loss of a single system (or overuse of a system compared with others) or to alterations in the environment in which the individual develops, can generate remarkable changes in the functional and anatomical organization of the neocortex.

How do alterations in connections occur?

Another question that arises from the current results is: What features of development are modified to account for alterations in thalamocortical and corticocortical connectivity? Evidence suggests that at least two possible, although not mutually exclusive, mechanisms are involved in this process. The absence of patterned activity from the retina could affect/shift molecular gradients, which could subsequently alter the trajectory of axons along the visual pathway. For example, recent evidence indicates that there is a substantial amount of molecular specificity in thalamocortical axonal development (e.g. Molnar et al., 1998a; Dufour et al., 2003, see Lopez-Bendito and Molnar, 2003; Vanderhaeghen and Polleux, 2004 for review).

Alternatively, there is good evidence that the failure to prune exuberant connections is responsible for the alterations in connectivity observed with the loss of a sensory system (see Innocenti and Price, 2005 for review). For example, studies on the development of geniculocortical projections to area 17 in hamsters indicate that initial projections are broadly distributed early in development and become more precise at later postnatal ages (Naegele et al., 1988; Krug et al., 1998). There is also a good deal of evidence that developing corticocortical connections are initially imprecise, and do not reflect the adult form until later in development. For example, studies of corticocortical connections in hamsters indicate that initial projection zones are exuberant, and subsequently become more restricted (Lent et al., 1990). Further, studies in monkeys and cats demonstrate that cortical connections of area 17 are initially broadly distributed, and that transient projections from inappropriate areas, such as primary auditory area and other cortical areas, are present (e.g. Dehay et al., 1984, 1988; Innocenti et al., 1988; Kennedy et al., 1989; Price et al., 1994; Caric and Price, 1999, see Innocenti and Price, 2005 for review). These data support the hypothesis that aberrant corticocortical connections reported in the present study could be the result of exuberant projections which fail to get pruned due to alterations in sensory driven activity between sensory systems.

It is clear that the developmental mechanisms that underlie the anatomical changes observed in these studies involve factors extrinsic to the immature neocortex and play a large role in the maintenance of connections and in the specification of cortical fields. It is likely that a combination of correlated activity and expression of molecular signals acts to direct the organization of sensory axons.

Acknowledgments—We wish to thank Dylan Cooke, Deborah Hunt, DeLaine Larsen, and Jeffery Padberg for their helpful comments on this manuscript. This work was supported by a McDonnell Foundation grant and an NINDS award (R01-NS35103) to Leah Krubitzer, by an NSF fellowship (DG-0202740) to Sarah Karlen, and by an Institutional NIH Training Grant to UC Davis (5 T32 MH19989).

REFERENCES

Ankaoua D, Malach R (1993) Evidence for plasticity of intrinsic horizontal connections in area 17 of the rat. Isr J Med Sci 29:555–569.

Asanuma C, Stanfield BB (1990) Induction of somatic sensory inputs to the lateral geniculate nucleus in congenitally blind mice and in phenotypically normal mice. Neuroscience 39:533–545.

Bavelier D, Neville HJ (2002) Cross-modal plasticity: where and how? Nat Rev Neurosci 3:443–452.

Berman NE (1991) Alterations of visual cortical connections in cats following early removal of retinal input. Brain Res Dev Brain Res 63:163–180.

Brunso-Bechtold JK, Florence SL, Casagrande VA (1983) The role of retinogeniculate afferents in the development of connections between visual cortex and the dorsal lateral geniculate nucleus. Brain Res 312:33–39.

Buchel C, Price C, Frackowiak RS, Friston K (1998) Different activation patterns in the visual cortex of late and congenitally blind subjects. Brain 121(Pt 3):409–419.

Caric D, Price DJ (1999) Evidence that the lateral geniculate nucleus regulates the normal development of visual corticocortical projections in the cat. Exp Neurol 156:353–362.

Carroll EW, Wong-Riley MT (1984) Quantitative light and electron microscopic analysis of cytochrome oxidase-rich zones in the striate cortex of the squirrel monkey. J Comp Neurol 222:1–17.

Catalan-Ahumada M, Deggouj N, De Volder A, Melin J, Michel C, Veraart C (1993) High metabolic activity demonstrated by positron emission tomography in human auditory cortex in case of deafness of early onset. Brain Res 623:287–292.

Catalano SM, Shatz CJ (1998) Activity-dependent cortical target selection by thalamic axons. Science 281:559–562.

Cohen LG, Celnik P, Pascual-Leone A, Corwell B, Falz L, Dambrosia J, Honda M, Sadato N, Gerloff C, Catala MD, Hallett M (1997) Functional relevance of cross-modal plasticity in blind humans. Nature 389:180–183.

Cooper HM, Herbin M, Nevo E (1993) Visual system of a naturally microphthalmic mammal: the blind mole rat, Spalax ehrenbergi. J Comp Neurol 328:313–350.

Dehay C, Bullier J, Kennedy H (1984) Transient projections from the fronto-parietal and temporal cortex to areas 17, 18 and 19 in the kitten. Exp Brain Res 57:208–212.

Dehay C, Giroud P, Berland M, Killackey H, Kennedy H (1996) Contribution of thalamic input to the specification of cytoarchitectonic cortical fields in the primate: effects of bilateral enucleation in the fetal monkey on the boundaries, dimensions, and gyrification of striate and extrastriate cortex. J Comp Neurol 367:70–89.

Dehay C, Horsburgh G, Berland M, Killackey H, Kennedy H (1989) Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input. Nature 337:265–267.

Dehay C, Kennedy H, Bullier J (1988) Characterization of transient cortical projections from auditory, somatosensory, and motor cortices to visual areas 17, 18, and 19 in the kitten. J Comp Neurol 272:68–89.

Djavadian RL, Bialoskorska K, Turlejski K (2001) Reorganization of the corticotectal projections introduced by neonatal monocular enucleation in the Monodelphis opossum and the influence of serotoninergic depletion. Neuroscience 102:911–923.

Doron N, Wollberg Z (1994) Cross-modal neuroplasticity in the blind mole rat *Spalax ehrenbergi*: a WGA-HRP tracing study. Neuroreport 5:2697–2701.

Drager UC (1978) Observations on monocular deprivation in mice. J Neurophysiol 41:28–42.

Dufour A, Seibt J, Passante L, Depaepe V, Ciossek T, Frisen J, Kullander K, Flanagan JG, Polleux F, Vanderhaeghen P (2003) Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. Neuron 39:453–465.

Dunn CA, Kahn DM, Krubitzer L (2001) Development of retinal connections in the short-tailed opossum (*Monodelphis domestica*). Soc Neurosci Abstr 27(2):1523.

Finlay BL, Sengelaub DR, Berian CA (1986) Control of cell number in the developing visual system. I. Effects of monocular enucleation. Brain Res 393:1–10.

Finney EM, Fine I, Dobkins KR (2001) Visual stimuli activate auditory cortex in the deaf. Nat Neurosci 4:1171–1173.

Frost SB, Milliken GW, Plautz EJ, Masterton RB, Nudo RJ (2000) Somatosensory and motor representations in cerebral cortex of a primitive mammal (*Monodelphis domestica*): a window into the early evolution of sensorimotor cortex. J Comp Neurol 421:29–51.

Gallyas F (1979) Silver staining of myelin by means of physical development. Neurology 1:203–209.

Godement P, Saillour P, Imbert M (1979) Thalamic afferents to the visual cortex in congenitally anophthalmic mice. Neurosci Lett 13:271–278.

Godement P, Saillour P, Imbert M (1980) The ipsilateral optic pathway to the dorsal lateral geniculate nucleus and superior colliculus in mice with prenatal or postnatal loss of one eye. J Comp Neurol 190:611–626.

Heumann D, Rabinowicz T (1980) Postnatal development of the dorsal lateral geniculate nucleus in the normal and enucleated albino mouse. Exp Brain Res 38:75–85.

Heumann D, Rabinowicz T (1982) Postnatal development of the visual cortex of the mouse after enucleation at birth. Exp Brain Res 46:99–106.

Hsiao K (1984) Bilateral branching contributes minimally to the enhanced ipsilateral projection in monocular Syrian golden hamsters. J Neurosci 4:368–373.

Huffman KJ, Nelson J, Clarey J, Krubitzer L (1999) Organization of somatosensory cortex in three species of marsupials, *Dasyurus hallucatus*, *Dactylopsila trivirgata,* and *Monodelphis domestica*: neural correlates of morphological specializations. J Comp Neurol 403:5–32.

Hunt DL, King B, Kahn DM, Yamoah EN, Shull GE, Krubitzer L (2005) Aberrant retinal projections in congenitally deaf mice: how are phenotypic characteristics specified in development and evolution? Anat Rec A Discov Mol Cell Evol Biol 287:1051–1066.

Innocenti GM, Berbel P, Clarke S (1988) Development of projections from auditory to visual areas in the cat. J Comp Neurol 272:242–259.

Innocenti GM, Frost DO (1980) The postnatal development of visual callosal connections in the absence of visual experience or of the eyes. Exp Brain Res 39:365–375.

Innocenti GM, Price DJ (2005) Exuberance in the development of cortical networks. Nat Rev Neurosci 6:955–965.

Izraeli R, Koay G, Lamish M, Heicklen-Klein AJ, Heffner HE, Heffner RS, Wollberg Z (2002) Cross-modal neuroplasticity in neonatally enucleated hamsters: structure, electrophysiology and behaviour. Eur J Neurosci 15:693–712.

Jeffery G (1984) Transneuronal effects of early eye removal on geniculo-cortical projection cells. Brain Res 315:257–263.

Kahn DM, Huffman KJ, Krubitzer L (2000) Organization and connections of V1 in *Monodelphis domestica*. J Comp Neurol 428:337–354.

Kahn DM, Krubitzer L (2002) Massive cross-modal cortical plasticity and the emergence of a new cortical area in developmentally blind mammals. Proc Natl Acad Sci U S A 99:11429–11434.

Kaiserman-Abramof IR, Graybiel AM, Nauta WJ (1980) The thalamic projection to cortical area 17 in a congenitally anophthalmic mouse strain. Neuroscience 5:41–52.

Kennedy H, Bullier J, Dehay C (1989) Transient projection from the superior temporal sulcus to area 17 in the newborn macaque monkey. Proc Natl Acad Sci U S A 86:8093–8097.

Kingsbury MA, Lettman NA, Finlay BL (2002) Reduction of early thalamic input alters adult corticocortical connectivity. Brain Res Dev Brain Res 138:35–43.

Krug K, Smith AL, Thompson ID (1998) The development of topography in the hamster geniculo-cortical projection. J Neurosci 18: 5766–5776.

Lent R, Hedin-Pereira C, Menezes JR, Jhaveri S (1990) Neurogenesis and development of callosal and intracortical connections in the hamster. Neuroscience 38:21–37.

Lent R, Mendez-Otero R (1980) Plasticity of the ipsilateral retinotectal projection in early enucleated opossums: changes in retinotopy and magnification factors. Neurosci Lett 18:37–43.

Levanen S, Jousmaki V, Hari R (1998) Vibration-induced auditory-cortex activation in a congenitally deaf adult. Curr Biol 8:869–872.

Lopez-Bendito G, Molnar Z (2003) Thalamocortical development: how are we going to get there? Nat Rev Neurosci 4:276–289.

Lund RD, Cunningham TJ, Lund JS (1973) Modified optic projections after unilateral eye removal in young rats. Brain Behav Evol 8:51–72.

Miller B, Chou L, Finlay BL (1993) The early development of thalamocortical and corticothalamic projections. J Comp Neurol 335:16–41.

Molnar Z, Adams R, Blakemore C (1998a) Mechanisms underlying the early establishment of thalamocortical connections in the rat. J Neurosci 18:5723–5745.

Molnar Z, Knott GW, Blakemore C, Saunders NR (1998b) Development of thalamocortical projections in the South American gray short-tailed opossum (*Monodelphis domestica*). J Comp Neurol 398:491–514.

Naegele JR, Jhaveri S, Schneider GE (1988) Sharpening of topographical projections and maturation of geniculocortical axon arbors in the hamster. J Comp Neurol 277:593–607.

Negyessy L, Gal V, Farkas T, Toldi J (2000) Cross-modal plasticity of the corticothalamic circuits in rats enucleated on the first postnatal day. Eur J Neurosci 12:1654–1668.

O'Brien BJ, Olavarria JF (1995) Anomalous patterns of callosal connections develop in visual cortex of monocularly enucleated hamsters. Biol Res 28:211–218.

Olavarria J, Malach R, Van Sluyters RC (1987) Development of visual callosal connections in neonatally enucleated rats. J Comp Neurol 260:321–348.

Olavarria J, van Sluyters RC (1984) Callosal connections of the posterior neocortex in normal-eyed, congenitally anophthalmic, and neonatally enucleated mice. J Comp Neurol 230:249–268.

Olavarria JF, Li CP (1995) Effects of neonatal enucleation on the organization of callosal linkages in striate cortex of the rat. J Comp Neurol 361:138–151.

Piche M, Robert S, Miceli D, Bronchti G (2004) Environmental enrichment enhances auditory takeover of the occipital cortex in anophthalmic mice. Eur J Neurosci 20:3463–3472.

Price DJ, Ferrer JM, Blakemore C, Kato N (1994) Postnatal development and plasticity of corticocortical projections from area 17 to area 18 in the cat's visual cortex. J Neurosci 14:2747–2762.

Rakic P (1981) Development of visual centers in the primate brain depends on binocular competition before birth. Science 214: 928–931.

Rakic P, Suner I, Williams RW (1991) A novel cytoarchitectonic area induced experimentally within the primate visual cortex. Proc Natl Acad Sci U S A 88:2083–2087.

Rehkamper G, Necker R, Nevo E (1994) Functional anatomy of the thalamus in the blind mole rat Spalax ehrenbergi: an architectonic and electrophysiologically controlled tracing study. J Comp Neurol 347:570–584.

Roder B, Rosler F, Neville HJ (1999) Effects of interstimulus interval on auditory event-related potentials in congenitally blind and normally sighted humans. Neurosci Lett 264:53–56.

Roder B, Rosler F, Neville HJ (2000) Event-related potentials during auditory language processing in congenitally blind and sighted people. Neuropsychologia 38:1482–1502.

Sadato N, Pascual-Leone A, Grafman J, Ibanez V, Deiber MP, Dold G, Hallett M (1996) Activation of the primary visual cortex by Braille reading in blind subjects. Nature 380:526–528.

So KF, Campbell G, Lieberman AR (1985) Synaptic organization of the dorsal lateral geniculate nucleus in the adult hamster. An electron microscope study using degeneration and horseradish peroxidase tracing techniques. Anat Embryol (Berl) 171:223–234.

Toldi J, Feher O, Wolff JR (1996) Neuronal plasticity induced by neonatal monocular (and binocular) enucleation. Prog Neurobiol 48:191–218.

Toldi J, Rojik I, Feher O (1994) Neonatal monocular enucleation-induced cross-modal effects observed in the cortex of adult rat. Neuroscience 62:105–114.

Trevelyan AJ, Thompson ID (1992) Altered topography in the geniculo-cortical projection of the golden hamster following neonatal monocular enucleation. Eur J Neurosci 4:1104–1111.

Trevelyan AJ, Thompson ID (1995) Neonatal monocular enucleation and the geniculo-cortical system in the golden hamster: shrinkage in dorsal lateral geniculate nucleus and area 17 and the effects on relay cell size and number. Vis Neurosci 12:971–983.

Vanderhaeghen P, Polleux F (2004) Developmental mechanisms patterning thalamocortical projections: intrinsic, extrinsic and in between. Trends Neurosci 27:384–391.

Weeks R, Horwitz B, Aziz-Sultan A, Tian B, Wessinger CM, Cohen LG, Hallett M, Rauschecker JP (2000) A positron emission tomographic study of auditory localization in the congenitally blind. J Neurosci 20:2664–2672.

Yaka R, Yinon U, Wollberg Z (1999) Auditory activation of cortical visual areas in cats after early visual deprivation. Eur J Neurosci 11:1301–1312.

(Accepted 19 June 2006)
(Available online 24 August 2006)