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Retinal Input Influences the Size and Corticocortical Connectivity of Visual Cortex During Postnatal Development in the Ferret

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Abstract

Retinal input plays an important role in the specification of topographically organized circuits and neuronal response properties, but the mechanism and timing of this effect is not known in most species. A system that shows dramatic dependence on retinal influences is the interhemispheric connection through the corpus callosum. Using ferrets, we analyzed the extent to which development of the visual callosal pattern depends on retinal influences, and explored the period during which these influences are required for normal pattern formation. We studied the mature callosal patterns in normal ferrets and in ferrets bilaterally enucleated (BE) at postnatal day 7 (P7) or P20. Callosal patterns were revealed in tangential sections from unfolded and flattened brains following multiple injections of horseradish peroxidase in the opposite hemisphere. We also estimated the effect of enucleation on the surface areas of striate and extrastriate visual cortex by using magnetic resonance imaging (MRI) data from intact brains. In BEP7 ferrets we found that the pattern of callosal connections was highly anomalous and the sizes of both striate and extrastriate visual cortex were significantly reduced. In contrast, enucleation at P20 had no significant effect on the callosal pattern, but it still caused a reduction in the size of striate and extrastriate visual cortex. Finally, retinal deafferentation had no significant effect on the number of visual callosal neurons. These results indicate that the critical period during which the eyes influence the development of callosal patterns, but not the size of visual cortex, ends by P20 in the ferret.

INDEXING TERMS

callosal connections; interhemispheric; visual cortex; plasticity; enucleation; deprivation; deafferentation; MRI

The highly organized patterns of visual corticocortical connections in adult animals represent the end point of developmental processes that reshape the widespread, immature distribution of connections present during ontogeny (reviewed by Innocenti, 1991; Innocenti and Price, 2005). The fact that visual corticocortical pathways in a variety of species undergo similar developmental changes suggests that common mechanisms guide the specification and maturation of these pathways across species. This idea receives support from the finding that input from the eyes plays an important role in the development of both intra- and interhemispheric visual corticocortical connections in virtually all species studied to date (Innocenti, 1991; Innocenti and Price, 2005).

Various experimental manipulations of visual input have been used for studying the role of the eyes in the development of central visual pathways, including removing retinal afferents through enucleation, dark rearing, and visual deprivation through eyelid suture. Compared with other approaches to induce visual deprivation, enucleation has the advantage that it can be performed long before the eyes open, which facilitates the study of the role of retinal input before the onset of visual experience. The interhemispheric connection through the corpus callosum has often been the system of choice in studies of the effect of enucleation on the development of corticocortical projections because the distribution of callosal cells and axonal terminations forms distinct patterns that extend over broad cortical regions and are consistent among individuals of the same species. Moreover, the overall distribution of callosal connections in one hemisphere can be readily revealed following multiple injections of anatomical tracers into the opposite hemisphere.

Studies in the rat have shown that development of normal visual callosal patterns requires retinal input during a brief time window extending from postnatal day 4 (P4) to P6 (Olavarria et al., 1987; Olavarria and Hiroi, 2003). Even though the callosal pathway is very immature at P6 (Olavarria and Van Sluyters, 1985a), removal of the eyes at this age or later does not prevent the development of normal callosal patterns. In contrast, removal of the eyes at P4 results in patterns that are strikingly abnormal in both their overall distributions and their point-to-point topography (Olavarria et al., 1987; Olavarria and Hiroi, 2003). The fact that virtually the same abnormalities observed in animals enucleated at P4 are also present in animals enucleated at birth, or even in anophthalmic rats (Olavarria et al., 1988), provides evidence that the eyes do not exert a significant influence on the development of corticocortical connections prior to P4. A critical period extending from P4 to P6 has also been described for the visual callosal connections in the mouse (Olavarria and Hiroi, 2003).

These studies show that lack of retinal input during this brief critical period induces permanent alterations in the overall distribution and topography of the visual callosal pathway. Thus, the critical period determined by delaying enucleation represents a unique developmental stage during which retinal input specifies the normal layout and topography of corticocortical projections. Mapping out this critical period is therefore important for investigating how the eyes guide the development of corticocortical projections. However, whereas early enucleation has been shown to affect the development of corticocortical connections in several other species, including monkeys (Dehay et al., 1989, 1996), cats (Innocenti and Frost, 1980; Olavarria and Van Sluyters, 1995), hamsters (Fish et al., 1991; O'Brien and Olavarria, 1995; Rhoades and Dellacroce, 1980), and opossum (Karlen and Krubitzer, 2009), there is little or no information about the beginning and end of the critical period in these or other species. This may in part be because in many species, the critical period for the effect of retinal input on the distribution and topography of corticocortical connections likely ends during gestation, making the experimental mapping of the critical period difficult.

Due to the lack of comparative data related to critical period timing, a question that remains unanswered is whether the critical period correlates with the same stage of central visual development in different species. This question is important because the finding that the critical period correlates with a specific developmental stage across species would suggest that the eyes guide the development of corticocortical connections through a similar mechanism in all species. In addition, this finding would facilitate prediction of the critical period in species in which this period occurs prenatally, as is likely the case in humans. Taking advantage of the fact that ferrets are born at relatively early stages of development (Jackson et al., 1989), we decided to explore the period during which the presence of the eyes is required for the normal development of the visual callosal pattern in this species. The pattern of visual callosal connections has been studied in normal adult ferrets (Grigonis et al., 1992; Innocenti et al., 2002; Rockland, 1985), and it is known that early bilateral enucleation induces marked abnormalities on the overall callosal pattern (Bock et al., 2010b).

Here we compared the callosal patterns in normal adult ferrets, and in adult ferrets that had been enucleated at P7 or P20. These ages were chosen because development in the ferret during this period corresponds approximately to development in the rat during the P4–P6 critical period. As suggested before (Olavarria et al., 1987), a factor that could control the beginning of retinal influences on the callosal pathway is the arrival of afferents from the dorsal lateral geniculate nucleus (dLGN). In the rat, thalamic fibers project to layer IV in visual cortex as early as P3 (Kageyama and Robertson, 1993), whereas in the ferret fibers of thalamic origin can be observed in cortical layer IV by the end of the first postnatal week (Herrmann et al., 1994). Similarly, by P6 in rats (Olavarria and Safaeian, 2006; Ruthazer et al., 2010) and P20 in ferrets (Juliano et al., 1996), corticocortical axons of simple morphology begin invading upper cortical layers just as supragranular layers differentiate from the cortical plate (Herrmann, 1996; Ignacio et al., 1995; Jackson et al., 1989). Motivated by a recent study showing that the size of striate cortex was significantly reduced in ferrets bilaterally enucleated at P1 (Reillo et al., 2010), we also analyzed magnetic resonance imaging (MRI) data from intact brains to assess the effect of enucleation at P7 and P20 on the size of visual cortex. We were interested in determining whether the effects of enucleation on the callosal pattern and on the size of visual cortex have comparable critical periods. Brief reports on some of these data have been published previously (Bock et al., 2010a, b).

MATERIALS AND METHODS

Our study is based on data obtained from a total of 13 ferrets (*Mustela putorius furo*) purchased from Marshall Bioresources (North Rose, NY) and delivered to the Oregon Health and Science University (OHSU) Department of Comparative Medicine on postnatal day 5. This study, and all procedures involved, were approved by the OHSU Institutional Animal Care and Use Committees, and were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Enucleation was performed either on postnatal day 7 (bilaterally enucleated [BE]P7, $n = 5$) or postnatal day 20 (BEP20, $n = 3$). Animals were placed under 2% isoflurane anesthesia, and arterial pulse oxygenation, pulse rate, respiration rate, and core body temperature were monitored throughout surgical in vivo procedures. Under aseptic conditions, slits were cut in both eyelids, the eyes were removed with iridectomy scissors, and the eyelids were sutured closed. Topical bupivacaine was administered intraoperatively immediately after removing the eyes. After full recovery from anesthesia, animals were returned to their mothers.

Tracer injections

Anatomical tracer injections were performed on nine animals (three control, three BEP7, and three BEP20) at adulthood (P120 or older). Animals were placed under 2% isoflurane anesthesia and intubated; arterial pulse oxygenation, pulse rate, respiration rate, end-tidal pCO₂, and core body temperature were monitored throughout surgical procedures. A 1 × 2-cm craniotomy was performed over the occipital and parietal lobes using a handheld bone drill. Then 65–90 0.01–0.02- μ l injections of the anatomical tracer horseradish peroxidase (HRP; Sigma, St. Louis, MO; 20% in saline), separated by 200–300 μ m, were pressure-delivered through glass micropipettes (50–100- μ m tip diameter) 600–800 μ m below the dura throughout the exposed visual cortex of one hemisphere. In some cases, portions of the ectosylvian gyrus were also injected with HRP. Following injections, the bone chip was replaced and the muscle and skin layers were sutured closed.

Histochemical processing

After a survival period of 2 days, animals were injected with 0.5 ml Euthasol (i.p.; Virbac, Fort Worth, TX), and heparinized phosphate-buffered saline (PBS) was injected into the left cardiac ventricle until the fluid from the right atria was clear. Phosphate-buffered paraformaldehyde (2%, pH 7.4) was perfused through the left ventricle for 8 minutes; brains were removed from the skull and sectioned along the midline; and the cortical mantles from the hemispheres contralateral to tracer injections were unfolded and flattened according to procedures described previously (Olavarria and Van Sluyters, 1985b, 1995). The subcortical tissue from these hemispheres was discarded. Prior to unfolding, digital images of the intact hemispheres were obtained to record the gyral and sulcal pattern. The exposed gyral crowns, but not buried sulcal walls, were stained with either a felt-tip marker or through immersion in a 1% solution of cresyl violet for 30–60 seconds in order to preserve the gyral and sulcal pattern in the flattened hemisphere. Superimposition of this surface pattern onto tangential sections aided in estimating the locations of cortical areas in the unfolded and flattened tissue (Fig. 1). Due to the intrinsic cortical curvature, the flattening procedure required that several cuts be made along the margin of the cortical mantle (indicated by curved arrows in Fig. 1B).

The patterns of retrogradely labeled somas and anterogradely labeled axon terminals in the hemispheres contralateral to the tracer injections were revealed with standard HRP protocols (Olavarria et al., 1987) in histological sections (70 μ m thick) cut tangentially to the cortical surface. The hemispheres injected with HRP were left overnight in phosphate-buffered paraformaldehyde (2%, pH 7.4) and 20% sucrose. This tissue was sectioned either in the coronal or sagittal plane, and a series of sections in each animal was reacted for HRP to examine the labeling patterns in the injected cortex, the dLGN, and other visual thalamic nuclei.

MRI data acquisition

Prior to unfolding and flattening, MRI data were collected from the hemisphere contralateral to the HRP injections in all nine brains injected with this enzyme. MRI data were also collected from one hemisphere in four additional brains (two control and two BEP7), for a total of 13 brains (five control, five BEP7, and three BEP20). In 11 of the 13 brains imaged, the hemispheres were separated along the midline and only one hemisphere was placed in a cylindrical container that fits within a 2-cm-diameter solenoidal radiofrequency (RF) transmit/receive coil for MRI data acquisition. For the remaining two brains (one BEP7 and 1 control, with HRP injections), MRI was performed prior to separating the hemispheres, and the entire brains were fit within a container and imaged by using a 3.5-cm-diameter RF coil. MRI data were acquired by using an 11.7 T MRI system interfaced with a 9-cm bore diameter magnetic field gradient coil (Bruker, Rheinstetten, Germany) following previously

described procedures (Bock et al., 2010b; Kroenke et al., 2009). T₂-weighted images were obtained by using a multislice single spin-echo pulse sequence with an echo time of 35 ms, a TR of at least 3 seconds, and a single “average.” Image resolution was isotropic, with 0.25-mm-sided voxels for the brain hemispheres imaged within the 2-cm-diameter RF coil, and 0.35-mm-sided voxels for the brains imaged using the 3.5-cm-diameter RF coil. Images acquired at 0.35-mm resolution were interpolated to 0.25-mm isotropic resolution prior to subsequent steps of the analysis. Following MRI, brain tissue was stored in PBS at 4°C.

Histological and MRI data analysis

Histological sections through the unfolded and flattened cortex were scanned to obtain digital images of the HRP labeling patterns at low magnification. Reconstructions of the callosal patterns were prepared by superimposing a series of tangential sections using Adobe Photoshop CS2 (Adobe Systems, San Jose, CA). Sections were carefully aligned with each other by using the edges of the sections, sulci, blood vessels, and other fiducial marks, and the final pattern of callosal connections was reconstructed by merging all images together. During the reconstruction process, all imaging processing used, including contrast enhancement and intensity level adjustments, were applied to the entire images, and all animals were analyzed similarly. Thresholded versions of the final patterns were prepared after the patterns were high-pass-filtered to remove gradual changes in staining density. The same filter parameters and thresholding levels were applied to all control and BE animals.

The same sections used to reconstruct the overall pattern of callosal connections were also examined at high magnification by using a microscope equipped with a motorized stage (LEPCO) controlled by a Dell XPS T500 computer running the graphic System Neurolucida (MicroBrightField, Colchester, VT), and each neuron was individually plotted. The complete distribution of labeled callosal cells was then reconstructed from all sections analyzed, over the entire visual cortex (see Fig. 7). The borders of area 17 in normal ferrets were estimated by using previous physiological (Law et al., 1988; Manger et al., 2002a) and anatomical (Innocenti et al., 2002; Reillo et al., 2010) data. In BEP7 ferrets, the borders of area 17 were estimated based on recent histological descriptions of the effect of early bilateral enucleation on the size of area 17 in the ferret (Reillo et al., 2010). These authors studied adult ferrets that had been bilaterally enucleated at P1 and found a significant reduction in the size of area 17. They also showed that the location of area 17 borders was the same as in control ferrets, and that the change in size was due to a reduction in the size of the splenial gyrus and sulcus.

To compare the distributions of callosal cells in striate cortex of control and enucleated ferrets, this area was divided into five bins of equal width drawn parallel to the 17/18 border, and the number of callosal cells in each bin was plotted as a histogram (see Fig. 7D). To perform a quantitative analysis of callosal connections in extrastriate cortex, the callosal patterns in control and enucleated ferrets were carefully aligned to one another by using gyral and sulcal landmarks, and a common extrastriate visual region containing callosal labeling in all animals was delineated and used for quantification purposes (see Fig. 8A). Within this common region, the average number of callosal cells per section contained in the thresholded callosal region, and the percentage of the common area occupied by the thresholded callosal region, were determined for each animal. Data from striate and extrastriate cortex were compared between groups by using ANOVA with α set at 0.05; post hoc comparisons were made by using the Tukey HSD test.

To estimate the effects of enucleation on the sizes of striate and extrastriate visual cortex, “fiducial” surface models of the outer (layer I to the pial surface) cortical mantle boundary were manually constructed by using MRI data and the CARET software package (Van Essen et al., 2001) following previously described procedures (Bock et al., 2010b; Kroenke

et al., 2009). Olfactory bulbs were excluded from the surface models at the position of the lateral olfactory tract and rostral to it. In order to accurately delineate cortical region boundaries, standard CARET functions were used to generate a flattened cortical surface model for each hemisphere. On the flat surface model, striate, extrastriate, and isocortex regions of interest were identified based on cortical landmarks (see Fig. 9). To take advantage of anatomical features easily identified in all brains, the borders of these regions were conservatively chosen, so the term “region” is used in place of “cortex” when referring to measurements associated with striate and extrastriate cortex. Thus, from medial to dorsal, the striate region was defined as the medial cortical surface extending from the fundus of the splenial sulcus to the occipital crest. Although the striate cortex extends about 1 mm past the occipital crest onto posterior regions of the lateral gyrus (Innocenti et al., 2002; Law et al., 1988; Manger et al., 2002a; Reillo et al., 2010), the occipital crest was used to ensure that the same region was identified in all ferrets. In addition, the anterior and posterior borders of the striate region were drawn in correspondence with previous studies of striate cortex in the ferret (Innocenti et al., 2002; Law et al., 1988; Manger et al., 2002a; Reillo et al., 2010).

The extrastriate region was defined as the lateral cortical surface extending from the occipital crest to the suprasylvian sulcus. At the dorsalmost portion of the suprasylvian sulcus, the anterior border of the extrastriate region was drawn as a perpendicular line extending from the suprasylvian sulcus to the midline crest (see Fig. 9). The isocortex region was defined as the entire cortical surface extending from the midline to the parahippocampal gyrus, and contained both the striate and extrastriate regions. Using the “Region of Interest Operations” tool in the CARET software package, the surface area of these three regions was determined for each animal. It should be noted that, for a given region of interest, its boundaries were delineated on the flattened surface models, but the surface area was determined from the corresponding region on the fiducial model. The striate and extrastriate surface area values were then subtracted from the isocortex surface area value to obtain a “nonvisual” isocortex surface area. The surface areas for both the striate and extrastriate regions were then normalized by dividing them by the “nonvisual” isocortex surface area. Statistical comparison of this ratio between groups was performed by using an ANOVA with α set at 0.05; post hoc comparisons were made by using the Tukey HSD test.

RESULTS

Following injections of HRP into the visual cortex of one hemisphere, the distribution of labeled cell bodies and axon terminals in adult animals was analyzed in control ($n = 3$), BEP7 ($n = 3$), and BEP20 ($n = 3$) ferrets. Previous studies of the visual callosal pattern in normal ferrets have used histological sections cut either transversally through the occipital pole (Grigonis et al., 1992; Rockland, 1985) or tangentially through a semiflattened, but not unfolded, preparation (Innocenti et al., 2002). In order to obtain more detailed information about fine features of the callosal pattern over the exposed surface of gyri, as well as in the hidden banks of sulci, we unfolded and flattened the cortical mantles before sectioning them in the tangential plane (Olavarria and Van Sluyters, 1985b). This approach also allowed a more detailed comparison of the callosal pattern in normal ferrets with that in BEP7 and BEP20 ferrets. The locations of visual areas described in previous physiological studies were estimated from the relation of these areas to gyral and sulcal landmarks (Innocenti et al., 2002; Manger et al., 2002a). In Figure 1A, the location of visual areas, as well as somatosensory and auditory areas, are indicated in the intact brain, whereas in Figure 1B, the borders of visual (fine dotted lines) areas have been drawn across gyri (in gray) and sulci (in white) on the unfolded and flattened cortical mantle.

Callosal pattern in normal ferrets

Although labeled callosal cells and terminations form a highly discontinuous pattern over much of the visual cortex, we found that the overall organization of visual callosal connections is remarkably conserved across cases, and that several features of the callosal pattern were consistently recognized in all control cases analyzed. Figure 2 shows that the callosal pattern observed in single histological sections (Fig. 2A) is very similar to that reconstructed by superimposing data from five sections (Fig. 2B), indicating that the callosal pattern is consistent across sections. Figure 2C illustrates that the thresholded version of the callosal pattern provides an accurate representation of the reconstructed pattern, and it was therefore used for calculating the percentage of the common visual area occupied by densely labeled callosal connections. Close inspection of Figure 2 indicates that the callosal pattern consists of densely labeled bands and patches that partially or completely surround largely acallosal areas. Comparison of the callosal pattern in Figure 2 with that from a second control case shown in Figure 6A illustrates our observation that the callosal pattern in normal ferrets is consistent from animal to animal.

In agreement with previous studies (Grigonis et al., 1992; Innocenti et al., 2002; Manger et al., 2002a; Rockland, 1985), we observed that the majority of callosal connections are found in extrastriate areas, with relatively little callosal labeling in primary visual cortex (area 17, V1). Describing the callosal pattern and comparing it with diagrams presented previously are facilitated by noting that the patchy, densely labeled callosal features are not distributed randomly over the cortex. Instead, these features tend to form larger, band-like configurations that are consistent across cases. In all normal ferrets we identified four callosal band-like regions of callosal connections, as illustrated in Figure 4A. Posteriorly, we observed two parallel band-like regions of callosal connections. One, referred to as the “posterior” band, straddles the borders between areas 17 and 18, whereas the other, referred to as the “intermediate” band, straddles the lateral border of area 19 (see Fig. 4A). These band-like regions merge at their medial and lateral ends, and at different intervals, partial or complete callosal “bridges” delineate several acallosal islands (marked with black stars in Figs. 2 and 6A) between the bands (Innocenti et al., 2002).

We observed that the callosal band straddling the 17/18 border is highly asymmetrical. Indeed, the labeling within area 17 is not apparent in the low-power images shown in Figures 2B and 6A. However, examination at higher magnification reveals a rather sparse population of labeled cells distributed close to the anterior border of area 17 (see Fig. 7A). In contrast, immediately anterior to the 17/18 border, we observed a series of densely labeled patches that give this band a beaded appearance (indicated by arrows in Figs. 2B and 6A). Another feature present in all normal ferrets is a callosal-free zone located on the suprasylvian gyrus (marked with black asterisks in Figs. 2 and 6A). This area was framed by the other two callosal band-like regions, namely, an “anterodorsal” band located at the dorsal/medial border of the suprasylvian gyrus, and an “anteroventral” band located at the ventral/lateral border of this gyrus (see Fig. 4A). At their posterior ends, both of these band-like regions merged with callosal patches located in the “intermediate” band-like region. From the anteroventral callosal band-like region, three or four densely labeled patches extended down the posteromedial bank of the suprasylvian sulcus until reaching (Fig. 2), or sometimes crossing (see Fig. 6A), the fundus of this sulcus. Finally, in more anterior and medial portions of the extrastriate cortex (parietal cortex), two or three acallosal areas of different sizes could be seen surrounded by patchy labeled regions.

Because HRP is transported both anterogradely and retrogradely, the callosal patterns revealed in HRP-reacted sections represent accumulation of reaction product in cells somas, dendrites, axon branches, and axon terminals. The same sections whose labeling patterns were scanned at low power to reconstruct the final callosal pattern were examined under a

microscope, and all labeled neurons were plotted individually. Figure 7A is a reconstruction of the labeled cells plotted at high magnification from the same five sections used in Figure 2B. Comparison of these two figures shows that the patterns are remarkably similar, indicating that regions densely labeled by HRP reaction product are also densely populated by labeled somas, whereas regions that appear devoid of HRP reaction product have at most a few labeled cells near their borders.

Callosal pattern in BEP7 ferrets

The patterns of callosal connections in ferrets binocularly enucleated on postnatal day 7 (BEP7) are shown in Figures 3 and 6B. Comparison of the callosal pattern in a single tangential section (Fig. 3A) to the reconstructed pattern using four tangential sections from the same animal (Fig. 3B) reveals that the callosal pattern of BEP7 animals is consistent across sections. All three BEP7 animals displayed a similar callosal connectivity pattern. Although callosal labeling in area 17 of BEP7 ferrets appeared as weak as in controls, plotting of individual neurons shows that significantly more cells are found in area 17 of BEP7 animals compared with controls (see Quantitative analysis, below), and that these are more widely distributed in area 17 (cf. Fig. 7A, B, D). In extrastriate cortex, the pattern of callosal connections in BEP7 ferrets is highly anomalous. Here, labeled cells and axon terminations accumulate into smaller patches that fill in many areas that are free of callosal labeling in control animals. This is particularly striking throughout visual areas 18, 19, and 21. In particular, the acallosal areas marked with black stars in normal ferrets (Figs. 2, 6A) are either absent or difficult to recognize in BEP7 ferrets. As a result, callosal band-like regions corresponding to the “posterior” and “intermediate” callosal band-like regions observed in controls are not apparent in BEP7 ferrets.

However, the major acallosal island found on the crown of the suprasylvian gyrus (black asterisks in Figs. 3 and 6B) in normal ferrets can be recognized in BEP7 ferrets, although it appears somewhat smaller and more irregular. As in normal ferrets, this acallosal island is framed by callosal regions that appear to correspond to the “anterodorsal” and “anteroventral” callosal band-like regions in normal ferrets (Fig. 4B). Figure 7B is a reconstruction of the labeled cells plotted at high magnification from the same four sections used in Figure 3B. Comparison of these two figures shows that, although very similar, the pattern of labeled somas appears somewhat more widespread, acquiring a “fuzzy” appearance. This probably reflects the fact that areas that appear lightly labeled at low power (Fig. 3B) do contain scattered labeled cells when viewed at high power.

Callosal pattern in BEP20 ferrets

We found that the patterns of callosal connections in ferrets binocularly enucleated on postnatal day 20 (BEP20; Figs. 5, 6C) not only are very similar among the three animals studied in this group, but also closely resemble the patterns in control ferrets. As with control and BEP7 animals, comparison of the callosal pattern of a single tangential section of a BEP20 animal (Fig. 5A) to the pattern reconstructed from four tangential sections (Fig. 5B) reveals that the callosal pattern of BEP20 animals is consistent across sections. As in control ferrets, only a few labeled cells are found close to the anterior border of area 17 in BEP20 animals (Fig. 7C). In extrastriate cortex, all major features of the callosal pattern in control ferrets can be readily recognized in BEP20 ferrets, including the string of densely labeled patches located immediately anterior to the 17/18 border (indicated with black arrows), the acallosal islands in areas 17 and 18 (marked with black stars in Figs. 5, 6C), and the acallosal area on the suprasylvian gyrus (marked with black asterisks in Figs. 5, 6C). As in control ferrets, this acallosal area is framed medially and laterally by two callosal bands, which extend posteriorly to merge with the callosal band-like region associated with the lateral border of area 19. The pattern revealed by plotting individual callosal cells from four

histological sections (Fig. 7C) is similar to that revealed in the final reconstruction using the scanned HRP labeling patterns from the same sections (Fig. 5B).

In all animals studied, analysis of HRP-labeled sections through the thalamus showed that the dLGN and adjacent visual thalamic nuclei were homogeneously labeled (data not shown), indicating that the relatively sparse distribution of labeled callosal cells in area 17 of all animals studied, and the patchy appearance of the pattern in area 18 and in other regions of extrastriate cortex, were not due to uneven administration, or uptake, of the tracer.

Quantitative analysis

Striate cortex—In striate cortex, we found a significant ($P = 0.001$) increase in the number of callosal cells in BEP7 ferrets ($M = 1339.17$, $SD = 302.10$) compared with controls ($M = 195.38$, $SD = 74.24$). In contrast, we found no difference ($P = 0.956$) in the number of callosal cells in area 17 between BEP20 ferrets and controls. The increase in callosal cells in BEP7 ferrets was not limited to the 17/18 border region. Area 17 was divided into five bins of equal width, running parallel to the 17/18 border (Fig. 7D). Whereas there was no difference in the number of callosal cells between groups for the most posterior bin “a,” we found a significant increase in the number of callosal cells in area 17 in BEP7 ferrets for bin “b” ($M = 81.08$, $SD = 43.93$, $P = 0.039$), bin “c” ($M = 193.33$, $SD = 91.98$, $P = 0.013$), bin “d” ($M = 370.35$, $SD = 44.69$, $P = 0.000$), and bin “e” ($M = 612.74$, $SD = 35.39$, $P = 0.000$) compared with control animals for bin “b” ($M = 5.50$, $SD = 5.41$), bin “c” ($M = 8.15$, $SD = 8.16$), bin “d” ($M = 53.33$, $SD = 42.69$), and bin “e” ($M = 124.46$, $SD = 30.07$). We found no difference between BEP20 ferrets and controls for any bin.

Extrastriate cortex—Confirming the impression that callosal connections tend to fill in spaces that appear callosal free in controls (Figs. 3, 6B), we found that the percentage of extrastriate visual cortex occupied by callosal connections was significantly ($P = 0.009$) increased in BEP7 ferrets ($M = 59.16\%$, $SD = 0.37$) compared with controls ($M = 49.81\%$, $SD = 2.14$) (Fig. 8B). In contrast, no significant ($P = 0.981$) differences in this percentage were observed in BEP20 ferrets ($M = 50.19\%$, $SD = 3.78$) compared with controls, supporting our observation that the callosal pattern in BEP20 ferrets (Figs. 5, 6C) closely resembles that in controls (Figs. 2, 6A). Within the common extrastriate visual region, we found that the average number of cells per section was not significantly ($P = 0.729$) different among control ($M = 1717.44$, $SD = 723.15$), BEP7 ($M = 1956.75$, $SD = 719.78$), and BEP20 ($M = 1794.21$, $SD = 1006.72$) ferrets (Fig. 8C).

MRI measurements—We used MRI data to compare the surface area of striate, extrastriate, and isocortex regions in control, BEP7, and BEP20 ferrets. In each animal, the areas measured for the striate and extrastriate regions were normalized by dividing them by the area of the “nonvisual” isocortex region (see Materials and Methods). We found that the normalized surface area of the striate region was significantly ($P = 0.001$) reduced in BEP7 ferrets ($M = 0.091$, $SD = 0.0084$) compared with controls ($M = 0.12$, $SD = 0.0098$) (Fig. 9G). On average, the striate region was reduced by 25.6% in BEP7 ferrets. The normalized striate region also experienced a significant ($P = 0.018$), if somewhat smaller (18.3% on average) reduction in BEP20 ferrets ($M = 0.10$, $SD = 0.0092$) compared with controls (Fig. 9G). In addition, we found that the normalized surface area of the extrastriate region was significantly ($P = 0.010$) reduced in BEP7 ferrets ($M = 0.21$, $SD = 0.015$), as well as in BEP20 ferrets ($M = 0.21$, $SD = 0.0033$; $P = 0.022$) compared with controls ($M = 0.24$, $SD = 0.020$) (Fig. 9G). On average, the reduction of the extrastriate region was 15% in these two groups. These data provide evidence that both striate and extrastriate cortices are reduced in BEP7 and BEP20 ferrets, and that the effect is stronger in BEP7 ferrets, at least in the striate region.

DISCUSSION

The pattern of visual callosal connections in normal ferrets

Our results from normal ferrets confirm and extend the results from previous studies in this species (Grigonis et al., 1992; Innocenti et al., 2002; Manger et al., 2002a; Rockland, 1985). In agreement with Innocenti et al. (2002), we observed that labeled callosal cells and terminations form a highly discontinuous pattern over much of the visual cortex. However, whereas these authors recognize three major band-like callosal regions in the extrastriate cortex, we recognize four such regions (Fig. 4A). The bands they call “posterior” and “intermediate” seem to correspond, respectively, to the bands we recognize at the 17/18 border, and the band at the lateral border of area 19. As in our material, they describe the “intermediate” band as corresponding laterally to the fundus, more medially to the posterior bank of the lateral sulcus and extending medially beyond this sulcus. Moreover, the band they call “anterior” seems to correspond to the band we call “anteroventral,” found at the lateral border of the acallosal region located on the suprasylvian gyrus (marked with black asterisks in Figs. 2, 6A). However, these authors do not explicitly recognize the band we identify as “anterodorsal,” forming the medial border of the acallosal region on the suprasylvian gyrus. Posteriorly, this band originates from the “intermediate” band at the lateral border of area 19 and extends anteriorly along the dorsal border of the suprasylvian gyrus. The triangular space between the “anterodorsal” and “intermediate” bands occupies portions of the lateral sulcus, and appears to form part of visual areas 21, posterior parietal caudal (PPc), and posterior parietal rostral (PPr) (Manger et al., 2002b).

The overall callosal pattern we observed bears some resemblance to that reconstructed in cats from tangential sections cut through the unfolded and flattened cortex (Olavarria and Van Sluyters, 1995), particularly in those callosal features associated with visual areas identified as areas 17, 18, 19, and 20 in both species (Manger et al., 2002a; Tusa et al., 1978, 1979). In ferrets, as in cats, two parallel bands of callosal connections straddle the border between areas 17 and 18, and the lateral border of area 19 (Fig. 4; see also Innocenti et al., 2002), and they correspond with representations of central visual fields in both species (Manger et al., 2002a; Payne, 1990). Between these bands a series of more or less complete callosal rings surrounds several acallosal islands (marked with black stars in Figs. 2 and 6A), which correspond to areas representing peripheral visual fields in areas 18 and 19 in both cats (Sanides and Albus, 1980) and ferrets (Manger et al., 2002a).

The callosal-free zone we observed on the suprasylvian gyrus in all control ferrets (marked with black asterisks in Figs. 2 and 6A) may be homologous with an area devoid of callosal connections on the suprasylvian gyrus of the cat (Olavarria and Van Sluyters, 1995). In the ferret, this area seems to be shared by several visual areas mapped recently, including middle portions of areas 21, and lateral portions of areas PPc and PPr (Manger et al., 2002b). In the suprasylvian sulcus we observed several patches of callosal labeling that might correspond to areas of central visual field representation in the suprasylvian visual area (SSY) (Cantone et al., 2006; Manger et al., 2008). Detailed anatomical and physiological experiments in the same animals will be required for determining with more precision how the pattern of callosal connections relates to the topography of visual areas in ferret extrastriate cortex.

At the start of our study we expected that, as in the cat (Bourdet et al., 1996; Innocenti and Frost, 1980; Olavarria and Van Sluyters, 1995), callosal connections would form a robust and relatively wide band straddling the 17/18 border. Surprisingly, in all three normal ferrets we failed to identify a distinct, densely labeled, callosal band such as that observed in the cat. Instead, in regions of V1 adjacent to its anterior border, callosal cells formed a sparse and relatively narrow distribution that was not sufficiently dense to be visible at low power,

as was the case in the extrastriate cortex (Figs. 2B, 6A). In contrast, in the area 18 portion of the 17/18 callosal zone, we observed numerous densely labeled callosal patches immediately anterior to the 17/18 border. These observations suggest that, unlike the cat, the 17/18 callosal zone in the ferret appears to consist of two parallel subzones separated by the 17/18 border: a weakly labeled subzone in anterior area 17, and a subzone in posterior area 18 made of densely labeled patches. These patches, not explicitly described in previous studies, were present in all three normal and all three BEP20 ferrets we analyzed. As in the cat (Olavarria, 2001a, b), and for reasons discussed in these previous studies, the patches of callosal connections in ferret area 18 likely correlate with ipsilateral ocular dominance columns that have been described in ferret area 18 by several laboratories (Innocenti et al., 2002; Redies et al., 1990; Ruthazer et al., 1999; White et al., 1999).

The possibility that our observation of weak callosal connections in anterior V1 was due to insufficient administration or uptake of HRP is unlikely for the following reasons. We observed that callosal connections were strong in extrastriate regions located around area 17 in the three normal animals we studied, and that HRP labeling in the dLGN ipsilateral to the injected hemisphere was uniformly dense. In addition, Innocenti et al. (2002) commented that callosal labeling at the anterior border of ferret V1 was relatively weak compared with that in area 18, and close observation of figures from other studies suggests a similar conclusion (Grigonis et al., 1992; Rockland, 1985). Finally, the sparseness of callosal connections in V1 may in part explain results suggesting that, unlike in cats, callosal connections in ferrets appear to play little or no role at the midline of the visual field (Nakamura et al., 2008).

Development of normal callosal connections depends critically on retinal influences during a postnatal period

We found that bilateral enucleation at P7 induces marked anomalies in the distribution of callosal connections. In area 17, the distribution of labeled callosal cells was still relatively sparse, but, unlike control ferrets, callosal neurons were not restricted to regions near the anterior border of area 17 and could also be observed in broader regions of this area. In normal adult monkeys, relatively few callosal cells can be found in striate cortex, and most of them form a sparse and narrow band adjacent to the V1/V2 border (Abel et al., 2000; Kennedy et al., 1986; Lund et al., 1975; Olavarria and Abel, 1996; Van Essen et al., 1982). Unlike our observations in the ferret, early prenatal enucleation in the monkey does not induce noticeable changes in either the number or distribution of callosal neurons in striate cortex (Dehay et al., 1989), perhaps because callosal connections are largely absent from monkey area 17 throughout development (Chalupa et al., 1989; Dehay et al., 1988). It is possible that the more widespread distribution of callosal cells that we observed in area 17 of BEP7 ferrets compared with control ferrets derives from an exuberant distribution of callosal cells present at early stages of normal development, as reported in other species (e.g., Olavarria et al., 1987). Testing this possibility will require study of the development of callosal connections in the ferret visual cortex.

In extrastriate cortex, the callosal pattern in BEP7 ferrets consisted of smaller patches of labeled cells and axon terminations compared with controls. These patches often occupied regions that were relatively free of callosal labeling in control animals, and, as a result, the percent area occupied by callosal connections was significantly greater in BEP7 than control ferrets. Several features that were readily seen in all control ferrets were therefore either absent or difficult to recognize in BEP7 ferrets, including the string of callosal patches located in area 18 close to the area 17 border, as well as the acallosal areas that were consistently present in areas 18 and 19 of control ferrets (marked with black stars in Figs. 2 and 6A). However, other features of the callosal pattern in control ferrets were recognizable in BEP7 ferrets, such as the acallosal area on the suprasylvian gyrus (marked with black

asterisks in Figs. 3 and 6B), although this appeared somewhat smaller in BEP7 ferrets. On either side of this acallosal region, callosal band-like regions resembling the “anterodorsal” and “anteroventral” band-like regions we identified in control ferrets could be recognized in BEP7 ferrets. Our results in extrastriate cortex of BEP7 ferrets bear some resemblance to those obtained by Dehay et al. (1989) following bilateral enucleation in fetal monkeys. These authors reported that, in area 18, callosally projecting neurons were more densely packed and stretched further rostrally from the 17/18 border than in normal animals.

In sharp contrast to our observations in BEP7 ferrets, we found that by P20 the development of callosal connections was no longer susceptible to disruption of visual input by binocular enucleation. Indeed, not only were the callosal patterns in ferrets enucleated at P20 virtually indistinguishable from those in control ferrets, but the percent of the cortical area occupied by callosal connections in these animals was not significantly different from that in control ferrets. These results indicate that the development of the overall pattern of callosal connections depends critically on retinal influences during a period between P7 and P20. The observation that bilateral enucleation at P21 had no significant effect on long-range horizontal connections in ferret striate cortex (Ruthazer and Stryker, 1996) further suggests that retinal influences specify the distribution of both inter- and intrahemispheric corticocortical connections during the same critical period. It is possible that the critical period in the ferret is somewhat shorter than the P7–P20 time window explored here, as suggested by the relative brevity of the critical periods in rats and mice (Olavarria and Hiroi, 2003).

This idea is also consistent with the critical period predicted for the ferret by translating the P4–P6 period in rats (Clancy et al., 2001, 2007). Studying ferrets enucleated at ages between P7 and P20 will be necessary to map with more precision the duration of the critical period in this species. Whether the influences exerted by the retinae during the critical period are mediated by retinal spontaneous activity or chemical cues is not currently known, but some clues come from previous studies in the rat. As in the rat (Olavarria and Hiroi, 2003), our results show that the critical period for the effect of the eyes on callosal development ends long before eye opening (about P30 in the ferret), suggesting that specification of the normal callosal pattern does not depend on visual experience. Consistent with this, Cusick and Lund (1982) showed in the rat that dark rearing failed to induce the abnormalities produced by enucleation at birth. Moreover, Chang et al. (1995) showed in rats that tetrodotoxin (TTX)-induced blockade of retinal activity in one eye during the critical period failed to reproduce the anomalies in the callosal pattern produced by early monocular enucleation, suggesting that at least some of the changes in the visual pathway brought about by monocular enucleation are not due to the imbalance of afferent activity caused by the removal of one eye. The present study opens the possibility of using the ferret to further investigate the relative roles of retinal activity and chemical cues in the development of normal callosal maps.

Effects of postnatal enucleation in ferrets vs. cats

The effects of neonatal enucleation on the callosal pattern in ferrets (this study) and rats (Cusick and Lund, 1982; Olavarria et al., 1987) appear much more severe than those observed in adult cats that had been bilaterally enucleated at birth (Innocenti and Frost, 1980; Olavarria, 1995; Olavarria and Van Sluyters, 1995). The studies in cats found that enucleation at P0 primarily reduces the number of callosal cells without significantly changing the overall callosal pattern at the 17/18 border and extrastriate cortex. The reduction of callosal neurons was more dramatic at the 17/18 callosal zone than in extrastriate cortex (Olavarria, 1995; Olavarria and Van Sluyters, 1995). By P6 in rats (Olavarria and Safaiean, 2006; Ruthazer et al., 2010) and P20 in ferrets (Juliano et al., 1996), corticocortical axons of simple morphology have begun invading upper layers of the

cortex just as supragranular layers differentiate from the cortical plate (Herrmann, 1996; Ignacio et al., 1995; Jackson et al., 1989). At birth, cats are at an approximately similar developmental stage (Aggoun-Aouaoui et al., 1996; Price and Zumbroich, 1989; Shatz and Luskin, 1986), suggesting that by P0 the critical period during which retinal influences specify the pattern of callosal connections has already ended in this species. A similar conclusion derives from translating to cats developmental stages from ferrets and rats (Clancy et al., 2001, 2007). It will be interesting to test this idea by investigating whether prenatal enucleations in cats induce the development of callosal patterns as anomalous as those we observed in BEP7 ferrets.

Although the idea that the critical period in cats has ended by P0 explains why the layout of the callosal pattern was largely undisturbed in cats enucleated at P0, it does not explain why enucleation after the critical period for callosal pattern formation leads to a significant loss of callosal cells. This loss in cell number is best explained as additional evidence that, in the cat, early visual experience is required to *maintain* the integrity of neuronal circuits and response properties that are attained during normal development independently from vision (Crair et al., 1998; Frégnac and Imbert, 1978; Innocenti et al., 1985; Zufferey et al., 1999). Surprisingly, in ferrets enucleated at P20 (this study) and rats enucleated at P6 (Olavarria et al., 1987; Olavarria and Hiroi, 2003), the mature callosal pattern closely resembles that in normal adult animals in both number and distribution of callosal neurons, indicating that, unlike cats, visual experience is not required for the maintenance of a normal complement of callosal neurons in these species. However, visual experience may prevent other deprivation-induced effects in these animals. For instance, studies in the hamster report that early visual experience prevents the deprivation-induced loss of refinement in the superior colliculus (Carrasco and Pallas, 2006). Additional comparative studies will be required to understand why different species depend on early visual experience to a different extent for maintaining normal complements of corticocortical neurons.

Postnatal influences of retinal input on the size of visual cortical areas and number of callosal neurons

Several studies have provided evidence that early enucleation leads to a reduction in the size of striate cortex (Dehay et al., 1996, 1989; Karlen and Krubitzer, 2009; Olavarria et al., 1987; Rakic, 1988; Rakic et al., 1991). Studies of the effect of fetal enucleation in macaques have concluded that enucleation leads to a reduction of size of the striate cortex, which becomes partly respecified into an additional, hybrid visual cortex, deemed area X (Rakic, 1988; Rakic et al., 1991), so that the overall surface area of the neocortex remains unchanged (Dehay et al., 1996). Recently, Reillo et al. (2010) showed in the ferret that bilateral enucleation at P1 induces a 35–40% reduction in the size of the striate cortex. However, unlike earlier findings in enucleated macaques, these authors found that the surface area of the cerebral cortex was also reduced, which led them to propose that enucleation at P1 does not lead to the respecification of striate cortex or enlargement of neighboring areas.

We performed measurements of the sizes of striate and extrastriate cortex from MRI data obtained from a number of brains that included those used in our analysis of the callosal pattern. We found that enucleation at P7, as well as at P20, caused a significant reduction in size of the striate cortex relative to controls (Fig. 9G). Compared with the reduction reported by Reillo et al. (2010) in BEP1 ferrets, our estimate of the reduction in striate cortex was on average smaller in BEP7 ferrets (25.6%), and smaller still for BEP20 ferrets (18.3%), suggesting that the effect of enucleation is greater for earlier enucleations, as previously reported for the macaque monkey (Dehay et al., 1996; Rakic et al., 1991). Although significant, the effect of enucleation on the size of the extrastriate visual cortex was smaller than for the striate cortex, and there was no obvious difference for enucleations performed at

P7 or P20. However, enucleation might have greater effects on smaller, specific regions of the extrastriate visual cortex, a possibility that is worth exploring in future studies. In agreement with Reillo et al. (2010), our data from the striate and extrastriate cortex suggest that the reduction in the striate cortex does not result in respecification of the striate cortex or enlargement of extrastriate areas. Additional studies will be required to explain why the visual cortex of macaques and ferrets responds differently to early binocular enucleation.

Our finding that enucleation at P20 causes a reduction in size of the visual cortex but does not affect development of the visual callosal pattern suggests that retinal influences continue to regulate the size of the visual cortex after the patterns of visual corticocortical connections have become specified. These observations further suggest that retinal influences regulate several aspects of cortical development through mechanisms that do not necessarily operate during the same time window. Previous studies in the monkey have shown that early enucleation reduces both the size of the dLGN and the number of fibers ascending from this nucleus (Dehay et al., 1996; Rakic, 1988). Subsequent studies have linked the effect of enucleation on the size of the striate cortex to the modulatory influences that thalamic afferents, by means of diffusible factors, exert over early stages of corticogenesis at the level of the ventricular zone (Dehay et al., 2001). Unfortunately, our material did not lend itself to carrying out accurate measurements of the size of the dLGN (see Materials and Methods), but Reillo et al. (2010) illustrated that enucleation at P1 induces a noticeable reduction in the size of the dLGN in the ferret. This observation raises the possibility that the reduction in size of the visual cortex we observed in BEP20 ferrets reflects a protracted effect of enucleation on the size of the dLGN. Further studies in the ferret are needed to determine the effects of enucleation at different ages on the sizes of both the dLGN and the striate cortex, and to compare these effects with corresponding data from monkeys (Dehay et al., 1996).

We found that although enucleation at P7 reduces the size of the extrastriate cortex and induces the development of abnormal patterns of callosal connections, it does not cause significant changes in the number of labeled callosal cells in this cortical region compared with control ferrets. This observation is consistent with our finding that the percentage of extrastriate visual cortex occupied by callosal connections is significantly larger in BEP7 than in control ferrets. It will be of interest to perform stereological studies to investigate whether this result applies only to callosal cells, or whether the total number of neurons is preserved in the extrastriate cortex of early enucleates, as appears to be the case in the striate cortex of BEP1 ferrets (Reillo et al., 2010). It is tempting to speculate that the marked changes we observed in the callosal pattern of BEP7 ferrets may be due, at least in part, to rearrangements imposed by the need to accommodate approximately the same number of callosal neurons in a significantly smaller cortical area.

In conclusion, our present results in the ferret and those from studies of the critical period in rats and mice (Olavarria and Hiroi, 2003) indicate that retinal influences are necessary for the development of normal callosal patterns. Although the nature of these influences remains to be fully understood, these studies further show that normal callosal development becomes immune to the effects of enucleation at a relatively early postnatal age. Because corticocortical connections are very immature at this age, these findings suggest that during a relatively brief critical period retinal influences are capable of laying down the normal blueprint of callosal connectivity and triggering cortical developmental processes that can continue independently from the eyes. Whether these processes depend primarily on chemical factors, or on patterns of cortical spontaneous activity that persist in the absence of retinal input (Chiu and Weliky, 2001) remains to be investigated. Understanding the mechanisms by which retinal influences specify the corticocortical connectivity of visual cortical areas thus remains an important challenge, but having determined the period during

which the retina exerts these effects will greatly facilitate this task by focusing attention on a well-defined developmental period.

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Figure 1.

A: Intact ferret brain showing the location of gyri, sulci, and various cortical areas. Dashed lines indicate cuts made to separate the posterior block that was unfolded and flattened. Posterior is left, and dorsal is up. **B:** Diagram showing an unfolded and flattened cortex. Curved arrows indicate further cuts made to fully flatten the cortex, dashed lines indicate fundi of sulci, gray areas indicate the surface of gyri, large dotted line indicates the outline of V1, and small dotted lines indicate the borders of other visual areas. A, auditory cortex; as, ansate sulcus; ls, lateral sulcus; PPc, posterior parietal caudal; PPr, posterior parietal rostral; pss, pseudosylvian sulcus; S, somatosensory cortex; spl, splenial sulcus; sss, suprasylvian sulcus; SSY, suprasylvian visual area. Scale bar = 1 cm in A, B.

Figure 2.

Visual callosal pattern in normal ferret. **A:** Single HRP-stained tangential section from a control ferret (case D). Dark areas correspond to callosal cell bodies and axonal terminations labeled with HRP reaction product. **B:** Data superimposed from five aligned HRP-stained tangential sections from the control ferret shown in A. **C:** Thresholded version of the callosal pattern shown in B. Black areas correspond to callosal regions densely labeled with HRP reaction product. Dashed lines indicate fundi of sulci, gray areas indicate the surface of gyri, and the dotted line indicates the outline of V1. Black stars on the lateral gyrus and asterisks on the suprasylvian gyrus indicate acallosal regions common to all control ferrets; black arrows indicate string of callosal patches located in area 18. Black X's in white circles in B indicate regions of artifactual labeling. Anatomical abbreviations as in Figure 1. Scale bar = 1 cm in A–C.

Figure 3.

A: Single HRP-stained tangential section from a BEP7 ferret (case D5). Dark areas correspond to callosal cell bodies and axonal terminations labeled with HRP reaction product. **B:** Data superimposed from four aligned HRP-stained tangential sections from the BEP7 ferret shown in A. **C:** Thresholded version of the callosal pattern shown in B; black areas correspond to callosal regions densely labeled with HRP reaction product. Dashed lines indicate fundi of sulci, gray areas indicate the surface of gyri, and the dotted line indicates the outline of V1. Black asterisks (suprasylvian gyrus) indicate acallosal regions common to all BEP7 ferrets. Black X's in white circles in B indicate regions of artifactual labeling. Anatomical abbreviations as in Figure 1. Scale bar = 1 cm in A–C.

Figure 4.

A: Diagram showing the four callosal band-like regions (dark gray bars) in a control ferret (case D). **B:** Diagram of the callosal pattern in a BEP7 ferret (case D5) showing two callosal band-like regions identified in BEP7 ferrets. Dashed lines indicate fundi of sulci, light gray areas indicate the surface of gyri, and the dotted line indicates the outline of V1. P, posterior band; I, intermediate band; A-V, anteroventral band; A-D, anterodorsal band. Scale bar = 1 cm in A, B.

Figure 5.

A: Single HRP-stained tangential section from a BEP20 ferret (case E3). Dark areas correspond to callosal cell bodies and axonal terminations labeled with HRP reaction product. **B:** Data superimposed from four aligned HRP-stained tangential sections from the BEP20 ferret shown in A. **C:** Thresholded version of the callosal pattern shown in B; black areas correspond to callosal regions densely labeled with HRP reaction product. Dashed lines indicate fundi of sulci, gray areas indicate the surface of gyri, and the dotted line indicates the outline of V1. Black stars on the lateral gyrus and asterisks on the suprasylvian gyrus indicate acallosal regions common to all BEP20 ferrets; black arrows indicate string of callosal patches located in area 18. Black X's in white circles in B indicate regions of artifactual labeling. Anatomical abbreviations as in Figure 1. Scale bar = 1 cm in A–C.

Figure 6.

Data superimposed from several aligned HRP-stained tangential sections from another control ferret (**A**, case D4), another BEP7 ferret (**B**, case D1), and another BEP20 ferret (**C**, case E4). Dark areas correspond to callosal cell bodies and axonal terminations labeled with HRP reaction product. Dashed lines indicate fundi of sulci; the dotted line indicates the outline of V1; black stars on the lateral gyrus and asterisks on the suprasylvian gyrus indicate acallosal regions common to ferrets of that group; black arrows indicate string of callosal patches in area 18. In C, arrowheads on the ectosylvian gyrus indicate several small, mediolaterally oriented callosal bands that likely correspond to features of the callosal pattern described previously in auditory cortex of the ferret (Wallace and Harper, 1997). Anatomical abbreviations as in Figure 1. Scale bar = 1 cm in A–C.

Figure 7.

Distribution of individual HRP-labeled callosal cells (black dots) reconstructed from the same tangential sections used to reconstruct the overall staining patterns. **A:** Normal ferret shown in Figure 2. **B:** BEP7 ferret shown in Figure 3. **C:** BEP20 ferret shown in Figure 5. Dashed lines indicate fundi of sulci, gray areas indicate the surface of gyri, and the dotted line indicates the outline of V1. **D:** Histogram quantifying the distribution of callosal cells in area 17 of control, BEP7, and BEP20 ferrets. Asterisks indicate a significant increase in the number of callosal cells in BEP7 ferrets compared with control and BEP20 animals for bins "c," "d," and "e," and a significant increase in the number of callosal cells in BEP7 ferrets compared with controls for bin "b." Error bars indicate standard error of the mean (SEM). Scale bar = 1 cm in A–C.

Figure 8.

A: Diagram showing the common extrastriate visual region (thin black line) used for quantitative analysis in all ferrets (see Materials and Methods). Dashed lines indicate fundi of sulci, gray areas indicate the surface of gyri, and the dotted line indicates the outline of V1. **B:** Histogram displaying the percentage of the common extrastriate visual region occupied by callosal labeling in control, BEP7, and BEP20 ferrets. Asterisk indicates that the percentage of the visual region occupied by callosal labeling in BE7 ferrets is significantly ($P < 0.05$) greater than in control and BEP20 ferrets. **C:** Histogram displaying the average number of labeled callosal neurons per section in the common visual region outlined in A. There were no significant differences among control, BEP7, and BEP20 ferrets. Error bars in B and C indicate SEM. Scale bar = 1 cm in A.

Figure 9.

Measurements of area size of visual cortex using surface reconstructions from MRI data. Medial views (left panels) show striate regions (dark gray) in (A) control ferret (case D4); (C) BEP7 ferret (case D1); and (E) BEP20 ferret (case E4). Lateral views (right panels) show extrastriate regions (dark gray) in (B) control ferret (case D4); (D) BEP7 ferret (case D1); and (F) BEP20 ferret (case E4). G: Histogram displaying the ratio of the striate (black bars) and extrastriate regions (gray bars) to the nonvisual isocortex regions in control, BEP7, and BEP20 ferrets. Asterisks indicate that the normalized sizes of striate and extrastriate regions in control ferrets were significantly ($P < 0.05$) larger than the sizes of the corresponding regions in BEP7 and BEP20 ferrets. Error bars indicate SEM. Scale bar = 1 cm in F (applies to A–F).