INTERNATIONAL ATOMIC ENERGY AGENCY
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
100 TRIESTE (ITALY) · P.O.B. 586 · MIRAMARE · STRADA COSTIERA 11 · TELEPHONE: 2240-1
CABLE: CENTRATOM · TELEX 460392-I

SMR/302-47

COLLEGE ON NEUROPHYSICS:
"DEVELOPMENT AND ORGANIZATION OF THE BRAIN"
7 November - 2 December 1988

"Ontogenetic Change in the Distribution of Callosal Projection Neurons in the Postcentral Gyrus of the Fetal Rhesus Monkey"

Herbert P. KILLACKEY
University of California
School of Biological Sciences
Department of Psychobiology
Irvine, CA
USA

THE JOURNAL OF COMPARATIVE NEUROLOGY 244:331-348 (1986)

Ontogenetic Change in the Distribution of Callosal Projection Neurons in the Postcentral Gyrus of the Fetal Rhesus Monkey

HERBERT P. KILLACKEY AND LEO M. CHALUPA
Department of Psychobiology and Anatomy, University of California, Irvine, Irvine, California 92717 (H.P.K.), Department of Psychology (L.M.C.) and California Regional Primate Center, (L.M.C., H.P.K.) University of California, Davis, Davis, California 95616

ABSTRACT

In the postcentral gyrus of the mature rhesus monkey the distribution of callosal projection neurons is discontinuous. The density of callosal projection neurons, which are mainly located in the supragranular layers, varies both within and across cytoarchitectonic areas (Killackey et al., '83). In the present study, we investigated the ontogeny of corpus callosum projections of the postcentral gyrus in five fetal rhesus monkeys, ranging in age from embryonic day (E) 108 to E 133. Multiple large injections of horseradish peroxidase that involved the underlying white matter were made into the postcentral gyrus of one hemisphere and the distribution of labeled neurons in the ipsilateral thalamus and the other hemisphere was determined. The pattern of thalamic label indicated that the tracer was effectively transported from all portions of the postcentral gyrus.

We found that the areal distribution pattern of labeled callosal projection neurons varied at the different fetal ages. At early fetal ages (E 108, E 111, and E 119) callosal projection neurons were continuously distributed throughout the postcentral gyrus. As in the adult animal, the vast majority of labeled callosal projection neurons were found in the supragranular layers, although a few labeled cells were located in the infragranular layers. From the earliest age, there was regional variation in the width of the band of labeled supragranular callosal projection neurons. The difference between the precentral and postcentral gyrus was most obvious, but there was also a difference between anterior and posterior portions of the postcentral gyrus. The first indication of some discontinuity in the distribution of callosal projection neurons was noted at E 126. By E 133, approximately 1 month before birth, the distribution of callosal projection neurons appeared remarkably mature.

On E 119 aggregations of anterograde label could be detected in restricted portions of the posterior postcentral gyrus beneath the cortical layers. By E 133 anterograde label was found within the cortical layers (most densely in layer IV) in these regions of the postcentral gyrus. Thus, the emergence of the discrete pattern of callosal projection neurons appears to be temporally correlated with the ingrowth of callosal afferents.

On the basis of these observations, as well as those of others (discussed in the text), we propose that the ontogenetic changes in the distribution of callosal projection neurons reflect the unique strategy employed by cortical projection neurons in establishing their patterns of connectivity. It is hypothesized that this strategy may involve multiple processes.

Key words: development, somatosensory cortex, corpus callosum, architectonic areas 3b, 1, 2, and 5

The postcentral gyrus of the adult rhesus monkey is composed of several striplike cytoarchitectonic areas that are known to be involved in the processing of somatosensory information (Powell and Mountcastle, '59A). From rostral to caudal these cytoarchitectonic areas are area 3b, which is located in the depth of the central sulcus on the anterior wall of the postcentral gyrus, areas 1 and 2, which are on the crown of the gyrus, and area 5, which is on the posterior wall of the postcentral gyrus in the depth of the intraparietal sulcus. At least three of these areas (3b, 1, and 2) are characterized by separate and complete representations of the body surface (Nelson et al., '80; Killackey et al., '83), while the somatotopic organization of area 5 appears to be more complex and is less well understood (Pons and Kaas, '85). This rostral to caudal progression in cytoarchitectonic areas is thought to reflect the sequence in which somatosensory information is processed in the postcentral gyrus. This supposition is based on two lines of evidence. First, the receptive field properties of neurons in the caudal cytoarchitectonic areas are more complex than the receptive field properties of neurons in the more rostral areas (Powell and Mountcastle, '59B; Iwamura et al., '80; Kalaska et al., '83). Second, the corticocortical connections of the postcentral gyrus appear to be arranged in a hierarchical fashion, such that a given area projects mainly to the areas that are caudal to it (Jones et al., '78; Shanks et al., '78, '85; Vogt and Pandya, '78; Pearson and Powell, '85).

The organization of callosal projections in the postcentral gyrus of the adult rhesus monkey has also been interpreted as reflecting sequential processing of somatosensory information (Killackey et al., '83). The areal distribution of neurons that project across the corpus callosum is not uniform (Jones et al., '79; Killackey et al., '83). The distribution of these callosal projection neurons varies both within a given cytoarchitectonic area and across the different cytoarchitectonic areas of the postcentral gyrus. In general, there is a gradient in the distribution of callosal projection neurons across the gyrus, such that they are least dense rostrally in area 3b and most dense caudally in area 5. Further, within the cytoarchitectonic areas characterized by a well-defined map of the body surface (areas 3b, 2, and 1) the distribution of callosal projection neurons is least dense in the regions where distal portions of the limbs are represented and denser in regions representing the proximal limbs, trunk, and head.

The nonuniform distribution of callosal projections is a common feature of the somatosensory cortex (Ebner and Meyers, '65; Ebner, '67; Jones and Powell, '68, '69; Karol and Pandya, '71; Yorke and Caviness, '75; Wise and Jones, '76; Akers and Killackey, '78; Gould and Kaas, '81; Cusick et al., '85). However, at the time of birth in the species studied to date, callosal projection neurons are distributed in a uniform areal fashion throughout cortex. The mature differential distribution of interhemispheric projection neurons is largely achieved during the early postnatal period. This phenomenon was first described in the visual cortex of the cat (Innocenti et al., '77). It has since been found to occur in the somatosensory cortex of the cat, rat, and opossum (Ivy et al., '79; Innocenti and Caminiti, '80; Ivy and Killackey, '81; Cabanna and Martin, '85), the auditory cortex of the cat (Feng and Brugge, '83), and the visual cortex of the hamster, rabbit, and rat (Rhoades and Dellacroce, '80; Chow et al., '81; Lund et al., '84; Olavarria and Van Sluyters, '85), suggesting that it is a general feature of cortical development.

Study of the ontogenetic change in callosal projection neuron distribution has been largely restricted to primary sensory cortices, portions of which, as noted above, are lacking in callosal connections in the mature animal. In the present study, we were interested in determining if the occurrence of this phenomenon is restricted to primary somatosensory cortex or if it occurs in all the cortical areas devoted to the processing of somatosensory information. The postcentral gyrus of the rhesus monkey affords a unique opportunity to study the development of callosal connections across a number of cytoarchitectonic areas that differ in the degree and organization of their callosal connectivity.

METHODS

Five fetal rhesus monkeys were employed in the present study. Their gestational ages were embryonic (E) days 108, 111, 119, 126 and 133. The gestational period in the rhesus monkey is about 165 days.

Breeding and estimation of gestation age

Timed pregnant animals were obtained by using the standard protocol of the California Primate Research Center. In brief, this consisted of determining the average menstrual cycle length of healthy female animals for 3 consecutive months. The average cycle was divided by two, and 2 days were subtracted from this value to indicate the optimal mating day. In order to maximize the probability of achieving conception, the animals were mated on the optimal day, as well as 2 days before and after this date. If a pregnancy occurred, the gestational age was calculated from the optimal day, so that our estimates of gestational age are accurate only to within 2 days. The 24-hour period following the optimal mating day is denoted as E 1.

Surgery and injections of tracer

Each pregnant monkey was premedicated with atropine (0.2 mg) and initially anesthetized with ketamine hydrobromide (10 mg/kg). The animal was then intubated and a surgical plane of anesthesia was maintained with Halothane and nitrous oxide. Blood pressure was monitored continually, and the level of anesthesia was varied to maintain a mean systolic pressure above 80–90 mm Hg. A balanced electrolyte and dextrose solution was delivered throughout the procedure.

For surgery the animal was placed in a recumbent position on a water circulation heating pad; the abdomen was prepared with povidone iodine, covered with sterile drapes, and a midline abdominal incision exposed the gravid uterus. The fetal head was identified by palpation and maneuvered to a nonplacental portion of the uterus, where uterotomy was performed. The uterotomy site was lifted, stabilized with tissue forceps, and 40–60 ml of amniotic fluid was removed and stored in a warm bath. The fetal head was externalized and maintained in a stable position with tissue clamps and sponges. A midline incision was made in the scalp with electrocautery, and the central and lateral sutures were exposed by blunt dissection. A craniatomy was performed by making an initial periosteal incision with electrocautery followed by cutting of the cranial bone and dura with dissecting scissors to form a cranial flap. This exposed an area of the cortex extending 20–30 mm from the midline and 10–15 mm rostral and caudal to the central sulcus that was discernible in all fetal animals.

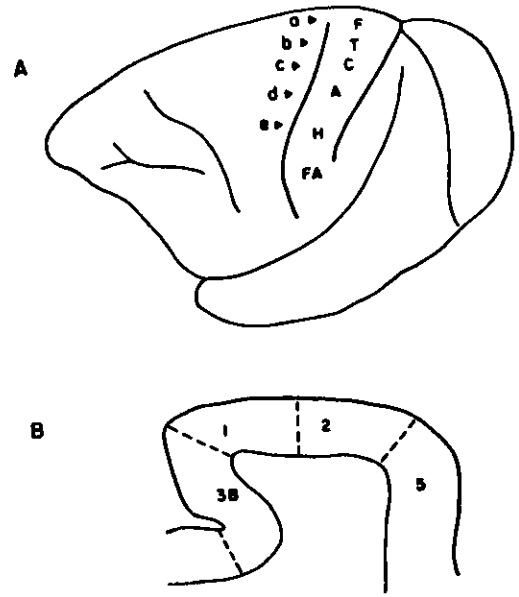


Fig. 1. A. A dorsolateral view of the rhesus monkey brain illustrating the major subdivisions of the representation of the body on the crown of the postcentral gyrus. The approximate levels of the parasagittal sections illustrated in Figures 3 through 6 are indicated by the letters a–e. Abbreviations: F, foot, T, trunk, C, cranium, A, arm, H, hand, and FA, f Schematic drawing of a parasagittal section through the postcentra illustrating the major cytoarchitectonic areas.

Injections of 50% horseradish peroxidase (HRP) were made by using either a 1- or a 5 μ Hamilton syringe with a short blunt needle to which a calibrated glass micropipette had been cemented. Multiple injections of approximately 0.5 μ HRP solution were made at spacings of about 1 mm so as to cover the entire postcentral gyrus. The cranial flap was then sutured back to normal position, the scalp was closed, and the head was returned to the amniotic cavity. The amniotic fluid was replaced, and all incisions were closed with routine procedures. After fetal viability was confirmed with a Doppler monitor, the pregnant monkey was returned to a standard primate cage and maintained on a postoperative analgesic (oxymorphone).

Twenty-four hours later, the pregnant monkey was reanesthetized as described above. The fetus was delivered by cesarian section, deeply anesthetized with barbituate, and then perfused through the heart with 500 ml of physiological saline followed by a 2.5% glutaraldehyde/1.25% paraformaldehyde fixative and then a 10% sucrose solution. All solutions were adjusted to a pH of 7.2 with 0.1 M phosphate buffer.

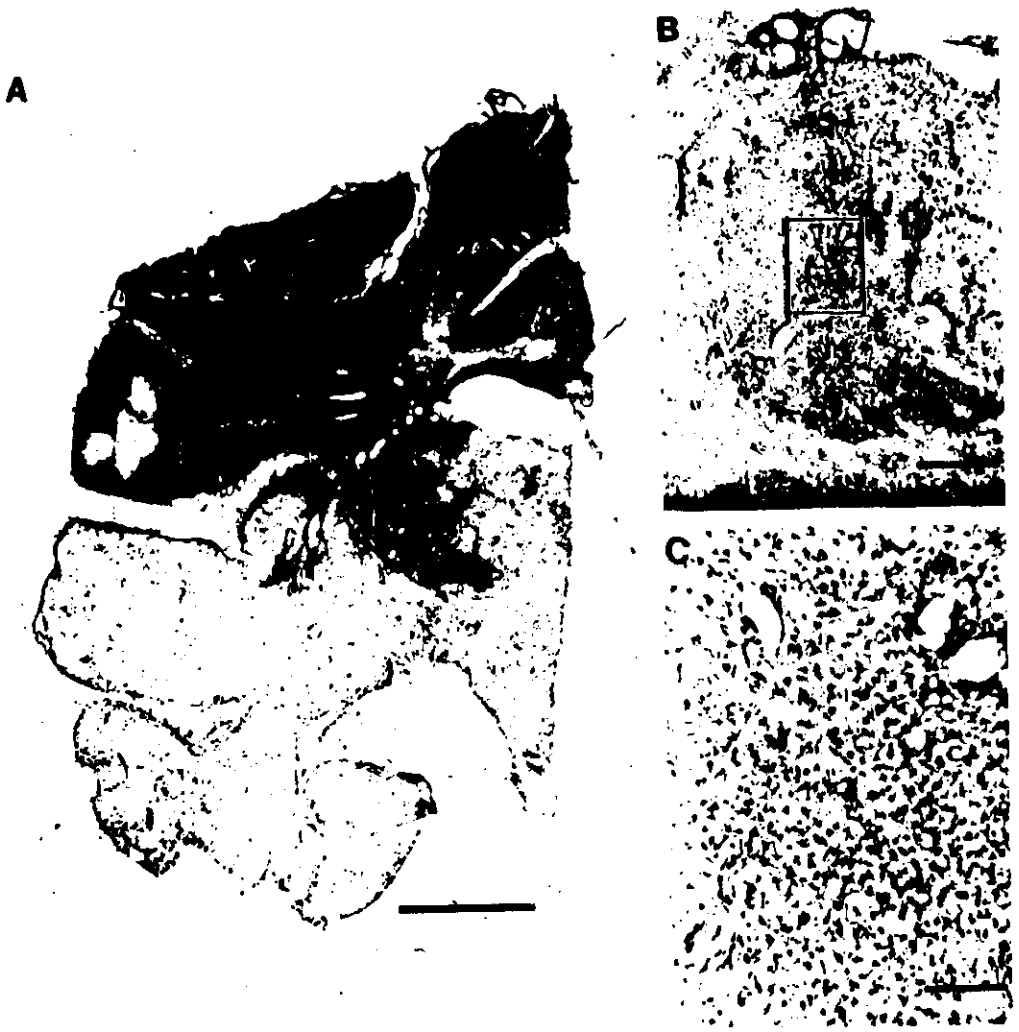
Histology and analysis

Within 1 hour of perfusion, the brain was removed from the cranium, and the two hemispheres were separated by a sagittal cut through the corpus callosum and then placed in 10% buffered sucrose solution for 24 hours. Each hemisphere was embedded in a gelatin sucrose medium and sectioned at 50 μm on a freezing microtome. The in hemisphere was cut in the coronal plane, while the c lateral side was sectioned in the parasagittal plane.

A series (of every fifth section) was treated for the p dase enzyme by using the tetramethyl benzidine prot Mesulam ('78). A second series of sections was prepa the same manner but counterstained with neutral r third series of sections was stained only with cresyl The resultant material was analyzed and photograph using routine light- and darkfield microscopy techniq

RESULTS

Before describing the development of callosal proj patterns it is necessary to summarize briefly their o zation within the postcentral gyrus of the adult monkey (see Killackey et al., '83). Figure 1A pres schematic dorsolateral view of the postcentral gyrus rhesus monkey. The major subdivisions of the body s representation that apply to areas 3b, 1, and 2 are on the crown of the gyrus and the approximate loca the parasagittal sections illustrated in Figures 4–7 dicated. The cytoarchitectonic areas 3b and 5 are buried in the depths of the central and intraparieta respectively. As illustrated in Figure 1B, area 3b is on the anterior wall of the precentral gyrus, while ar on the posterior wall. [Area 5 is often referred to superior parietal lobule (e.g., Caminiti and Sbricco but this designation cannot be applied to the fetal



g. 2. Coronal sections through the injected hemisphere of the E 133 l monkey illustrating the extent of the label. A. Low-power photomicro-ɔh illustrating the extent of label in the postcentral gyrus and the amus. Scale bar equals 4 mm. B. A higher-power photomicrograph illustrating dense label in the ventroposterior complex and surrounding nuclear groups. Scale bar equals 1 mm. C. High-power photomicrograph illustrating dense label in individual neurons of the ventroposterior complex. Scale bar equals 20 μm.

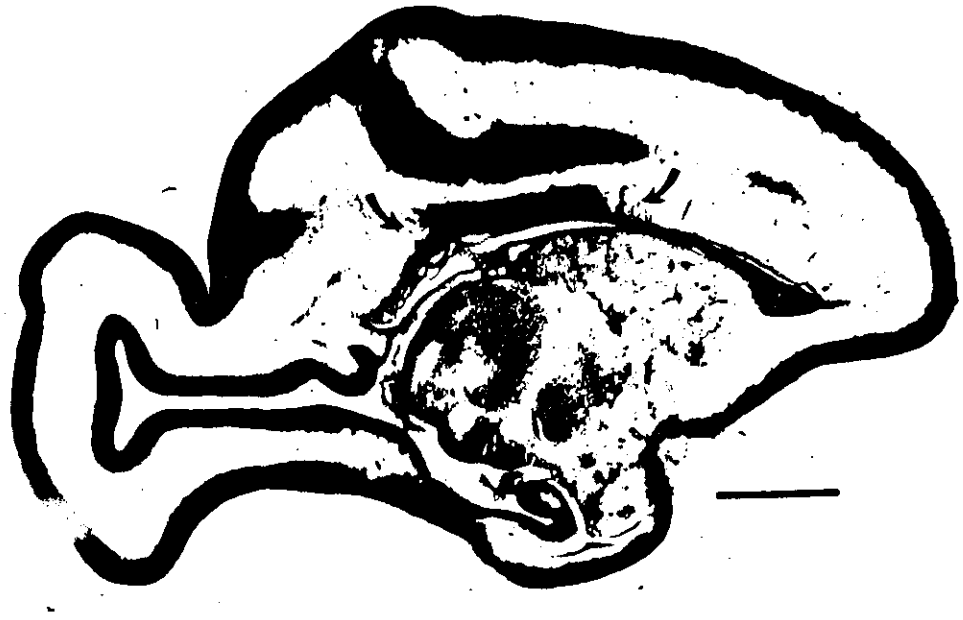


Fig. 3. Low-power photomicrograph of a parasagittal section through the cerebral hemisphere of an E 106 rhesus monkey fetus. Note the labeled fibers in the middle portion of the corpus callosum indicated by the arrows. Scale bar equals 5 mm.

ause the defining landmarks are not apparent at the lier stages of development studied.] The development of losal projection patterns in these two areas (3b and 5) is interest for several reasons. First, the mature distribu-n pattern of callosal projection neurons in area 3b shows greatest heterogeneity. In area 3b, the hand region is atively free of callosal projection neurons while the dis-ɔution of callosal projection neurons in the region of the nk representation is moderately dense. Second, the ma-ture pattern of callosal projection neurons in area 5 is the most specialized. In area 5, callosal projection neurons are distributed in vertical arrays that span both the supragranular and infragranular layers, while afferent callosal terminations are concentrated in the fourth layer. Third, the position of these two areas on the anterior (area 3b) and posterior (area 5) walls of the postcentral gyrus allows them to be localized in the fetal rhesus monkey at a time when it is difficult to make precise cytoarchitectonic distinctions.

Multiple injections of HRP into the entire postcentral gyrus resulted in dense peroxidase reaction product in all cortical layers and the underlying white matter. This is illustrated for the oldest fetal animal (E 133) in Figure 2A. In all cases the ventral posterior nucleus contained densely labeled neurons throughout the nucleus (see Fig. 2B,C). In addition, neurons in both the lateral posterior nucleus and the anterior pulvinar were labeled. These are the thalamic nuclei that project to the areas studied (Pons and Kaas, '85), and thus, this pattern of thalamic label indicates that the HRP was effectively transported from all portions of the postcentral gyrus.

Such injections resulted in restricted dense labeling of fibers within the middle portion of the corpus callosum. This is illustrated in Figure 3, which depicts a low-power photomicrograph of a parasagittal section close to the midline of the E 119 monkey. In this case and in all the other fetal animals, the hemisphere opposite to the site of the injection contained labeled neurons that projected across the corpus callosum. The cell bodies of the vast majority of these labeled corpus were in the supragranular layers, although a small number of labeled cells were also found in the deeper cortical layers. The areal distribution pattern of the labeled callosal projection neurons varied at the different fetal ages. This ontogenetic change in areal distribution was most obvious for the callosal projection neurons of the supragranular layers and is described below. In addition, we present observations on the callosal projection neurons of the infragranular layers and on the ingrowth of callosal afferents.

Areal distribution changes in the supragranular layers

In the youngest animal (E 108), the incipient postcentral gyrus could be clearly identified as a moderate-sized protuberance on the cortical surface. As may be seen in Figure 4, it is bounded rostrally by a shallow central sulcus and caudally by a shallow intraparietal sulcus. At this age the neocortical laminae have not yet fully formed. Cortical layers III–VI could be identified, but superficial to layer III a cortical plate was still present. This immature laminar organization, as well as the laminar pattern of labeled callosal projection neurons, is illustrated in Figure 8a and b. Following injections of HRP into the opposite hemisphere all labeled neurons were located deep to the cortical plate in layers III and V along the anterior wall of the postcentral gyrus and in layers III–V in the more posterior portions of the gyrus. At higher power, it was apparent that these labeled neurons have elongated perikarya typical of imma-

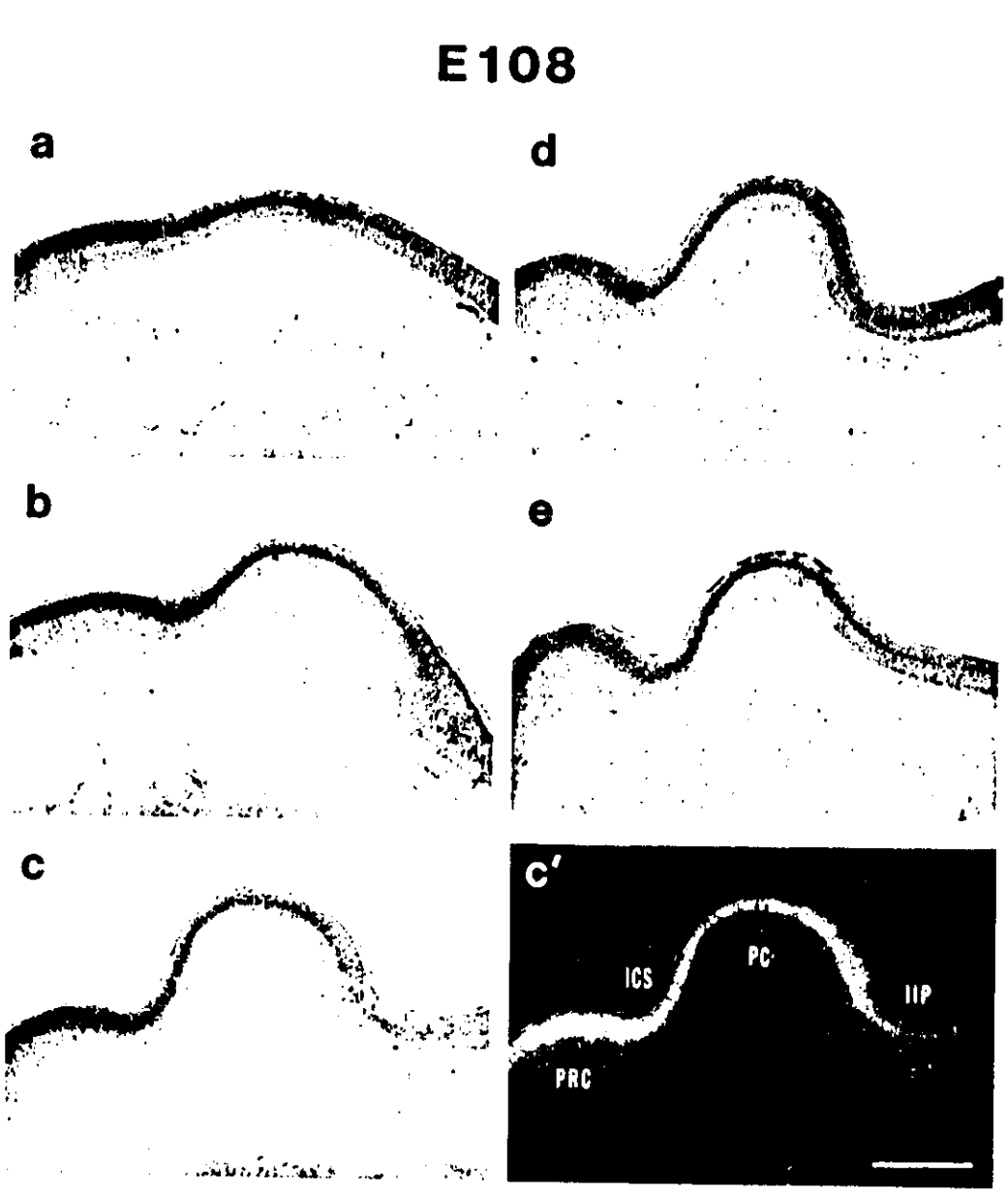


Fig. 4. Low-power photomicrographs of the postcentral gyrus of an E 108 fetal monkey. Note the continuous distribution of labeled callosal projection neurons. In this and the subsequent three figures the medial postcentral gyrus is illustrated in a and progressively more lateral portions of the postcentral gyrus are illustrated in b through e. c' is a darkfield photomicrograph of the section illustrated in c. Scale bar equals 2 mm in Figures 4–7. Abbreviations: ICS, incipient central sulcus, IIP, incipient intraparietal sulcus, PRC, precentral gyrus, PC, postcentral gyrus.

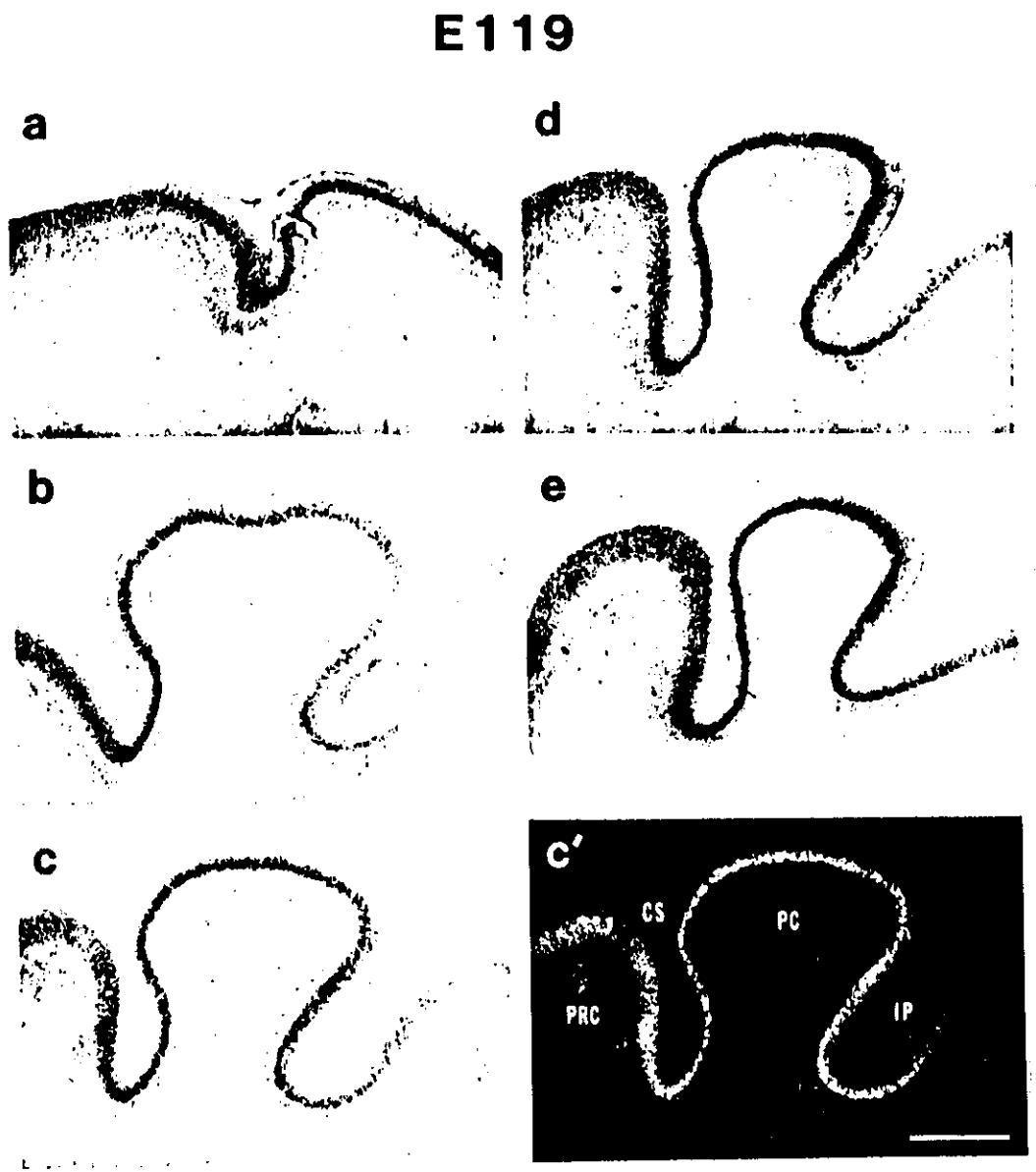


Fig. 5. Low-power photomicrographs of the postcentral gyrus of an E 119 fetal monkey. The distribution of labeled callosal projection neurons is still continuous. Abbreviations: CS, central sulcus, IP, intraparietal sulcus.

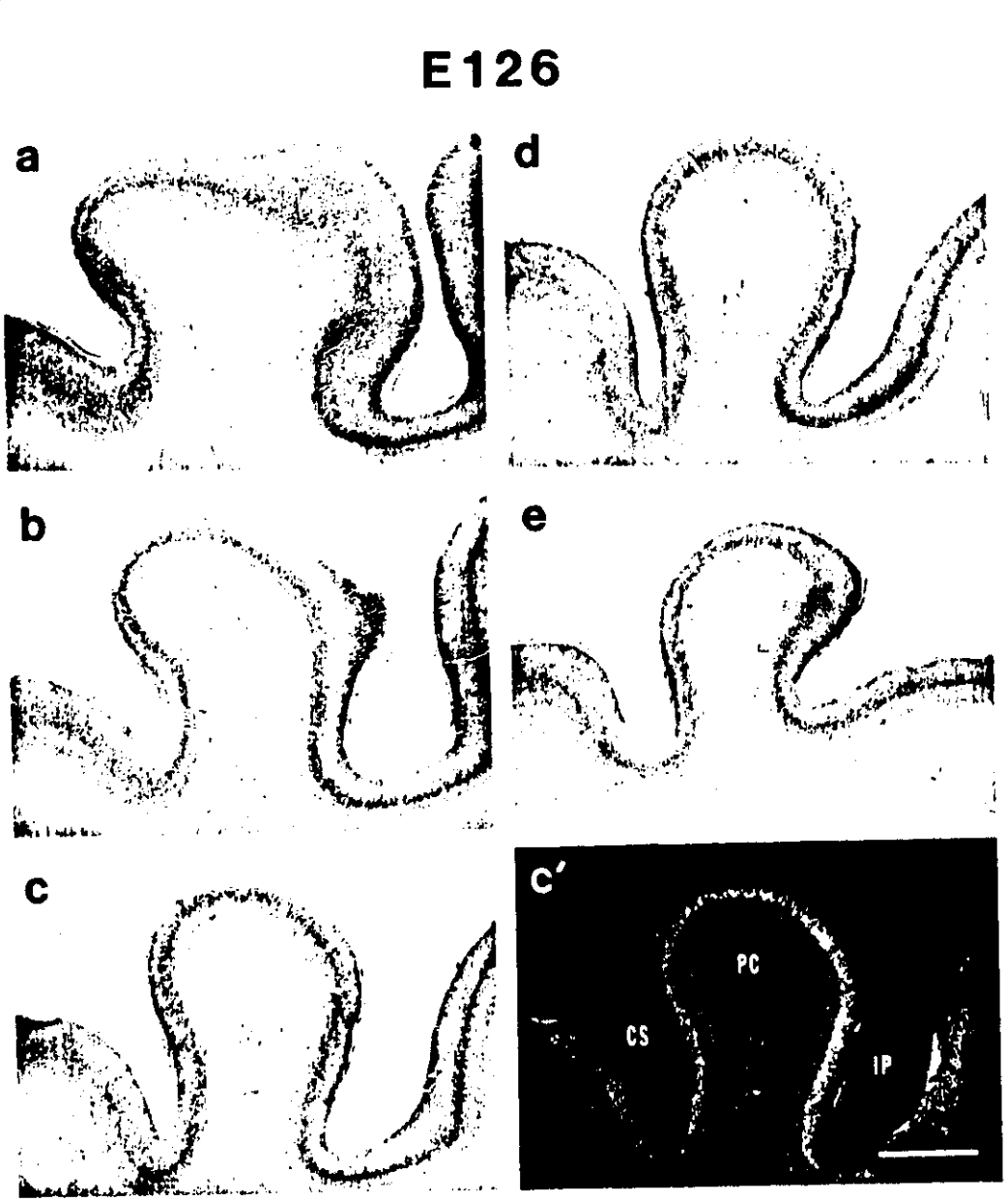


Fig. 6. Low-power photomicrographs of the postcentral gyrus of an E 126 fetal monkey. Note the reduction in density of labeled callosal projection neurons.

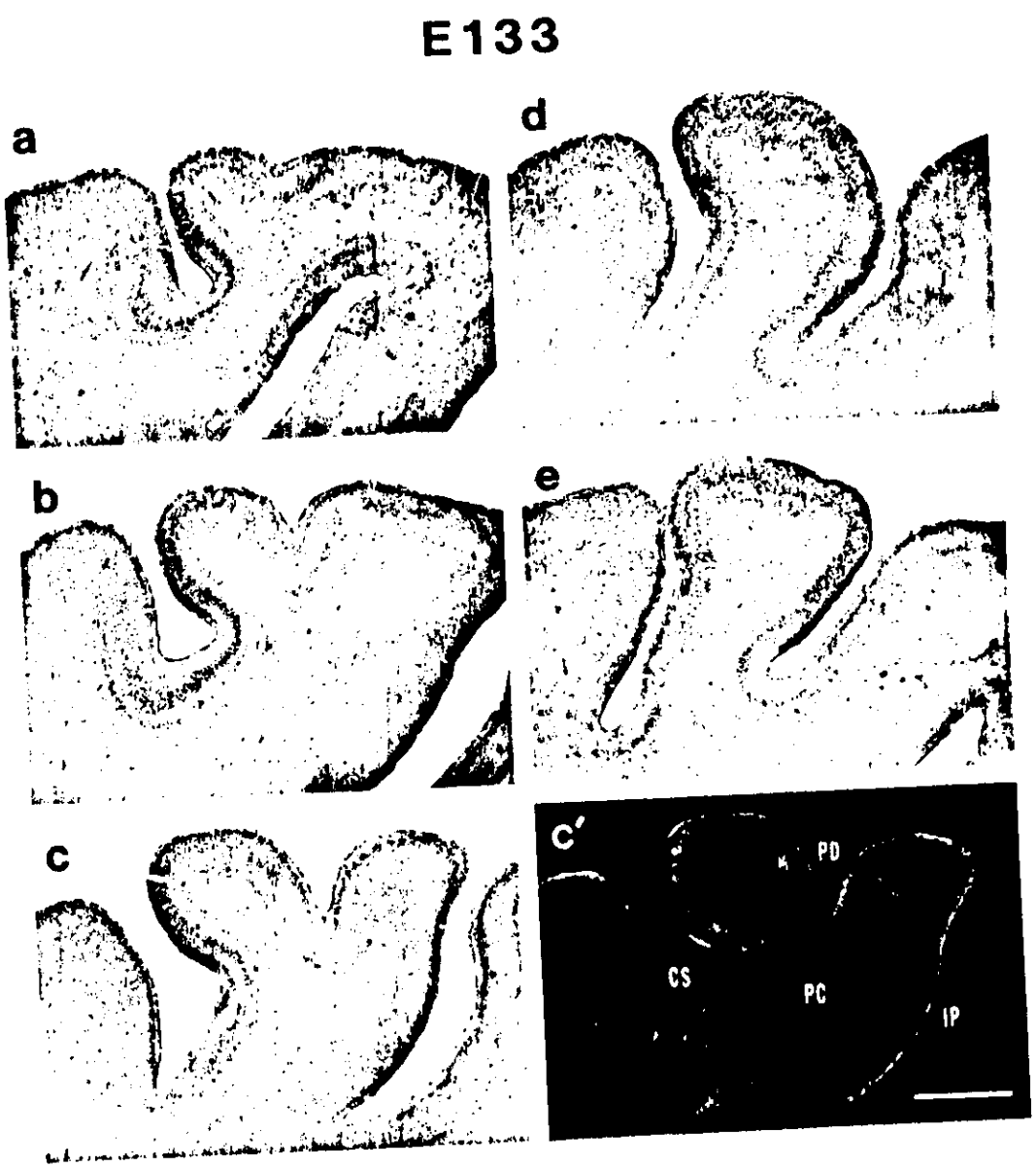


Fig. 7. Low-power photomicrographs of the postcentral gyrus of an E 133 fetal monkey. Labeled callosal projection neurons are distributed in the mature discontinuous pattern.

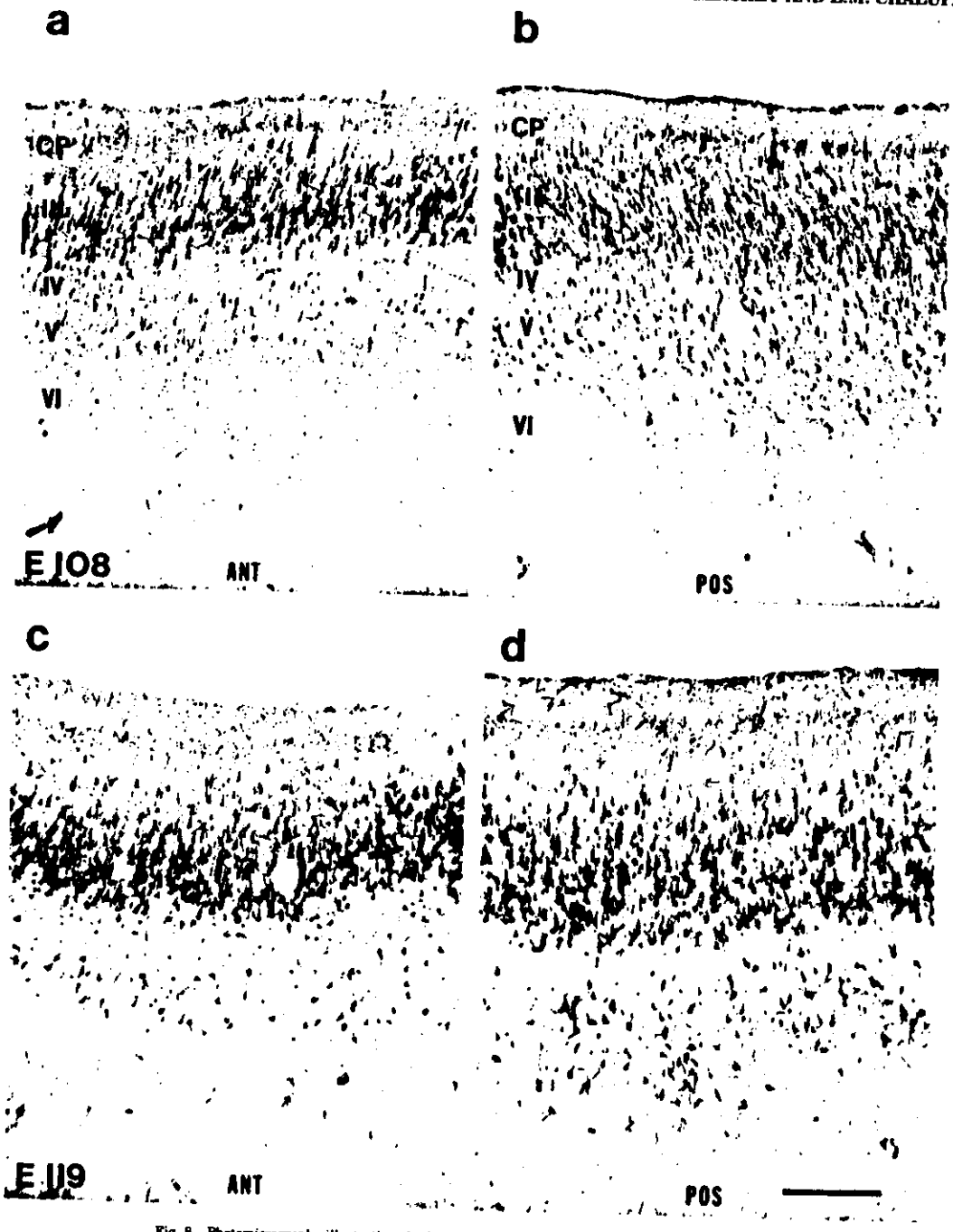


Fig. 8. Photomicrographs illustrating the laminar distribution of labeled callosal projection neurons. a and b illustrate labeled neurons along the anterior and posterior walls of the postcentral gyrus of an E 108 fetal monkey. c and d illustrate similar positions in the postcentral gyrus of an E 119 fetal monkey. Scale bar equals 250 microns in Figures 8 and 9. Abbreviations: ANT, anterior, POS, posterior.

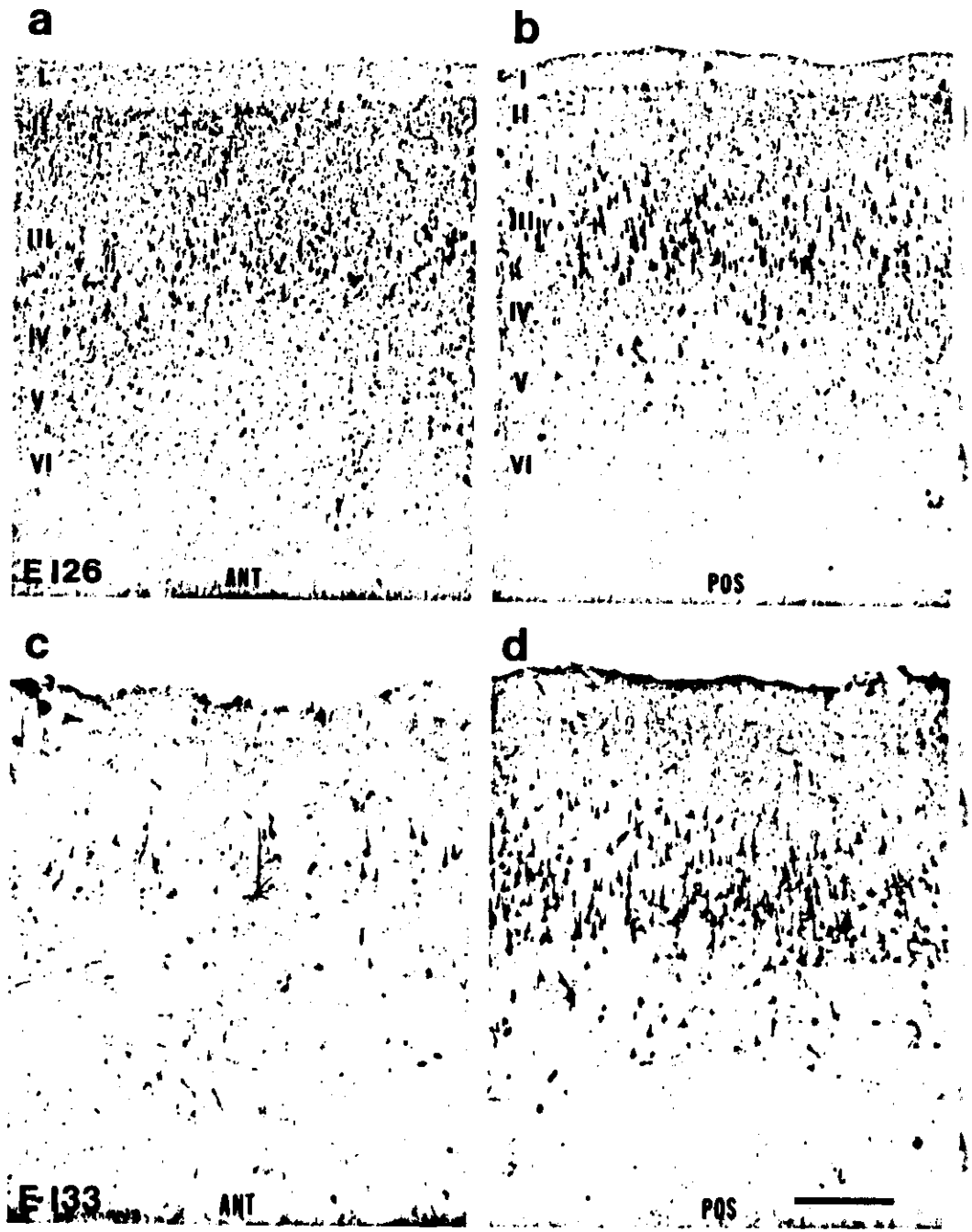


Fig. 9. Photomicrographs illustrating the laminar distribution of labeled callosal projection neurons. a and b illustrate labeled neurons along the anterior and posterior walls of the postcentral gyrus of an E 126 fetal monkey. c and d illustrate similar positions in the postcentral gyrus of an E 133 fetal

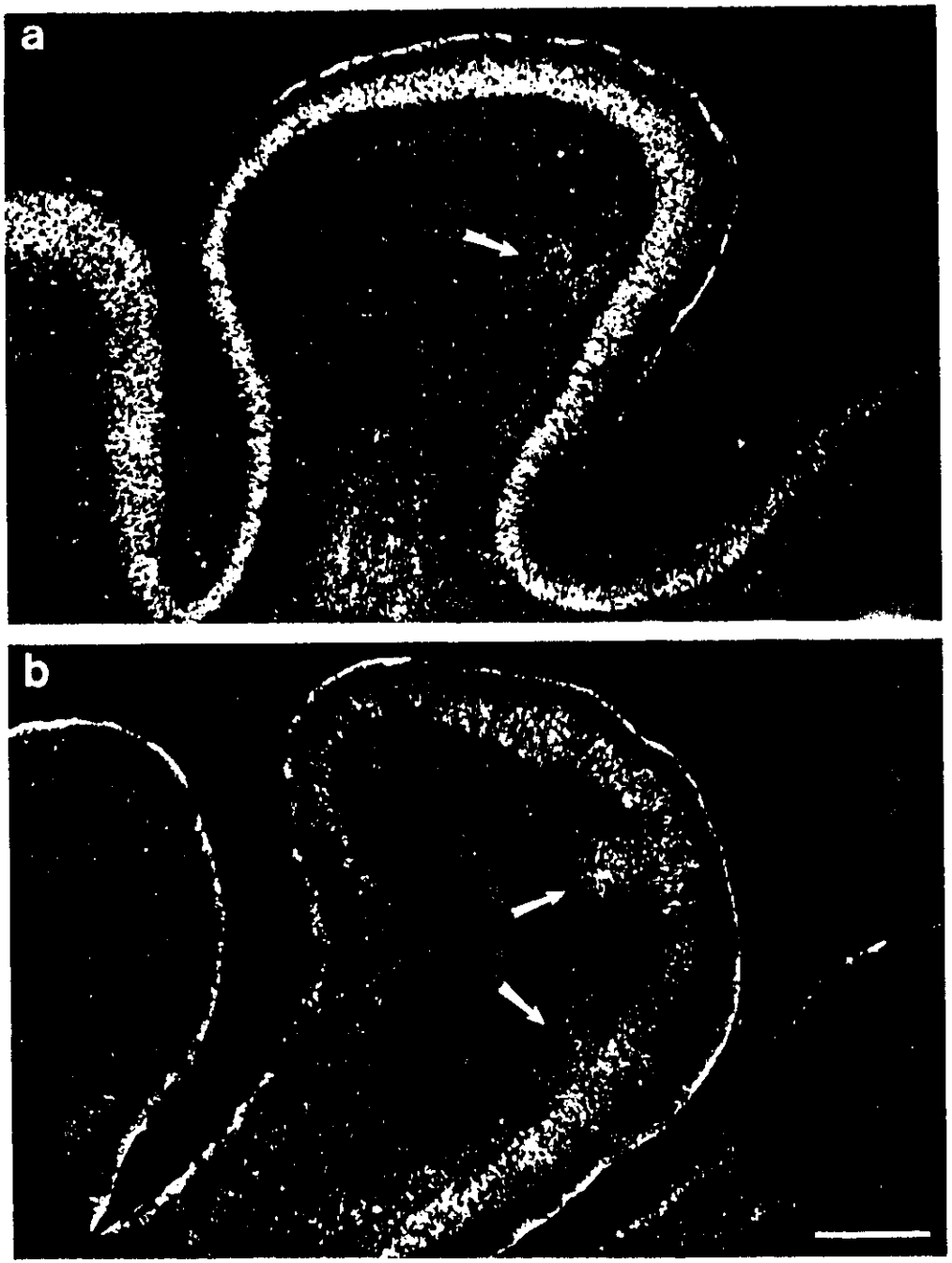


Fig. 10. Darkfield photomicrographs illustrating the change in the distribution of callosal projection neurons and the ingrowth of callosal afferents (arrows). At E 119 (a) labeled afferents are

ture neurons. Reaction product was also seen in the apical processes of these cells but no labeled basilar processes could be detected.

The areal distribution of callosal projection neurons at this age (E 108) was quite different from the mature pattern. Within the postcentral gyrus there was a dense continuous band of labeled callosal projection neurons that extended from the rostral to the caudal border of the gyrus (see Fig. 4). This dense band of labeled cells was largely confined to the supragranular layers although labeled cells were also present in the deeper layers (see details below). Regional variation in the width of this superficial band of labeled cells could be detected along the rostrocaudal axis. First and most obvious, the labeled band of supragranular callosal neurons was clearly wider in the motor cortex rostral to the incipient central sulcus. Second, the labeled band was narrower along the anterior wall of the postcentral gyrus. It gradually widened along the crown of the gyrus and became widest in the posterior wall of the postcentral gyrus. This is evident in both the low-power darkfield photo micrograph illustrated in Figure 4c' and in higher-power photomicrographs (Fig. 8a,b) in which the anterior and posterior walls of the postcentral gyrus can be directly compared. However, at this age there was no indication of any regional variation in either the density of labeled neurons or in the width of the band of labeled neurons along the mediolateral axis in any part of the postcentral gyrus. Presumably, it is this axis that corresponds to that of the body representation.

The distributions of labeled callosal projection neurons in the postcentral gyrus at E 110 and E 119 were essentially the same as described above. This is illustrated for the E 119 animal in Figure 5. At this age, a deeper sulcal pattern has emerged and the postcentral gyrus is more clearly demarcated. Labeled callosal projection neurons were still distributed in a continuous band throughout the superficial layers and there was no indication of the adult discontinuous pattern. The difference in the width of the band of labeled callosal neurons along the rostrocaudal axis noted at the earlier age was even clearer at this age. The wide pattern of label in the superficial layers of motor cortex clearly differed from the pattern in the postcentral gyrus where the band was narrowest along the anterior wall of the gyrus (Fig. 5d,e). The difference in laminar labeling pattern along the anterior and posterior wall of the postcentral gyrus is illustrated in Figure 8c and d. These figures also demonstrate that individual labeled neurons appear more mature at this age than at E 108. Note that the perikarya of many of the labeled cells are triangular, indicating that they are pyramidal neurons.

At E 119, there was still no variation in the pattern along the mediolateral axis (see Fig. 5). In particular, note the continuous pattern of labeled neurons along the caudal bank of the central sulcus in Figure 5E. This section is from the lateral portion of the postcentral gyrus near the end of the intraparietal sulcus. Therefore, it is most likely to be through the area of the hand representation. In the adult rhesus monkey, the caudal bank of the postcentral sulcus in the area of the hand representation of area 3b is almost entirely devoid of callosal projection neurons.

At E 126 the pattern of distribution of callosal projection neurons in the supragranular layers appears to be at an intermediate stage. At this age there are two major differences in the areal pattern of callosal projection neurons compared to the earlier ages. First, the overall density of distribution restricted to the deeper portions of layer III. This was particularly evident along the anterior wall of the postcentral gyrus (compare Fig. 6c' with Figs. 4c', 5c'). The pattern of labeled neurons along the posterior wall of the postcentral gyrus remains both denser and wider than that along the anterior wall (Fig. 9a,b). Second, the distribution of callosal projection neurons, while still relatively continuous in comparison to the mature pattern, is not entirely uniform. Local heterogeneities in the distribution pattern of labeled neurons could be detected within a single cytoarchitectonic area for the first time. Figure 6 demonstrates that the distribution of callosal projection neurons along the anterior wall of the postcentral gyrus is not uniform. Specifically, there are fewer labeled neurons in the region of the presumed hand representation of area 3b (Fig. 6e) than there are in other portions of presumptive area 3b (for example, Fig. 6b illustrates a section through the presumed trunk representation). It is also important to note that at this age there were many fewer labeled neurons in the presumed hand representation than was the case at the earlier ages (compare Figs. 5e, 6e).

By E 133 the distribution pattern of callosal projection neurons clearly approximates the mature pattern. Further, the morphology of individual labeled callosal projection neurons appeared quite mature. The majority of labeled cells were distinguishable as pyramidal neurons and in many instances reaction product could be detected in the proximal parts of both apical and basilar dendrites. Occasionally, a particularly well-filled neuron was seen to exhibit the morphological properties of adult pyramidal cells (see Fig. 9c). The overall density of the pattern of labeled cells was roughly the same as it was on E 126, but was more clearly discontinuous, and regional variations in the density of labeled neurons were readily apparent both across and within cytoarchitectonic areas. The variation across cytoarchitectonic areas can be seen in Figure 7d. Along the anterior wall of the postcentral gyrus labeled neurons form a thin, labeled band in the supragranular layers that ends at about the anterior crown of the gyrus. Further posteriorly on the crown of the gyrus, a second band of labeled cells begins and extends down the posterior wall of the postcentral gyrus. This second posterior band is both wider and denser than the anterior band. This difference in the labeling pattern along the anterior and posterior walls of the postcentral gyrus can be readily discerned in the higher-power photomicrographs of Figure 9c and d. The variation within a single cytoarchitectonic area can be detected by comparing Figure 7b and e. The distance from the midline and the presence of the intraparietal dimple suggests that this section (Fig. 7b) is at the level of the trunk representation. Note the continuous band of labeled cells in the supergranular layers of the anterior wall of the postcentral gyrus. In a more lateral section (Fig. 6d) through the presumed hand region (presumption based on the position of the section relative to the end of the intraparietal sulcus), there are relatively few labeled cells along the midportion of the anterior wall of the gyrus. Thus, a region of the anterior wall of the postcentral gyrus that at earlier ages contained numerous labeled callosal neurons is now relatively free of labeled cells.

The infragranular layers and the ingrowth of callosal afferents

At all fetal ages there was a small number of labeled callosal projection neurons in the infragranular layers of

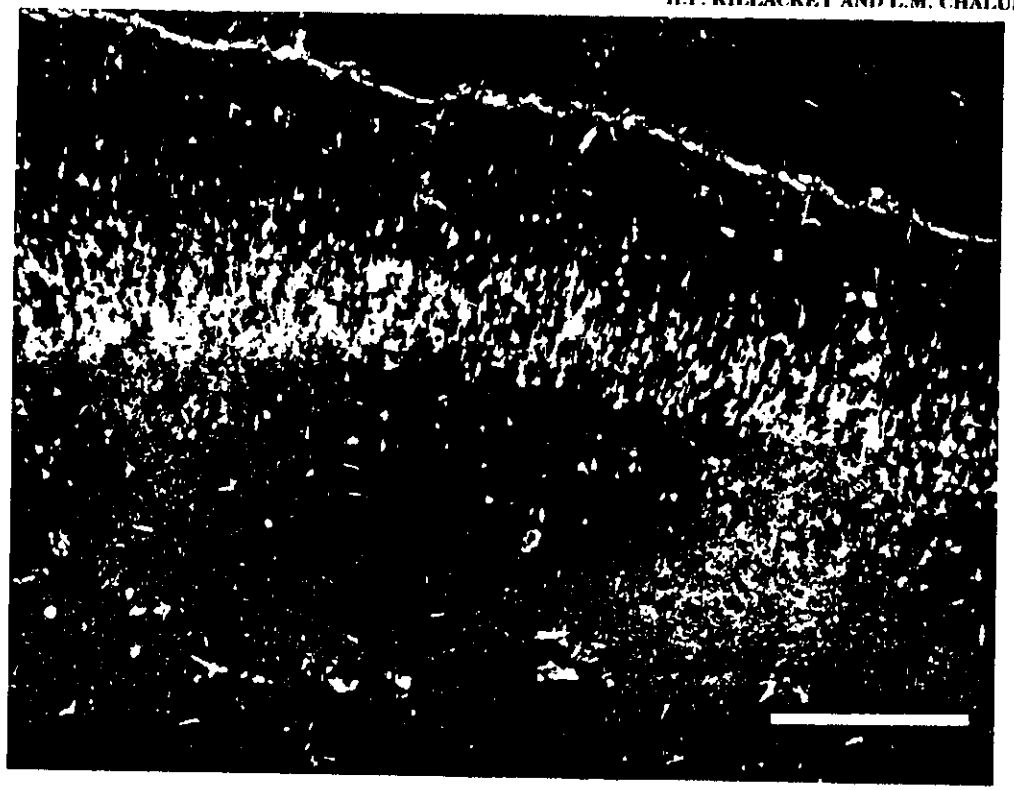


Fig. 11. Higher-power photomicrograph illustrating the distribution of afferent label in the posterior postcentral gyrus at E 133. Scale bar equals 0.5 mm.

neurons were distributed in faint but relatively uniform band throughout the postcentral gyrus. Certain aspects of this infragranular distribution pattern mirrored the pattern in the overlying supragranular layers. In the infragranular, as in the supragranular layers, the pattern was least dense and narrower along the anterior wall of the postcentral gyrus, and it increased in both width and density from the midpoint of the gyral crown and along the posterior wall of the gyrus. This difference between the anterior and posterior wall of the gyrus is clearly visible in the higher-power photomicrographs illustrated in Figure 8. At older fetal ages, E 126 and E 133, there were very few labeled callosal neurons in the infragranular layers along the anterior wall of the postcentral gyrus (Fig. 9a,c). However, even in the older fetal animals callosal projection neurons could still be found in the deeper layers of posterior postcentral gyrus, where they tended to be grouped in clusters (see Fig. 9b,d).

In two of the cases in the present study HRP was clearly transported in the anterograde as well as the retrograde direction. In the younger case (E 119) labeled fibers could be traced into the postcentral gyrus where they ended in restricted portions of the posterior part of the postcentral gyrus. At this age, the fibers terminated in well-defined clusters deep to the cortical layers which they appeared not to invade. This is illustrated in Figure 10a. At a later age (E 133) the fibers did invade the cortical [illegible]

vertical arrays in restricted portions of the postcentral gyrus (see Fig. 10b). As shown at higher power in Figure 11, these afferent fibers appear to have reached layer III, although their distribution is clearly densest in cortical layer IV. Regions in which callosal afferent to cortical layer IV were particularly conspicuous were characterized by vertical arrays of callosal projection neurons extending through both the supragranular and infragranular layers. Thus, the presence of labeled callosal projection neurons in the infragranular layers appears to be coextensive with regions of restricted afferent fiber ingrowth.

DISCUSSION

We have provided evidence that during development the callosal projection neurons of the postcentral gyrus of the rhesus monkey undergo a restriction in their areal distribution. This restriction process occurs early in the last trimester of pregnancy and it occurs in all portions of the postcentral gyrus. Thus, the restriction process is both independent of postnatal sensory experience, and a facet of the development of higher-order cortical processing areas as well as primary sensory cortex.

Several of our observations deserve particular emphasis. First, in the fetal rhesus monkey like in the adult, the vast majority of callosal projection neurons are located in the supragranular layers, and it is these supragranular callosal in their distribution. Second, there are regional differences in the width of the band of labeled supragranular callosal projection neurons from the earliest age studied. Third, callosal afferents appear to grow into restricted areas of cortex and the change in the areal distribution of callosal projection neurons appears to be temporally correlated with this ingrowth.

Comparison with other species

The ontogenetic changes in the distribution of callosal projection neurons in the postcentral gyrus of the rhesus monkey are similar to those that have been described to occur in the somatosensory cortex of the rat, cat, and opossum (Innocenti and Caminiti, '80; Ivy and Killackey, '81; Cabana and Martin, '85).

There is one striking species difference in the organization of callosal projections. In the rhesus monkey, the vast majority of callosal projection neurons are located in the supragranular layers, while in the rat, an approximately equal number of callosal projection neurons is located in the supragranular and infragranular layers. Indeed, when callosal projection neurons are labeled on the day of birth in the rat, the supragranular layers have not yet formed and dense, continuous bands of callosal projection neurons are found in the infragranular layers (Ivy and Killackey, '81). In the rat, callosal projection neurons in the supragranular layers cannot be labeled until several days after birth. One possible way this difference between the two species could be achieved is through the selective ontogenetic elimination of infragranular callosal projection neurons in the primate. However, the present results suggest that this is not the case. At the earliest age examined in the present study (E 108), the striking difference in the density of labeled callosal projection neurons in the supragranular and infragranular layers was already obvious. While the partial differentiation of the supragranular layers in the E 108 animal would suggest that the labeling was performed at a more mature state than in the rat experiment, we think it is unlikely that a large population of infragranular callosal projection neurons could have been eliminated earlier. We base this supposition on the fact that the distributional changes in the less-dense band of infragranular callosal projection neurons mirrored the changes in the supragranular layers. This is also the case in the somatosensory and visual cortices of the cat where supragranular callosal projection neurons also predominate (Innocenti and Caminiti, '80), and in the rat where the supragranular and infragranular callosal projection neurons are of equal density (Ivy and Killackey, '81). Thus, the evidence would suggest that the mature distribution of callosal projection neurons is shaped by similar mechanisms in all species, but that there is significant species variation in the genetic programs that determine the laminar distribution of callosal projection neurons.

At present, it is only possible to speculate on the significance of this species difference in the laminar origin of callosal projection neurons. it is reasonable to assume that there is a greater number of cytoarchitectonic areas involved in the processing of sensory information for a single sensory modality in the rhesus monkey than in the rat (Kaas, '83; Killackey, '83). Processing that in the rat is accomplished within a single vertical array of cortical tissue is probably distributed in the rhesus monkey over several interconnected vertical arrays of cortical tissue such as areas 3b, 1, and 2. This difference in processing may require less variability in the laminar position of classes of projection neurons. In this context, the infragranular callosal projection neurons of the rhesus monkey probably compose a distinct class. It has been suggested by others that their functional role is to provide "feedback" to previous processing stages (Maunsell and Van Essen, '83; Shanks et al., '85). This contention is supported by their greater frequency in area 5, which received inputs from the other cortical areas (Jones et al., '78; Pearson and Powell, '85).

Regional variation in the continuous pattern

While the results of the present study, as well as those dealing with the interhemispheric connections of other species, clearly show that the mature callosal projection pattern emerges from a continuous distribution of these neurons, it must be emphasized that a substantial degree of specificity can be recognized in this system very early in development. In our material regional variations in the pattern of callosal projection neurons were detectable as early as E 108 when their distribution was clearly continuous. The difference between the precentral and postcentral gyrus was most obvious, but there were also clear differences between the anterior and posterior postcentral gyrus. It has been previously noted that it is at this age that cytoarchitectonic distinctions between the precentral and postcentral gyrus first become apparent (Powell and Mountcastle, unpublished observations noted in Mountcastle, '78). Thus, the underlying basis for the regional variation in the neocortical mantle, which is so conspicuous in the adult, is probably programmed in the neuroepithelium.

It has been well documented that the laminar location of a neuron is correlated with its time of origin (Angevine and Sidman, '61; Rakic, '74). Further, the time of origin is also correlated with the ultimate projection of a cortical neuron (Jensen and Killackey, '84). In this context, the greater width of the band of labeled neurons in the superficial layers of particular regions can be interpreted as suggesting that a given class of projection neurons can be produced for differing lengths of time in different portions of the neuroepithelium. Rakic ('76a) has noted that there is a slight but significant difference in the time of origin of the neurons that compose the corresponding layers of areas 17 and 18. However, it remains to be determined if such a temporal gradient exists in the postcentral gyrus.

Relationship of ingrowth of afferent input to distribution changes

In the primate, afferent callosal fibers that have crossed the midline can be detected in the white matter of the frontal cortex at E 94 (Goldman-Rakic, '81); thalamocortical afferents can be detected in the white matter underlying visual cortex at an even earlier age (E 78) (Rakic, '76b). Thus, the major extrinsic afferents to cortex arrive before the cycle of cortical cell proliferation and migration has been completed and well before the laminar differentiation of the neocortex takes place. However, at this early time these afferents do not invade the developing cortical layers; rather they remain in a "waiting compartment" beneath it (Rakic, '81). Thalamocortical afferents have begun their invasion of the neocortex by E 91 (Rakic, '76B). Callosal afferents do not invade the cortex until a much later time. In frontal cortex of rhesus monkey, few if any callosal fibers have entered the cortical layers by E 123 (Goldman-Rakic, '81). The results of the present study are in accord with

bservations. On E 119 aggregations of callosal afferuld be detected in the posterior portions of the postgyrus beneath the cortical layers. Further, the ution of these afferents appears to be restricted to tical regions that they later invade. This suggests llosal afferents, or at least some subpopulation of re already organized with respect to their target at when the overall distribution of callosal projection s is still continuous. By E 133, the growth of callosal ts into the cortical layers at specific locations is ay and a mature distribution pattern of callosal ion neurons has emerged. These observations sugat the selection factors that govern the mature disn of callosal projection neurons are operating during iod of the "waiting compartment." Similar observan the formation of the mature pattern of callosal ions in the rat somatosensory cortex support this tion (Wise and Jones, '78; Ivy et al., '79; Ivy and ey, '81).

A proposed developmental strategy

oted above, ontogenetic change in the distribution of l projection neurons occurs in a variety of mammaecies. It has also been demonstrated to occur in r major class of cortical projection neurons—those oject subcortically (Stanfield et al., '82; Leong, '83; and Killackey, '84; Tolbert and Panneton, '83). In ases, there is an initial overabundant (but not necesdiffuse) outgrowth of afferent processes toward mulargets, followed by retraction of a subset of processes ome targets. It is important to emphasize that while ath does occur in the neocortex (Finlay and Slattery, does not appear to be an important component of ocess (Innocenti, '81; O'Leary et al., '81; Ivy and key, '82; Panneton and Tolbert, '84). In this regard tical projection neurons appear to be unique. In most ces, for example during the development of the optic in the fetal cat (Lia et al., '85), axonal elimination is plished by retinal ganglion cell loss rather than proimination. Perhaps cortical projection neurons are cious a resource to be squandered in this fashion. It en hypothesized that the initial overproduction and etraction of axonal processes is the most economical f matching subsets of a class of cortical projection as with specific inputs and targets without the geoding of specific guidance cues (Bates and Killackey,

same hypothesis can be applied to callosal projection ns. However, before doing so it should be pointed out he context within which a set of cortical projection ns forms an ordered array of connections differs conbly from that of more peripherally located projection ns, such as retinal ganglion cells. A set of cortical tion neurons, which must possess an intrinsic order, posed on their perikaryal distribution a topographic determined by the source of afferent input, and in rojects to another cortical region that is not necessarlered in the same fashion. Thus, during ontogeny the l projection neuron faces the dual task of coming into r with both the source of its afferent input and its . The execution of this task may require multiple pmental mechanisms. In the adult rhesus monkey, ially all supragranular projection neurons in the posparietal cortex project to either a single ipsilateral or lateral cortical target and the areal distributions of these two subclasses of cortical projection neurons overlap considerably (Andersen et al., '85). Indeed, there is evidence that each subclass projects to a single target by E 133 (Schwartz and Goldman-Rakic, '82). However, this may not be the case early in development when we have shown primate callosal projection neurons to be continuously distributed. In rat somatosensory cortex at this stage, individual callosal projection neurons project to ipsilateral targets as well (Ivy and Killackey, '82). Assuming that this is also the case in the primate, a given immature supragranular callosal projection neuron in area 3b, for example, would have a number of potential ultimate targets. It might project to the opposite hemisphere in a homotopic fashion to area 3b or heterotopically to area 1, 2, or 5. The same neuron might also project to area 1, 2, or 5 of the same hemisphere.

In primary somatosensory cortex (area 3b) callosal projection neurons are selectively eliminated from these regions of the somatotopic map where the most specializ ` tactile receptor surfaces are represented (the vibrissae pad of the rat or the glabrous skin of the monkey's digits). This suggests that some factor associated with specific thalamocortical projections may play a role in the initial decision of whether or not a given supragranular projection neuron maintains a callosal projection. In the rat, the ventral posterior nucleus projects to primary somatosensory cortex (Killackey, '73; Donaldson et al., '75) while in the rhesus monkey portions of this nucleus project to areas 3b, 1, 2, and parts of area 5 (Pons and Kaas, '85). Indeed, the gradient in distribution of callosal projection neurons both across and within the cytoarchitectonic areas of the postcentral gyrus seems to be inversely related to the distribution and density of the ventroposterior complex projections in the rhesus monkey. Thus, the same thalamic nucleus might play a similar role in both species.

While the distribution pattern of callosal projection neurons is remarkably mature by E 133, the time at which corpus callosum axons are actually lost in the rhesus monkey appears to be after birth rather than during fetal development (LaMantia and Rakic, '84). Indeed, these authors report that the number of callosal axons increases during the 2 months prior to birth. We can offer no clear-cut explanation of this paradox. However, it is likely to result from one of the following alternatives. Neurons that can no longer be labeled at E 133 may still extend a process into the corpus callosum, or alternatively, callosal projection neurons that can be labeled at E 133 may have several callosal processes. This second alternative would imply that new collateral processes are being added to the corpus callosum at the same time that processes related to inappropriately located cells are being eliminated. Perhaps the addition of callosal processes during this period is related to the establishment of patterns of connectivity between specific cortical areas. Thus, there may be postnatal changes in the density of callosal projections after their distribution has matured. Such density changes may be influenced by sensory experience, as has been shown in a recent study of the callosal projections of the cat visual system (Innocenti et al., '85).

On the basis of the evidence reviewed above, we would hypothesize that the formation of patterns of callosal projection neurons and connectivity involves multiple processes. In the rhesus monkey, the mature pattern of callosal projection neurons emerges from the immature continuous pattern during fetal development. This may be related to the ingrowth of specific thalamocortical afferents. At a later time and continuing into the postnatal period, specific patterns of callosal connectivity are established by processes that may be influenced by sensory experience.

ACKNOWLEDGMENTS

We express our sincere appreciation to Dr. Andrew G. Hendrickx, associate director, and Dr. Charles E. Cornelius, director, of the California Primate Research Center (C.P.R.C.) for their support and encouragement. We are indebted to Dr. John Anderson, associate veterinarian of the C.P.R.C. for performing the fetal surgery. We also acknowledge the excellent technical support of Ms. Debrah van der List, and the assistance of Dr. Louis Ostrach and Dr. Gary Belford. Finally, we thank Dr. E.G. Jones for his critical comments of an earlier version of this manuscript. This research was supported by NIH grants RR 00169 and NS 17234.

LITERATURE CITED

Akers, R.M., and H.P. Killackey (1978) Organization of corticocortical connections in the parietal cortex of the rat. J. Comp. Neurol. *181*:513–538.

Andersen, R.A., C. Asanuma, and W.M. Cowan (1985) Callosal and prefrontal associational projecting cell populations in area 7A of the macaque monkey: A study using retrogradely transported fluorescent dyes. J. Comp. Neurol. *232*:443–455.

Angevine, J.B., and R.L. Sidman (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. Nature *192*:766–768.

Bates, C.A., and H.P. Killackey (1984) The emergence of a discretely distributed pattern of corticospinal projection neurons. Dev. Brain. Res. *13*:265–273.

Cabana, T., and G.F. Martin (1985) The development of commisural connections of somatic motor-sensory areas of neocortex in the north american opossum. Anat. Embryol. *171*:121–128.

Caminiti, R., and Sbriccoli, A. (1985) The callosal system of the superior parietal lobule in the monkey. J. Comp. Neurol. 237:85–99.

Chow, K.L., H.D. Baumbach, and R. Lawson (1981) Callosal projections of the striate cortex in the neonatal rabbit. Exp. Brain Res. *42*:122–126.

Cusick, C.G., M.G. MacAvoy, and J.H. Kaas (1985) Interhemispheric connections of cortical sensory areas in tree shrews. J. Comp. Neurol. *235*:111–128.

Donaldson, L., P.J. Hand, and A.R. Morrison (1975) Corticothalamic relationships in the rat. Exp. Neurol. *47*:448–458.

Ebner, F.F. (1967) Afferent connections to neocortex in the opposum. J. Comp. Neurol. *129*:241–268.

Ebner, F.F., and R.E. Meyers (1965) Distribution of corpus callosum and anterior commissure in cat and racoon. J. Comp. Neurol. *124*:353–366.

Feng, J.Z., and J.F. Brugge (1983) Postnatal development of auditory callosal connections in the kitten. J. Comp. Neurol. *214*:416–426.

Finlay, B.L., and M. Slattery (1983) Local differences in the amount of early cell death in neocortex predict adult local specializations. Science *219*:1348–135.

Goldman-Rakic, P.S. (1981) Development and plasticity of primate frontal association cortex. In F.O. Schmitt, F.G. Worden, G. Adelman, and S.G. Dennis (eds): The Organization of the Cerebral Cortex. Cambridge, MA: MIT Press, pp. [illegible]–97.

Gould, H.J., and J.H. Kaas (1981) The distribution of commisural terminations in somatosensory areas I and II of the grey squirrel. J. Comp. Neurol. *196*:489–504.

Innocenti, G.M. (1981) Growth and re-shaping of axons in the establishment of visual callosal connections. Science *212*:824–827.

Innocenti, G.M., and R. Caminiti (1980) Postnatal shaping of callosal connections from sensory areas. Exp. Brain Res. *38*:381–394.

Innocenti, G.M., L. Fiore, and R. Caminiti (1977) Exuberant projection into the corpus callosum from the visual cortex of newborn cats. Neurosci. Lett. *4*:237–242.

Innocenti, G.M., D.O. Frost, and J. Illes (1985) Maturation of visual callosal connections in visually deprived kittens: A challenging critical period. J. Neurosci. *5*:255–267.

Ivy, G.O., and H.P. Killackey (1981) The ontogeny of the distribution of callosal projection neurons in the rat parietal cortex. J. Comp. Neurol. *195*:367–389.

Ivy, G.O., and H.P. Killackey (1982) Ontogenetic changes in the projections of neocortical neurons. J. Neurosci. *2*:1–17.

Ivy, G.O., R.M. Akers, and H.P. Killackey (1979) Differential distribution of callosal projection neurons in the neonatal and adult rat. Brain Res. *173*:532–537.

Iwamura, Y., M. Tanaka, and O. Hikosaka (1980) Overlapping representation of fingers in area 2 of the somatosensory cortex of the conscious monkey. Brain Res. *197*:516–520.

Jensen, K.F., and H.P. Killackey (1984) Subcortical projections from ectopic neocortical neurons. Proc. Natl. Acad. Sci. U.S.A. *81*:964–968.

Jones, E.G., and T.P.S. Powell (1968) The commissural connections of the somatosensory cortex in the cat. J. Anat. *103*:433–456.

Jones, E.G., and T.P.S. Powell (1969) Connexions of the somatosensory cortex of the rhesus monkey. II. Contralateral cortical connections. Brain *92*:717–730.

Jones, E.G., J.G. Coulter, and S.H.C. Hendry (1978) Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys. J. Comp. Neurol. *181*:291–348.

Jones, E.G., J.D. Coulter, and S.P. Wise (1979) Commissural columns in the sensory-motor cortex of monkeys. J. Comp. Neurol. *188*:113–136.

Kaas, J.H. (1983) What, if anything, is S-I? The organization of the "first somatosensory area" of cortex. Physiol. Rev. *63*:206–231.

Kalaska, J.F., R. Caminiti, and A.P. Georgopoulus (1983) Cortical mechanisms related to the direction of two-dimensional arm movements: Relations in parietal area 5 and comparison with motor cortex. Exp. Brain Res. *51*:247–260.

Karol, E.A., and D.N. Pandya (1971) The distribution of the corpus callosum in the rhesus monkey. Brain *94*:471–486.

Killackey, H.P. (1973) Anatomical evidence for cortical subdivisions based on vertically discrete thalamic projections from the ventral posterior nucleus to cortical barrels in the rat. Brain Res. *51*:326–331.

Killackey, H.P. (1983) The somatosensory cortex of the rodent, T.I.N.S. *6*:425–429.

Killackey, H.P., H.J. Gould, C.G. Cusick, T.P. Pons, and J.H. Kaas (1983) The relation of corpus callosum connections to architectonic fieldsand body surface maps in sensorimotor cortex of new and old world monkeys. J. Comp. Neurol. *219*:384–419.

LaMantia, A.-S., and P. Rakic (1984) The number, size, myelination and regional variation of axons in the corpus callosum and anterior commissure of the developing rhesus monkey. Soc. Neurosci. Abs. *10*:1081.

Leong, S.K. (1983) Localizing the corticospinal neurons in neonatal, developing and mature albino rat. Brain Res. *265*:1–9.

Lia, B., R.W. Williams, and L.M. Chalupa (1985) Overproduction of optic nerve fibers in the fetal cat does not reflect axonal branching within the optic nerve. Suppl. Invest. Ophthal. Vis. Sci. *26*:286.

Lund, R.D., F.L.F. Chang, and P.W. Land (1984) The development of callosal projections in normal and one-eyed rats. Dev. Brain Res. *14*:139–142.

Maunsell, J.H.R., and D.C. Van Essen (1983) The connections of the middle-temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey. J. Neurosci. *3*:2563–2586.

Mesulam, M.-M. (1978) Tetramethtl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. *26*:160–177.

Mountcastle, V.B. (1978) An organizing principle for cerebral function: The unit module and the distributed system. In G.M. Adelman and V.B. Mountcastle (eds): The Mindful Brain. Cambridge, MA: MIT Press, pp. 1–50.

Nelson, R.J., M. Sur, D.J. Felleman, and J.H. Kaas (1980) Representation of the body surface in postcentral parietal cortex of macaca fasicularis. J. Comp. Neurol. *192*:611–643.

O'Leary, D.D., B.B. Stanfield, and W.M. Cowan (1981) Evidence that the early postnatal restriction of origin of the callosal projection is due to the elimination of axonal collaterals rather than the death of neurons. Dev. Brain Res. *1*:607–617.

Olavarria, J., and R.C. Van Sluyters (1985) Organization and postnatal development of callosal connections in the visual cortex of the rat. J. Comp. Neurol. *239*:1–26.

Olavarria, J., R.C. Van Sluyters, and H.P. Killackey (1984) The pattern of callosal connections within the barrel field of rat somatosensory cortex. Brain Res. *291*:364–368.

Panneton, W.M., and D.L. Tolbert (1984) The collateral origin of a transient cerebrocerebellar pathway in kittens. A study using fluorescent double-labeling techniques. Dev. Brain Res. *14*:247–254.

Pearson, R.C.A., and T.P.S. Powell (1985) The projection of the primary somatic sensory cortex upon area 5 in the monkey. Brain Res. Rev. *9*:89–107.

Pons, T.P., and J.H. Kaas (1985) Connections of area 2 of somatosensory cortex with the anterior pulvinar and subdivisions of the ventroposterior complex in macaque monkeys. J. Comp. Neurol. *240*:16–36.

Powell, T.P.S., and V.B. Mountcastle (1959a) The cytoarchitecture of the postcentral gyrus of the monkey macaque mulatta. Bull. Johns Hopkins Hosp. *105*:108–131.

Powell, T.P.S., and V.B. Mountcastle (1959b) Some aspects of the functional organization of the cortex of the postcentral gyrus of the monkey: A correlation of findings obtained in a single unit analysis with cytoarchitecture. Bull. Johns Hopkins Hosp. *105*:133–162.

Rakic, P. (1974) Neurons in rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. Science *183*:425–427.

Rakic, P. (1976a) Differences in the time of origin and in eventual distribution of neurons in areas 17 and 18 of visual cortex in rhesus monkey. Exp. Brain Res. Suppl. *1*:244–248.

Rakic, P. (1976b) Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. Nature *261*:467–471.

Rakic, P. (1981) Developmental events leading to laminar and areal organization of the neocortex. In F.O. Schmitt, F.G. Warden, G. Adelman, and S.G. Dennis (eds): The Organization of the Cerebral Cortex. Cambridge, MA: MIT Press, pp. 7–28.

Rhoades, R.W., and D.D. Dellacroce (1980) Neonatal enucleation produces an asymmetric pattern of visual callosal connections in hamsters. Brain Res. *202*:189–196.

Schwartz, M.L., and Goldman-Rakic (1982) Single cortical neurons have axon collaterals to ipsilateral and contralateral cortex in fetal and adult primates. Nature *99*:154–155.

Shanks, M.F., R.C.A. Pearson, and T.P.S. Powell (1978) The intrinsic connections of the primary somatic sensory cortex. Proc. R. Soc. Lond. [Biol.] *200*:95–101.

Shanks, M.F., R.C.A. Pearson, and T.P.S. Powell (1985) The ipsilateral cortico-cortical connexions between the cytoarchitectonic subdvisions of the primary somatic sensory cortex in the monkey. Brain Res. Rev. *9*:67–88.

Stanfield, B.B., D.D. O'Leary, and C. Fricks (1982) Selective collateral elimination in early postnatal development restricts cortical distribution of rat pyramidal tract neurons. Nature *298*:371–373.

Tolbert, D.L., and W.M. Panneton (1983) Transient Cerebrocerebellar projections in kittens: Postnatal development and topography. J. Comp. Neurol. *221*:216–228.

Vogt, B.A., and D.N. Pandya (1978) cortico-cortical connections of the somatic sensory cortex (Areas 3, 1, and 2) in the rhesus monkey. J. Comp. Neurol. *172*:179–192.

Wise, S.P., and E.G. Jones (1976) The organization and postnatal development of the commissural projection of the somatic sensory cortex of the rat. J. Comp. Neurol. *168*:313–343.

Wise, S.P., and E.G. Jones (1978) Developmental studies of thalamocortical and commissural connections in the rat somatic sensory cortex. J. Comp. Neurol. *178*:187–208.

Yorke, C.H., and V.S. Caviness (1975) Interhemispheric neocortical connections of the corpus callosum in the normal mouse: A study based on anterograde and retrograde methods. J. Comp. Neurol. *164*:233–246.