Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1076–1079, February 1989
Neurobiology

Process elimination underlies ontogenetic change in the distribution of callosal projection neurons in the postcentral gyrus of the fetal rhesus monkey

(corpus callosum/prenatal development/primate brain)

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Communicated by James M. Sprague, November 3, 1988 (received for review August 4, 1988)

ABSTRACT **During fetal development, the regional distribution of callosal projection neurons in the rhesus monkey's postcentral gyrus changes from a uniform to a discontinuous pattern. To determine if this developmental change reflects the retraction of transient callosal projections, two different fluorescent tracers were injected into the brain of fetal monkeys of known gestational ages. Fast blue was injected into the entire postcentral gyrus of one hemisphere, whereas a second tracer (rhodamine latex beads or diamidino yellow) was injected into the caudal portion of the postcentral gyrus of the other hemisphere. The rostral portion of the postcentral gyrus (contralateral to the hemisphere injected with fast blue) was subsequently examined for the presence of labeled cells. In animals injected early in fetal development, on embryonic day 110 or younger and sacrificed 4 weeks later, there were numerous cells labeled with both tracers. In contrast, very few double-labeled cells were found in fetuses injected at an older age, embryonic day 135. We interpret these findings as showing that early in fetal development, when callosal projection neurons in the postcentral gyrus show a continuous distribution pattern, single cells in the rostral portion of this gyrus possess at least two collaterals, one projecting to the contralateral hemisphere and the other to the caudal portion of the gyrus. Subsequently, many of these neurons retract callosal collaterals while maintaining ipsilateral projections. Thus, process elimination accounts for the establishment of the discontinuous distribution of callosal neurons found in the postcentral gyrus of the mature primate.**

The corpus callosum interconnects the two cerebral hemispheres. Although all the major sensorimotor areas of the cortex contain callosal connections, at maturity the distributions of callosal projection neurons and their terminals vary markedly within and across cortical fields. This is clearly evident in the postcentral gyrus of the adult rhesus monkey, which contains four cytoarchitectonic areas involved in processing of somatosensory information (1, 2). In general, there is a gradient in the distribution of callosal projection neurons across these areas, such that they are least dense rostrally in area 3b and most dense caudally in area 5 (3). Furthermore, within a given somatosensory area, the distribution of callosal projection neurons is most dense in regions containing representations of the proximal limb, trunk, and head and least dense where the distal portion of the limbs is represented.

How are such regional variations in the distribution of callosal connections established? In recent years this issue has received considerable attention (e.g., refs. 4–10), and as a consequence some general principles are beginning to be formulated. It is known that, with the possible exception of area 17 in the monkey (10), the discontinuous distribution pattern of callosal projection neurons found at maturity arises from a relatively uniform distribution of these neurons. In the postcentral gyrus of the rhesus monkey, we (11) have recently shown that this change takes place during fetal life, commencing about embryonic day 119 (E119) and achieving a state resembling that of a mature animal by E133, about 1 month before birth.

The establishment of mature callosal projection patterns could be due to cell death or to process elimination. In the present study, we sought to determine whether or not callosal projection neurons in the postcentral gyrus of the fetal rhesus monkey have multiple projections during the period of their early widespread distribution. This would indicate that process elimination is involved in the establishment of the primate's callosal distribution patterns. Such a mechanism has been implicated in the development of callosal connections in the rat (12) and the cat (13).

In the adult rhesus monkey, the available evidence (14, 15) indicates that there are very few cortical cells that project to more than one cortical area. Schwartz and Goldman-Rakic (14) have also reported that there are very few such neurons in the fetal rhesus monkey. Their observations were made on association cortices, and the youngest age studied, E132, was near the time that the callosal projection pattern in the postcentral gyrus already resembles that of the mature animal. Hence, the possibility that retraction of transient collaterals underlies the development of callosal connections in the primate is still open and warrants further investigation.

METHODS AND MATERIAL

A detailed description of breeding, estimation of gestational age, and fetal surgical procedures has been provided elsewhere (11). Here we emphasize that surgery was carried out under sterile conditions and that a surgical plane of anesthesia was maintained with halothane and nitrous oxide. Following externalization of the fetal head, a midline incision and blunt dissection revealed the central and lateral sutures in the skull. Bilateral bone flaps exposed the postcentral gyri of both hemispheres. Following retraction of the dura, multiple injections of fluorescent tracers were made into each hemisphere. Fast blue was injected extensively throughout the postcentral gyrus of one hemisphere, whereas in the other hemisphere rhodamine latex beads or diamidino yellow injections were restricted to the posterior portion of the crown of the postcentral gyrus. The fast blue injections always involved the white and gray matter, but in no case was there spread of tracer to the contralateral side. Following a survival period, which varied for different cases (see Table 1), the pregnant monkey was reanesthetized and the fetus was removed by cesarean

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Abbreviation: E*xxx*, embryonic day *xxx*.

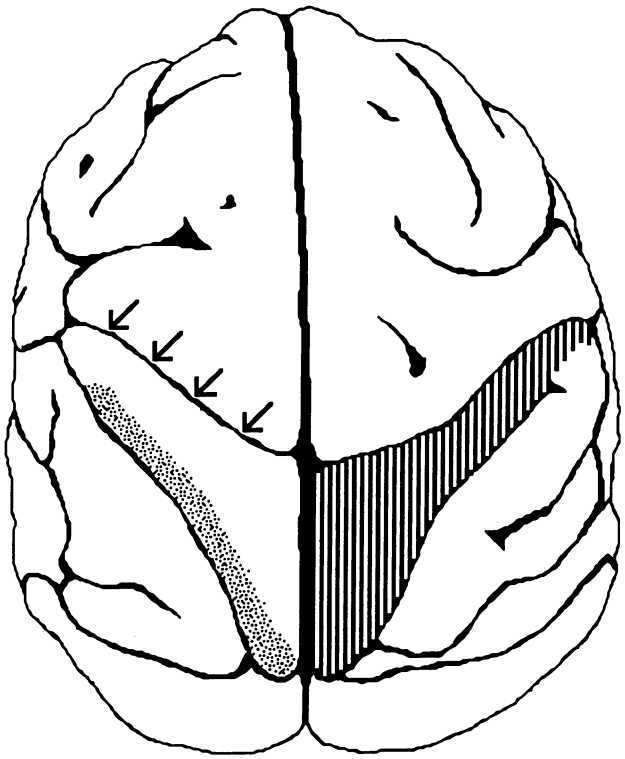

FIG. 1. Drawing of the dorsal surface of the fetal monkey brain illustrating the regions injected with fluorescent tracers. The hatched region is the postcentral gyrus, which received multiple injections of fast blue. The stippled area in the contralateral hemisphere was injected with either rhodamine latex beads or diamidino yellow. The arrows point to the central sulcus. The rostral portion of the postcentral gyrus in this hemisphere was subsequently examined for the presence of labeled cells.

section. The fetal animal was deeply anesthetized with an intraperitoneal injection of barbiturate and perfused transcardially with physiological saline followed by 2.5% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Subsequently, the rostral portion of the postcentral gyrus (corresponding in the adult to areas 3b and 1) in the hemisphere ipsilateral to the rhodamine bead or diamidino yellow injections was examined for the presence of labeled cells with a Leitz epifluorescent microscope. This experimental paradigm is summarized in Fig. 1, while Table 1 indicates the fetal ages of the animals, their survival period, as well as the tracers we injected.

Table 1. Ages of fetuses, their survival period, and tracers injected

Age		
Injection	Sacrifice	Tracers
E111	E114	DY/FB
E115	E120	DY/FB
E107	E135	RLB/FB
E110	E138	RLB/FB
E135	E150	RLB/FB
E135	E151	RLB/FB

DY, diamidino yellow; FB, fast blue; RLB, rhodamine latex beads.

RESULTS

The pattern of retrogradely labeled neurons we observed was dependent on the fetal age at which the injections were made. Injections in fetuses younger than E110 resulted in a continuous band of fast blue-labeled neurons throughout the postcentral gyrus. This was the case in the two animals that survived at least until day E135 as well as in two others injected at an early age with fluorescent tracers and sacrificed several days later. In contrast, equivalent injections in two older fetuses, at E135, resulted in a discontinuous or patchy pattern of fast blue-labeled neurons, which varied regionally within the gyrus. At all ages the vast majority of labeled neurons were confined to the supragranular layers. By E135 the labeled neurons could be clearly identified as pyramidal cells with prominent dye-filled apical dendrites extending towards the cortical surface (see Fig. 2).

The presence of a continuous band of labeled cells after the early injections confirms our previous observations (11) made with a different tracer (horseradish peroxidase) and short survival periods in fetuses younger than E133. Importantly, the long survival periods employed in the present study extend our previous findings by providing evidence that these early callosal projection neurons are not eliminated by a wave of cell death.

After restricted injections with rhodamine beads or diamidino yellow, there were pockets of labeled neurons along the

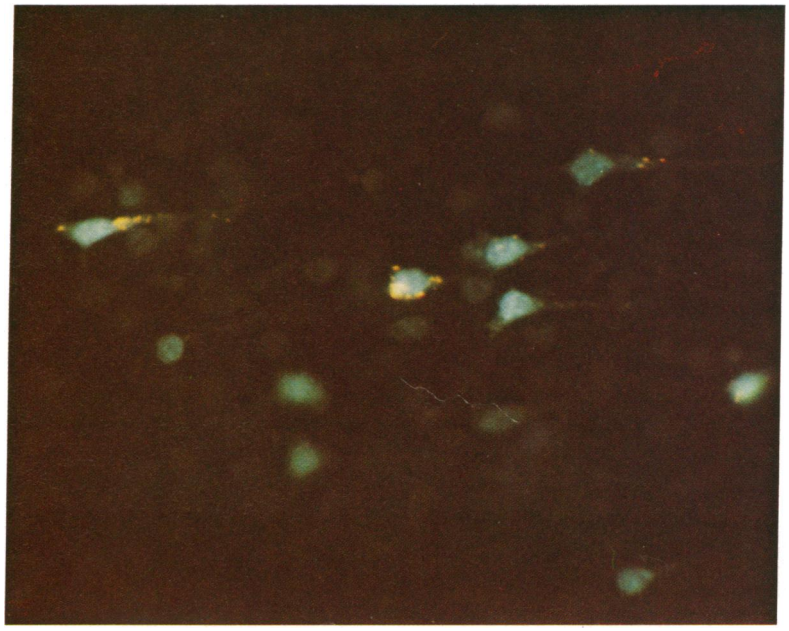

FIG. 2. Examples of double-labeled cells in the rostral portion of the postcentral gyrus of a fetal monkey injected with rhodamine latex beads (punctate orange label) and fast blue (blue cytoplasmic label) at E110 and sacrificed at E138. The tissue was illuminated with a broad band ultraviolet light source (filter bandpass, 340–380 nm).

rostral bank of the postcentral gyrus (area 3b). As was the case with the callosal projection neurons, the vast majority of these labeled neurons were in the supragranular layers. The granular nature of the rhodamine label and the resultant partial filling of the labeled neurons made it more difficult to specify the morphological characteristics of these cells. In a number of fortuitous instances, however, it was evident that these were also pyramidal neurons.

From the perspective of the main objective of the present study, the most dramatic difference between the fetal material labeled at early and late times was the incidence of double-labeled neurons. In the animals injected early in fetal development and sacrificed at or after E135, numerous cells were backfilled with both fast blue and the rhodamine beads (or diamidino yellow). In contrast, very few such neurons were observed when the injections were made in the older fetal monkeys, at E135. This is in agreement with the paucity of double-labeled cells observed in other cortical areas in older fetuses and in adult monkeys (14, 15).

As shown in Fig. 2, the double-labeled cells could be clearly identified by the continuous cytoplasmic fast blue label punctuated by intense rhodamine label. It can also be seen that such neurons were interspersed among pockets of neurons singly labeled with either tracer. This finding is further documented in Fig. 3, which depicts diagrammatically the distribution of single- and double-labeled cells within the rostral bank of the postcentral gyrus (areas 3b and 1) of an animal injected at E107 and sacrificed at E135. No attempt was made to count the number of single- and double-labeled neurons because, as has been discussed in detail by others (e.g., ref. 16), such quantification of cells backfilled with fluorescent tracers is of questionable validity.

Finally, it should be noted that due to technical limitations we were unable to document the presence of double-labeled cells in the two animals injected early (E107 and E115) and sacrificed after a short survival period. At these young fetal ages, most of the cell bodies of pyramidal neurons in the supragranular layers are composed of nuclear material with very little cytoplasm. Consequently, it was difficult to identify unequivocally two tracers within an individual neuron. For this reason, we shifted to the alternate strategy of using relatively long survival periods following injections of tracers in the early fetuses.

DISCUSSION

The present results, in conjunction with our earlier work on the postcentral gyrus (11), allow several points to be made

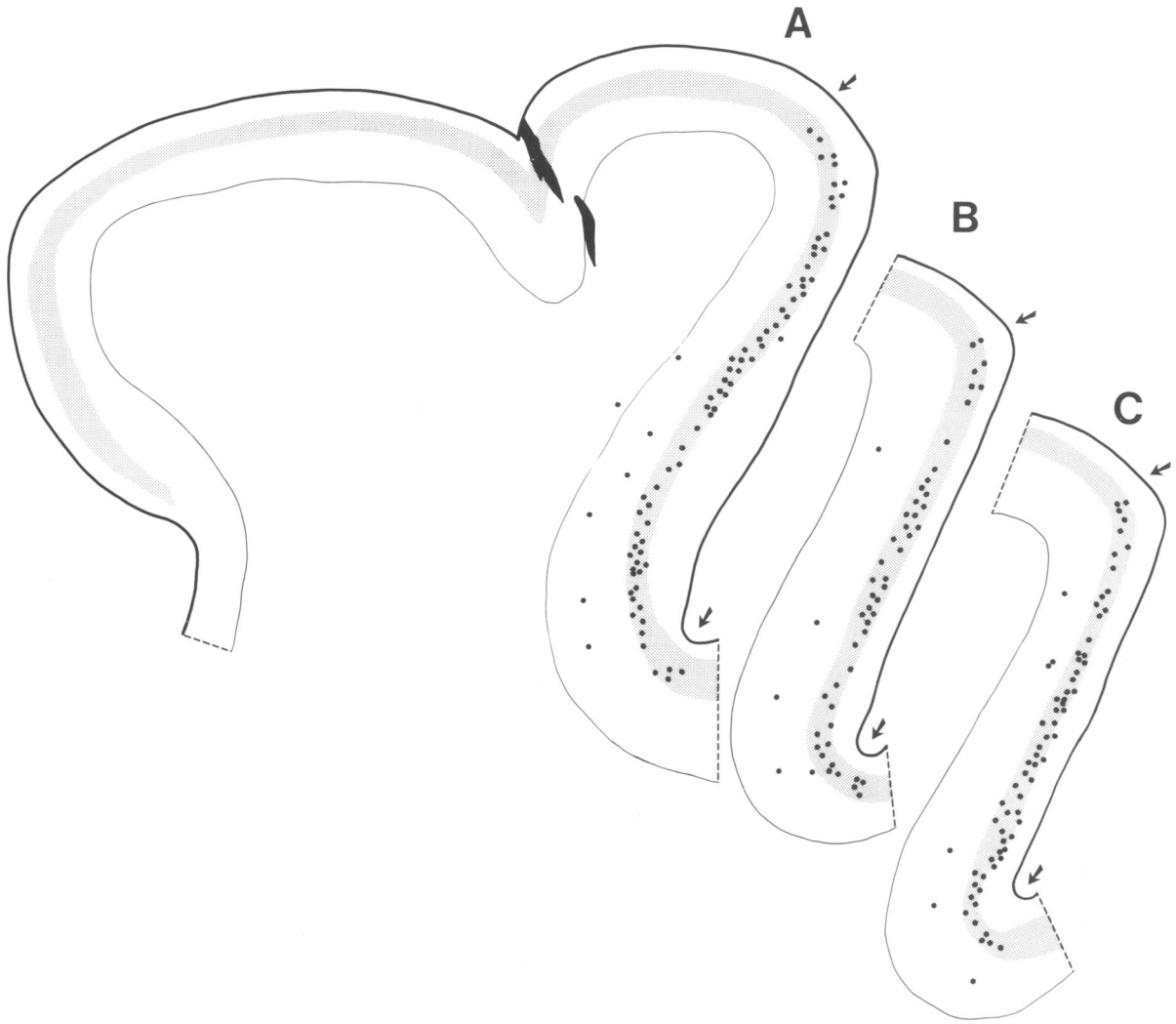

FIG. 3. Parasagittal sections through the postcentral gyrus of an E135 fetal rhesus monkey that received multiple injections of fast blue throughout the entire contralateral postcentral gyrus and restricted injections of rhodamine beads in the caudal portion of the ipsilateral gyrus at E107. (*A*) Entire postcentral gyrus. (*B* and *C*) Rostral bank of the gyrus in more lateral sections. Black denotes the locus of rhodamine bead injections. The stippling indicates the continuous band of fast blue-labeled neurons in the supragranular layers, whereas the dots indicate individual double-labeled neurons, which were plotted in the regions between the arrows.

regarding the prenatal development of callosal connections in the postcentral gyrus of the rhesus monkey. First, early in development, callosal projection neurons are distributed continuously throughout this region of the neocortex, whereas by about 1 month before birth the discontinuous distribution pattern characteristic of the mature animal can be identified. Second, in the early fetuses as in the adult, callosal projection neurons are pyramidal cells, the vast majority of which are confined to the supragranular layers. Third, the establishment of the discontinuous projection pattern is due to the retraction of axon collaterals that projected during early fetal development to the contralateral hemisphere. In the postcentral gyrus, this occurs sometime between E119 and E133 (11). Fourth, at young fetal ages, a substantial number of callosal neurons in the rostral portion of the postcentral gyrus also project to the posterior segment of the crown of this gyrus. The ipsilateral projection is maintained after prenatal retraction of callosal collaterals.

The experimental paradigm we employed does not permit us to ascertain whether or not two axonal branches stemmed from a single neuron during the same developmental period. Since the fluorescent dyes we employed probably remained available for uptake at the injection sites throughout the survival period, we cannot rule out the possibility that, in the cells we found to be double labeled, retraction of a callosal process may have occurred prior to the outgrowth of a new axon directed ipsilaterally. We believe that such a scenario is unlikely but, even so, it would not invalidate our contention that process elimination underlies the redistribution of callosal projection neurons in the primate.

The other mechanism that could underlie the restructuring of early callosal projection patterns is cell death. Cell death has been observed in the developing neocortex (17), but the significance of this phenomenon for the formation of cortical connections is still unclear. The apparent maintenance of the continuous projection pattern after a long survival period in the fetuses we injected early in development indicates that cortical cell death cannot be a primary factor in establishing callosal projections. Our results, however, do not rule out the possibility that cell death could contribute to some minor degree to the establishment of the mature callosal projection pattern.

Our findings differ from the observations of Schwartz and Goldman-Rakic (14), who reported that in the fetal monkey cortex, as in the adult, there are very few cells that project to more than one cortical area. We can think of several possible explanations for this discrepancy. First, the two fetal rhesus monkeys Schwartz and Goldman-Rakic studied were injected at ages (E132 and E137) when the adult distribution of callosal projection neurons has already been achieved in the postcentral gyrus. Second, the ipsilateral collaterals of callosal projection neurons we double labeled were quite limited in length; they extended from the rostral to the caudal portion of the postcentral gyrus. This was also the case in the two other studies (12, 13) that reported ontogenetic changes in the incidence of double-labeled callosal cells. In contrast, the Schwartz and Goldman-Rakic study was concerned with cells with more extensive collateral branches, which interconnect three widely separated areas of association cortex. Third, these investigators examined areas of frontal cortex, but it is yet to be established whether or not the cortical areas in the frontal cortex exhibit exuberant distribution of callosal projection neurons. There is good reason to believe, as discussed below, that there are variations among cortical areas in the early distribution of callosal projection neurons.

Dehay *et al.* (10) have reported recently, and we have confirmed (18), that primary visual cortex (area 17) of the fetal monkey is largely free of callosal projection neurons. These observations are in sharp contrast with the continuous distribution of callosal cells observed in area 18 of the prenatal monkey and with our findings on the early development of callosal projections in the postcentral gyrus (ref. 11 and this paper). Thus, although our findings clearly indicate that process elimination plays a role in the maturational restriction of callosal projection neurons in the somatosensory areas of the monkey's postcentral gyrus, another mechanism appears to be responsible for the difference in the distribution of callosal neurons between areas 17 and 18 of the primate visual cortex. Among the issues to be resolved are the factors underlying the formation of callosal projections in different cortical areas and the mechanisms by which the mature pattern of callosal projection neurons is achieved in a particular cortical area.

We are indebted to Dr. Andrew G. Hendrickx, director of the California Primate Research Center, for his support of this work. We thank Dr. John Anderson for performing the fetal surgeries and Barry Lia and Cara Snider for assistance in making the injections and tissue processing. This research was supported by National Institutes of Health Grants RR 00169 and NS 17234.

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