Callosal projections in rat somatosensory cortex are altered by early removal of afferent input

(development/corpus callosum/thalamic ablation/nerve section)

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ABSTRACT During the first postnatal week, the distribution of callosal projection neurons in the rat somatosensory cortex changes from a uniform to a discontinuous pattern. To determine if this change is influenced by afferent inputs to the somatosensory cortex, the effect of both early unilateral infraorbital nerve section and unilateral removal of the dorsal thalamus on the distribution of callosal projections in the somatosensory cortex was examined. One month after either of the above manipulations at birth, the tangential distribution of callosal projections in the somatosensory cortex was examined using the combined retrograde and anterograde transport of horseradish peroxidase. Both manipulations alter the distribution of callosal projection neurons and terminations in the somatosensory cortex. After infraorbital nerve section, the distribution of callosal projections is altered in the contralateral primary somatosensory cortex. The abnormalities observed are consistent with the altered distribution of thalamocortical projections. In addition, consistent abnormalities were observed in the pattern of callosal projections of the second somatosensory area of both hemispheres. Most notably, they are absent in a portion of the region that contains the representation of the mystacial vibrissae and sinuses hairs in this area. Thalamic ablation resulted in highly aberrant patterns of callosal projections in the somatosensory cortex on the operated side, where abnormal bands and clusters of callosal projections were observed in apparently random locations. These results are interpreted as evidence that both peripheral and central inputs influence the maturational changes in the distribution of callosal projection neurons.

In the rat somatosensory system, early peripheral and more central damage has a profound effect on neuronal organization at succeeding levels of the neural axis up to and including primary somatosensory cortex (1-5). Similar effects have also been reported in the visual system (for review, see refs. 6 and 7) but in this case an additional effect of early damage on the distribution of interhemispheric projections that interconnect cortical visual areas by way of the corpus callosum has also been reported (8-12). The one study (13) that examined the effect of neonatal damage to afferent cortical input in the rat somatosensory system reported that direct thalamic damage in the neonatal rat has no effect on the distribution of callosal projections in somatosensory cortex. This result is somewhat surprising for two reasons. (i) In most other respects the central effects of peripheral injury in the somatosensory system are very similar to those in the visual system. (ii) The early distribution of callosal projection neurons is very widespread throughout all of the somatosensory cortex, and during the first postnatal week or so they are largely eliminated from regions of primary somatosensory cortex that receive dense thalamic input from the ventral posterior nucleus (14, 15). In light of this, we decided to further assess the effect of early peripheral injury and thalamic damage on the distribution of callosal projections in rat somatosensory cortex.

MATERIALS AND METHODS

Thirteen litters of Sprague-Dawley or of Long-Evans rats were used in this study. All neonatal surgery was performed under cryogenic anesthesia, after which rats were revived on a heating pad and returned to their dams. Rats from 4 litters were subjected to unilateral section of the infraorbital nerve on the day of birth. Rats from 7 litters had their dorsal thalami removed unilaterally by aspiration through a small-diameter glass pipette that was inserted into the cranium from a posterior approach. Rats from 2 additional litters were used as age-matched controls. The pattern of callosal projections as determined by the combined retrograde and anterograde transport of horseradish peroxidase and patterns of staining for succinic dehydrogenase of all rats were examined when they were 1 month old. In addition, the distribution of horseradish peroxidase label in the thalamic ipsilateral to the cortical injections was examined to verify the uniformity of tracer uptake in the cortex (see Fig. 2D). In this context, both the absence of the ipsilateral thalamus for verifying cortical injections and the difficulty of combining complete thalamic ablations with large horseradish peroxidase injections for labeling the entire callosal pathway precluded study of the effects of thalamic ablation in the hemisphere contralateral to thalamic removal.

Under ketamine/xylazine anesthesia, rats received multiple injections of horseradish peroxidase [50% (wt/vol); Sigma, type VI] evenly distributed over the surface of the cortex. After a 24- to 36-hr survival period, rats were deeply anesthetized and perfused transcardially with saline followed by a 1.25% (vol/vol) glutaraldehyde/1% paraformaldehyde buffered fixative solution. The brains were removed, the cortices were detached, and the noninjected cortex was held flattened between glass slides during post-fixation and sucrose infiltration. The thalami were sectioned in the coronal plane and the cortices were sectioned in the tangential plane on a freezing microtome. Sections were processed for horseradish peroxidase histochemistry according to the protocol of Mesulam (16), mounted on gelatin-coated slides, and air-dried before being covered with a coverslip. Sections through the thalami were counterstained with neutral red.

One rat from each litter was used to determine cortical patterns of succinic dehydrogenase staining. These rats were deeply anesthetized and perfused transcardially with 10% (vol/vol) glycerol. The brains were removed and the cortices were detached and held flattened between glass slides as they were immersed in isopentane cooled to −40°C. Tangential sections were then cut in a cryostat, collected on gelatin-
coated slides, and reacted for succinic dehydrogenase histochemistry as described by Killackey and Belford (17).

RESULTS

In layer IV of rat primary somatosensory cortex (SI), the distribution of afferent terminations arising from the ventral posterior nucleus is reflected in the pattern of succinic dehydrogenase activity (17, 18), as in Fig. 1A. Overall, this pattern is a caricature map of the body surface of the rat. Note the exaggerated representation of densely innervated portions of the head such as the mystacial vibrissae and smaller sinus hairs on the muzzle. Lateral to SI, there is a second cortical area that also contains a representation of the body surface of the rat. Although this representation is not detectable with succinic dehydrogenase histochemistry, it can be demonstrated with physiological (19, 20) and anatomical (ref. 21; unpublished observations) techniques. In this area (SII), the map is organized as a rough mirror image of the first representation. Thus, there are two adjoining representations of the vibrissae and upper sinus hairs in the rat somatosensory cortex. The infraorbital nerve innervates both the vibrissae and sinus hairs of the upper jaw of the rat. If this nerve is sectioned at birth, as shown in Fig. 1B, the regions in SI in which these structures are normally represented are highly abnormal. Instead of rows of punctate clusters in SI, there are bands as well as areas of irregular staining in which there is no discernible pattern. This altered succinic dehydrogenase pattern reflects the altered distribution of thalamocortical afferents (5). Other portions of the map (e.g., lower jaw, forelimb, and hindlimb representation) are normally organized and there is no detectable change in enzyme staining in SII.

The normal pattern of callosal projections to rat SI cortex is largely complementary to the pattern described above (22). Labeled axons and somata surround and interdigitate with the regions that receive dense thalamic input from the ventral posterior nucleus (see Fig. 1C). In the region of the head representation, labeled callosal neurons and terminations tend to outline the representations of individual vibrissae and sinus hairs. There is also a dense band of callosal projections that overlaps the border between SI and SII. Adjoining this band within SII, there is a caudomedial to rostralateral alignment of “holes” that contain very sparse callosal projections separated by labeled “bridges” of callosal cells and terminations. Such bridges are continuous with a further lateral and caudal band of labeled callosal projections in SII.

Manipulations of the peripheral input to the somatosensory cortex result in profound modifications of the pattern of callosal projections. In rats whose infraorbital nerves were sectioned at birth, the band of densely labeled callosal cells and terminations that straddles the border of SI and SII is disrupted (see Fig. 1D–F). The major anomaly is a large gap in this band of callosal projections in a constant location largely within SII. In addition, smaller more variable gaps in the band are seen further caudally and medially in SII. These anomalies in the pattern of callosal projections are seen in both hemispheres although they tend to be more pronounced in the hemisphere contralateral to the infraorbital nerve section. Further, they are found in the portion of SII where the vibrissae and sinus hairs are represented. In the hemisphere contralateral to the nerve section (see Fig. 1D and F), the pattern of callosal projections within SI is also perturbed. Abnormal clusters of label are sometimes found within the region in which the mystacial vibrissae and sinus hairs would normally be represented. In portions of SI in which the succinic dehydrogenase pattern was not affected normal patterns of callosal connectivity were observed. Note in particular the normal pattern of callosal projections in the cortex associated with the lower jaw in all the cases illustrated.

Manipulation of the thalamic input to somatosensory cortex affects the distribution of callosal projections even more profoundly. The cortices of three rats in which the thalamus was removed at birth as well as a coronal section through the thalamus from one of these rats, indicating the extent of the lesions, are illustrated in Fig. 2. In these cases, aberrant patterns of callosal projections are found throughout the neocortex on the side of the lesion. In most cases, abnormal bands, patches, and clusters of labeled axons and somata are seen in presumptive somatosensory cortex. The location of this label varies from rat to rat and the apparently random patterns formed bear no resemblance to any portion of the patterns observed in normal rats or in rats subjected to infraorbital nerve section at birth. In some cases, aberrant patterns (which are not illustrated), the label is more diffuse and, in yet others, large regions of the neocortex are devoid of callosal projections. This change in the distribution of callosal projections appeared to be contingent upon complete lesions of the ventral posterior nucleus. In cases in which a portion of the ventral posterior nucleus was left intact callosal projections were largely normal.

DISCUSSION

The present results provide evidence that the pattern of callosal projections in somatosensory cortex is dramatically altered by removal of thalamic afferents to this cortex and is more subtly altered by section of the nerve innervating the periphery represented in a portion of this cortex. These results are best discussed in terms of the effect of these experimental manipulations on the different cortical areas composing the rat somatosensory cortex, namely, SI and SII, and in the context of developmental change in the distribution of callosal projection neurons. Initially, callosal projection neurons are uniformly distributed throughout somatosensory cortex although located in their correct laminar position (14, 15). At approximately the end of the first postnatal week, the mature disjunctive pattern of callosal projections is achieved by a process that apparently involves the retraction, or elimination, of the axonal processes of inappropriately located neurons rather than cell death (23, 24). In the rodent visual cortex, a similar change in the distribution of callosal projection neurons occurs (25) and various early manipulations have been found to result in callosal projections within primary visual cortex in areas from which they are normally eliminated (9–11). On this basis, it has been suggested that peripheral input acting through the thalamus plays a role in the maturation of the callosal pattern. The altered pattern of callosal projections in SI after infraorbital nerve section can be interpreted in a similar fashion. Infraorbital nerve section at birth results in alterations in neuronal projection patterns at each succeeding level of the neural axis (5, 26, 27). Thus, the periphery can be regarded as providing an extrinsic template that is passed from a given level of the neuroaxis to the next within the confines of a preexisting topographic order (28). At the cortical level, the chief effect of infraorbital nerve section seems to be on the terminal arbors of the thalamocortical projection fibers (5). After infraorbital nerve section, terminal arbors are more variable and larger in size. They also tend to be more elongate in form and the extend of overlap between adjacent arbors is much greater than normal. The net result of this is a “smearing” of the normally punctate somatotopic map (compare the face representation in Fig. 1 A and B). In this context, the altered distribution of callosal projections in SI after infraorbital nerve section is a direct consequence of the altered distribution of thalamocortical projections in somatosensory cortex that in turn results from an altered extrinsic template. Thus, in SI the distribu-
Fig. 1. Photomicrographs of tangential sections through the flattened somatosensory cortex. (A) Pattern of succinic dehydrogenase staining in a normal rat. The representation of various portions of the body surface (F, forepaw; H, hindpaw; L, lower jaw; T, trunk; U, upper jaw; V, vibrissae) in the primary somatosensory cortex and the location of the second somatosensory area (SII) are indicated. (B) Pattern of succinic dehydrogenase staining in somatosensory cortex contralateral to the side on which the infraorbital nerve was sectioned at birth. Note the anomalous pattern of staining in the portions of SI in which the vibrissae and upper jaw are represented but not in the area of the representation of the lower jaw or limbs. (C) Dark-field photomicrograph illustrating the distribution of horseradish peroxidase-labeled callosal neurons and terminations in the somatosensory cortex of a normal rat. The pattern of callosal projections in the somatosensory cortex contralateral (D and F) and ipsilateral (E) to the neonatal infraorbital nerve section. The arrows in C and D point out the normal pattern of callosal projections in a portion of the face region at the SI/SII border and the altered pattern after infraorbital nerve section, respectively. (Bars = 1 mm.)
tion of callosal projections after nerve section are determined by the same mechanism(s) that operates in the normal animal; however, the extrinsic cues provided to this mechanism are different.

The pattern of callosal projections after thalamic ablation, on the other hand, can be interpreted as a result of an expression of the more intrinsic organizational properties of these projections. In this context, thalamic ablation may be regarded as the ultimate way of removing organizing influences that are extrinsic to the neocortex. If an extrinsic template such as alluded to above was the only source of organization available to the maturing callosal projections, one would have expected this manipulation to result in a maintenance of the initial widespread and uniform distribution of callosal projection neurons. This is clearly not the case in the somatosensory cortex (both SI and SII). Rather, the clustered nature of callosal projections in these cases demonstrates a residual tendency toward aggregation. Similarly, the lack of a uniform distribution suggests that some process elimination is occurring. The factors guiding this aggregation and elimination may be intrinsic to callosal neurons or related to remaining inputs to the cortex—most notably, callosal projections originating in the opposite hemisphere. Another point to consider in relation to SI is the apparent discrepancy between the current results and the report of Wise and Jones (13) that neonatal thalamic ablation does not alter the pattern of callosal projections in somatosensory cortex. These authors focused on the laminar pattern of callosal terminations that is best viewed in the coronal plane. They reported that the laminar pattern of terminations is unchanged and that these terminations form discrete patches in the less granular portions of somatosensory cortex as in the normal rat. This is not inconsistent with the present results as we also observe discrete patches of callosal label in somatosensory cortex. However, the distribution of these patches is radically altered. While this is readily apparent in the tangential plane of section, it is less obvious in the coronal plane. Finally, it should also be mentioned that thalamic ablation is a relatively massive manipulation that may have other effects on the neocortex in addition to altering the distribution of callosal projection neurons. For example, it has been reported (29) that lesions of the thalamic input nucleus result in increased

Fig. 2. (A–C) Dark-field photomicrographs of tangential sections through the flattened cortex after the removal of the ipsilateral dorsal thalamus at birth. The distribution of callosal projections in all three rats bears no resemblance to either the normal pattern or altered patterns illustrated in Fig. 1. (D) A coronal section through the thalamus of the rat whose cortex is shown in C, illustrating the extent of the ipsilateral lesion. (Bars = 1 mm.)
cell death in the visual cortex. This effect, however, seems to be largely focused on cortical layers IV and VI, which directly receive thalamic input, and not on the layers in which callosal projection neurons and their terminations are located. Similarly, although the present thalamic ablations also severed the subcortical projections emanating from the neocortex, there is evidence that the response of callosal projections and subcortical projections to peripheral manipulations are independent and not interactive. Rhoades and Fish (30) have reported that whereas bilateral enucleation alters the distribution of callosal projection neurons, this same manipulation does not alter the distribution of either corticotectal or corticogeniculate projections.

In SII, the pattern of callosal projections is also altered by neonatal infraorbital nerve section. The major effect of this manipulation is a lack of callosal projections in the somatotopically appropriate part of SII, portions of which are normally heavily interconnected by the corpus callosum. Further, this effect is also detectable in SII in the hemisphere ipsilateral to the nerve section. In relation to the hypothesized role of the periphery in establishing central patterns of neural organization, the result suggests that this role extends beyond primary sensory areas of cortex into cortical areas less directly associated with receptor surfaces, perhaps even into the other hemisphere. A second aspect of the result is that the nerve section resulted in an absence of normally occurring callosal projections suggesting that either abnormal process elimination or cell death has occurred. Most studies in the rodent visual cortex (9–11) generally report a more widespread distribution of callosal projections after peripheral manipulation. This difference may be attributable to the fact that these studies focused on primary visual cortex, which may be considered to be more closely related to its respective receptor surface than is SII, and the fact that primary visual cortex receives a bilateral retinal input. In any case, the present results establish that a peripheral manipulation can result in both abnormal distributions of callosal projection neurons in SI and a failure to maintain callosal projections in SII. The presence of an effect in the hemisphere ipsilateral to the lesion that receives normal peripheral input from the contralateral body surface suggests that peripheral input per se is not sufficient to maintain callosal projections in SII. In this area, the failure to maintain callosal projection neurons is most likely attributable to anomalies in the contralateral SII. Is this failure due to callosal processes from this hemisphere finding an abnormal target contraterally or, conversely, is it due to the inappropriate withdrawal of callosal processes originating in the anomalous hemisphere, their failure to provide the appropriate signal for maintenance, or both? This conundrum illustrates the basic problem in understanding the maturational changes in the distribution of callosal projection neurons. At present, it is unclear whether the mechanism(s) that underlies process elimination operates by maintaining projections in “appropriate” locations, by eliminating them from “inappropriate” locations, or by both.

In summary, the present experiments provide evidence that the mature distribution of callosal projections is shaped by multiple influences. One such influence is the periphery acting by way of thalamic afferents to the cortex. In addition, reciprocal interactions between the hemispheres appear to play some role in these maturational events. The role of such reciprocal interactions is more obvious in cortical areas further removed from the periphery.

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