The Organization and Postnatal Development of the Commissural Projection of the Rat Somatic Sensory Cortex

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ABSTRACT Anterograde and retrograde tracing experiments have been used to demonstrate the origin and terminal distribution of commissural fibers in the first somatosensory cortex (SI) of the rat. The commissural fibers originate from pyramidal cells of all layers, but predominantly from layers III and V. The fibers terminate in a series of approximately vertical bands. In each of these there are concentrations of terminals extending from the inner portion of the molecular layer to the deep portion of layer III as well as in the superficial part of layer V, and in layer VI. Discrete vertical bands of cortex are reciprocally connected across the midline to give both the origin and terminal regions of the projection a patchy or "columnar" appearance. The commissural fibers arise from and terminate in areas of the cortex that lie between and alongside the aggregations of granule cells that distinguish SI of the rat. No commissural fibers terminate within the aggregations of layer IV cells themselves but the more superficial terminal ramifications may come to overlie these aggregations. A heterotopic projection to the contralateral second somatosensory cortex has been observed and is similar in form to the homotopic projection to SI.

Many commissural fibers have crossed the midline in the corpus callosum by the day of birth but lie in the underlying white matter and do not enter the cortical plate until at least the third postnatal day. During the first postnatal week these fibers grow somewhat diffusely into the maturing cortex and their topographic and laminar pattern of distribution attains its adult characteristics by the end of the first week. Commissural axons, thus, arise from immature cells but the maturation of cell form seems to precede the ingrowth of these axons and the acquisition of commissural synapses.

The anatomical organization of the commissural pathways joining the cortex of the two cerebral hemispheres has been studied in considerable detail in a number of species. Apart from the work of Jacobson ('65, '70), however, little attention has been devoted to these pathways in the rat and there has been no detailed study of the commissural connections of individual cortical areas in this species.

The first somatic sensory area of the rat cortex is composed of a number of anatomically distinct cellular subdivisions, each representing a different portion of the receptive periphery (Welker, '76) and each apparently receiving a segregated thalamic input (Killackey, '75). The present study was undertaken to determine the relationship of the commissural pathway to these subdivisions and to determine whether, as in other animals, certain parts of the body representation are not interconnected by commissural fibers (Ebner and Myers, '65; Jones and Powell, '68, '69; Pandya and Vignolo, '69, '71). Furthermore, the relatively immature state of the commissural pathway at birth in the rat (Auroux, '64) provides the opportunity to examine the postnatal development of the callosal fibers and their relationship to the maturing cortex. Since the fibers of the allocortical commissures have been reported to have the ability to sprout and

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form new connections in response to injury, (Lynch, Mosko, Parks and Cotman, '73) and since the sprouting observed is quantitatively greater if the injury occurs in immature animals (Lynch, Stanfield and Cotman, '73), such a developmental study forms a necessary basis for investigations that will seek to determine whether similar plastic phenomena can be induced in the neocortical commissures.

Some of these data have been reported previously in preliminary form (Wise, '75).

MATERIALS AND METHODS

A total of 91 albino rats of the Holtzman strain were used in these experiments. The brains were prepared for the study of the commissural pathway by a variety of neuroanatomical methods.

Autoradiography

58 rats were prepared for the autoradiographic fiber tracing method. Fifteen adult animals and 43 neonatal animals ranging in age from a few minutes to twenty-one days were injected with tritiated amino acids. Tritiated proline and leucine (5-[^3]H-L-proline, specific activity 17 Ci/mmole and 4,5-[^3]H-L-leucine specific activity 46 Ci/mmole) were evaporated and rediluted in 0.9% saline to give an equal parts solution of proline and leucine containing 25–50 μCi/μ1 of activity. Single or multiple injections of 0.05 to 0.1 μl were made through a 31-gauge needle attached to a one microliter Hamilton syringe or with air pressure through glass micropipettes having tip diameters of 20–50 μm. In one immature animal a survival time of five days was used, but in the remainder the survival periods were 12 hours to two days. The animals were perfused with 10% neutral formalin and paraffin sections of the brains were prepared for autoradiography by the method of Cowan et al. ('72). The exposure was two weeks at 4°C and the developed autoradiographs were counterstained through the emulsion with thionin. Prior to embedding, two of the brains were flattened between glass slides as described by Welker and Woolsey ('75) in order to bring most of the first somatic sensory cortex (SI) into one parasagittal plane. In two animals, 2.25 μl of 3H-adenosine (2,8-[^3]H adenosine, 50 Ci/mmole) in a concentration of 20 μCi/μl was injected into the SI cortex. After a survival period of one day, the brains were treated by the same autoradiographic procedure as outlined above. In all cases the cortical location of the injection was verified on the basis of cytoarchitecture and its restriction to the somatic sensory area could be confirmed by the presence of a demonstrable projection restricted to the somatic sensory nuclei of the thalamus.

In order to present the data on the laminar distribution of commissural fibers in graphic form, counts of silver grains per unit area were made in 120 to 307.5 μm wide traverses across the thickness of selected areas of the cortex. This was done by aligning one side of an eyepiece reticule parallel to the pia, and counting the grains that fell within it as it was moved stepwise across the thickness of the cortex.

Horseradish peroxidase

Single or multiple injections of horseradish peroxidase (HRP, Sigma, Type VI) in concentrations of 500 μg/ml were made into the somatic sensory cortex of eleven adult and ten neonatal (1–2 days old) rats. After survival periods of 1–3 days, the animals were perfused with a mixture of 0.4–4.0% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer. Fifty μm frozen sections of the brains were cut and treated according to the method of LaVail et al. ('73; see also Jones and Leavitt, '74). Fixative solutions containing 1% paraformaldehyde and 1.25% glutaraldehyde were found to be optimal.

Combined HRP and 3H-amino acids

Large (1 μl) injections of a solution containing 500 μg/ml of horseradish peroxidase and 50 μCi/μl of 3H-proline and 3H-leucine were made into somatosensory cortex of four adult rats. The brains were fixed either with 2% paraformaldehyde or with 1% paraformaldehyde and 1.25% glutaraldehyde. Fifty μm frozen sections were prepared as described above for HRP histochemical staining, but then the slides were subsequently coated with emulsion and exposed for autoradiography.

Axonal degeneration

In six mature animals the corpus callosum was cut or the whole neocortex was removed on one side. After a survival period
of five days, the brains were prepared for 
the demonstration of terminal axonal de-
generation by the Wiitanen ('69) modifica-
tion of the Nauta technique. In four of 
the six cases, the non-damaged hemispheres 
were first flattened as described above and 
then sectioned parasagitally at 20 or 30
μm. The other two brains were sectioned 
frontally or horizontally. Alternate sections 
through the whole hemisphere were stained 
by the Wiitanen technique or with thionin.

RESULTS

General organization of the commissural 
projection

Injections of tritiated amino acids that 
appear to be confined to the first somatic 
sensory area of one side lead to labeling 
of large numbers of commissural axons 
that may be seen traversing the anterior 
portion of the corpus callosum to reach 
the white matter of the opposite side. On 
the side opposite the injection, the labeled 
fibers turn perpendicularly into the cortex 
as a series of distinct bundles. The major 
bundles are situated dorsally and an-
teriorly and enter the cortex of the clearly 
identifiable granular, first somatic sensory 
area (SI) (Woolsey and Fairman, '46; Welk-
er '71, '74). A second group of fibers 
descends in the white matter towards the 
rhinal sulcus and enters a region of the 
cortex that corresponds to the second 
somatic sensory area (SII; see Welker and 
Sinha, '72).

The distribution of the commissural 
fibers in the SI area is not uniform. Follow-
ing large injections of tritiated amino 
acids which spread beyond the confines of 
SI, and after hemidecortication or commis-
surotomy in which the total complement 
of commissural fibers can be expected to 
be labeled either by transported radioac-
tivity or by degeneration, it is found that 
certain significant parts of the body repre-
sentation lack commissural fiber terminals. 
Within those parts of the representation 
that are connected, the distribution of the 
commissural fibers is patchy.

The most useful preparation for exam-
ining the topographic and three-dimen-
sional relationships of the commissural 
projection was found to be the flattened 
hemisphere, tangentially sectioned and 
stained by the Wiitanen technique subse-
quent to commissurotomy or hemidecortica-
tion. This procedure allows the mapping of 
clusters of degeneration (figs. 1, 4) in rela-
tion to the aggregation of granule cells 
(figs. 5, 6) known to contain the somato-
topic representation of each body part 
(Welker, '76). Flattened hemisphere prepa-
rances that were prepared by the autoradiographic method yielded similar 
results, but one can be more confident that 
the full complement of commissural fibers 
is "labeled" after a lesion than after an 
injection or even a series of injections of 
labeled amino acids.

Examination of the pattern of degener-
ation in relation to the clearly visible 
aggregates of layer IV cells seen in SI in 
alternate, Nissl stained sections reveals 
that three significant regions do not re-
ceive commissural fibers. The first of these 
is very large and corresponds to the several 
aggregates of cells that represent the vari-
ous sinus hairs of the face, jaws, and head —the vibrissal representation (Welker, 
'71, '74, '76; Welker and Woolsey, '75). 
None of the granule cell aggregates that 
form the several subfields of this region 
(Welker and Woolsey, '75) receive commis-
sural fibers. Other granule cell aggregates 
that lack commissural fibers are the two 
situated anterodorsally that, according to 
Welker, represent the limbs. Granule cell 
aggregates that represent the trunk and 
limb regions and the dorsal and ventral 
aspects of the head representation have 
bundles of commissural fibers associated 
with them but these bundles lie between 
them or at their edges and do not penetrate 
the center of the aggregates. Dark field 
photomicrographs of the Nauta stained 
preparations, therefore, reveal the re-
presentation of the rat's body form out-
lined by clusters of degenerating axons 
and axon terminals (figs. 1, 4).

The existence of areas without commis-
sural connection has been confirmed with 
the autoradiographic tracing technique. 
In three animals with small injections con-
fined to the SI cortex, no commissural 
fibers could be demonstrated. In each of 
these animals, technical difficulties could 
be ruled out and the topographic site of 
the injection could be confirmed by the 
distribution of labeled corticothalamic 
fibers in the ventrobasal complex. In all three 
cases a very dense mass of labeling was 
found in the arcuate nucleus of the ven-
Fig. 1 From an experiment in which the corpus callosum was cut five days previously and one hemisphere flattened prior to sectioning parasagittally at 30 μm. Lines near the frontal pole indicate the portion of the brain that was not flattened sufficiently to bring it into the same plane of section as the rest of the hemisphere. Alternate sections were stained by the Witanen method and with thionin. A is a camera lucida drawing of a section stained by the Witanen method in which stipple indicates degenerating bundles of commissural fibers and terminals (cf. fig. 4). B is a composite made from camera lucida drawings of five thionin stained sections lying deep and superficial to A. The aggregates of layer IV granule cells seen in these sections are indicated by hatching. Letters indicate parts of the body surface represented by the various aggregates (after Welker, '76). The part represented by the unlabeled aggregates are yet unknown. Subunits such as the “barrels” representing mystacial and other vibrissae are also indicated (cf. figs. 5 and 6). C is a tracing of a figure from Welker ('76) showing the distorted image of the body surface as represented by the granular aggregates. Figure is drawn at approximately the same magnification and orientation as the sections in A and B. Only three of the five rows of cell groups representing the five rows of mystacial vibrissae are indicated in Welker’s figure. D shows A and B superimposed. Note the manner in which the commissural bundles ascend around and between the layer IV aggregates but barely encroach upon them.
trobasal complex which is known to contain the thalamic vibrissal representation (Emmers, '65). When commissural fibers were labeled, the corticothalamic terminal labeling was invariably found to extend into parts of the ventrobasal complex representing proximal portions of the head and limbs and the trunk.

Commissural fibers entering SI, when stained by the Witanen method or labeled autoradiographically, form a series of closely packed vertical bundles of variable size. These enter the deeper layers of the cortex and ascend to layer I, spreading out as they approach it. The majority of these bundles ascend between the aggregations of granule cells in the trunk, head, and limb representations but, in expanding in the more superficial layers, some of their terminal ramifications come to overlie these aggregates. Patches of terminals related to different commissural fiber bundles may, therefore, fuse in layers I and II (fig. 15). The bundles entering the cortex adjacent to the vibrissal and distal limb representation ascend along the edges of the relevant granule cell aggregates (fig. 1) in zones where layer IV, though present, contains a much reduced density of cells. The commissural fiber bundles collectively, therefore, seem to follow the ventral and dorsal surfaces of the body representation.

The patches of terminals formed by each ascending bundle of commissural fibers vary greatly in size (figs. 4, 9). The smallest, situated near the trunk and proximal hindlimb representation, are 200 μm in diameter and are clearly separated from one another by gaps of variable dimension, at least 100 μm, which contain either extremely sparse or no degeneration. Other, larger patches of commissural fibers are also found. The largest patch seen in the dorsal part of SI is approximately 200 by 1,000 μm. Another large bundle, outlining the dorsal head representation, sweeps ventrolaterally for a distance of 3 mm. The density of this band, as demonstrated by degeneration and autoradiographic methods is not uniform suggesting that it is the product of the fusion of several columnar patches of commissural terminals each similar to the discrete, small patches seen in relation to the trunk representation.

Other areas of the cortex also receive a patchy distribution of commissural fibers (figs. 1, 4). These include the posterior parietal areas, the SII cortex, and a narrow strip which probably represents the border between areas 17 and 18 of the visual cortex (Dräger, '75). Other areas such as the motor regions contain a more homogeneous distribution of commissural fibers.

**Laminar distribution of commissural fiber bundles**

Not every commissural fiber bundle in SI was necessarily labeled in each autoradiographic experiment because of variation in the size of the injections of isotopically labeled amino acids. However, the autoradiographic experiments give a very clear demonstration of the terminal distribution of the individual bundles, especially in relation to the layers of the cortex. From counts of the silver grains such as those shown in figure 2, the relative terminal density in each layer can be indicated.

Each commissural bundle clearly has as its basis a relatively compact central bundle of fibers which are represented by dense linear chains of silver particles. As it ascends through the layers of the cortex, the central bundle can be observed to be associated with less regular labeling that very probably represents clusters of terminals and preterminal ramifications in the cortex. It has been consistently noted that the terminal labeling in the supragranular layers is considerably more dense than at any other level and extends for the greatest distance on all sides of the central bundle. Although the terminal labeling becomes progressively wider from layer III up to layer I, the greatest density of silver grains is usually found over layer III, and a lesser amount over the superficial half of layer I. Depending on the size of the incoming fiber bundle, the total lateral spread of terminal labeling in the deep half of layer I may be 200–1,000 μm. A short distance from the central bundle it appears that the commissural terminals are mostly in layers I to III (fig. 2; traverses 1A, 1B, 2B). Closer to the central bundle, the grain density over all the supragranular layers increases and a significant peak now appears in the upper half to two-thirds of layer V (figs. 2; traverses 1C, 2C, and fig. 15). Within the central bundle itself grains are dense over all layers, since
Fig. 2 From an experiment in which an injection of (3H) leucine and proline was made in the contralateral SI (cf. fig. 10). Sections 1-5 are in antero-posterior order and dots indicate the multiple column-like distribution of the labeled commissural fiber terminals in both SI and SII (section 5 lower). Note how each column-like mass has a central zone with wider terminal zones in layer V and particularly in the supragranular layers. Grain counts (top right, bottom left) across the cortex, therefore, show different patterns of terminal distribution depending on their position relative to the column. In every case, however, layer IV is virtually devoid of terminal labeling. Stipple in the graphs represents background grain density (cf. figs. 7, 8, 11–16).

there is heavy fiber labeling (figs. 7, 8, 11, 12). There is often a small peak in grain density over layer VI, though it is difficult in this layer to distinguish termin-

al from fiber labeling (fig. 7). The most striking and consistent feature of these observations is the decrease in grain density over layer IV, (the internal gran-
ular layer) relative to both the immediately deep and superficial layers (figs. 2, 11, 15).

An identical pattern of terminal distribution is seen in sections stained for degenerating fibers and terminals by the Witanen method. In these preparations (figs. 11, 15), especially those cut in the horizontal or frontal planes, the fiber degeneration appears fine but extremely dense. In this material it is often easier to distinguish an apparent terminal zone in layer VI (figs. 11, 12), since the contrast between fiber and terminal degeneration is more distinct, but in all other respects the pattern is the same as that shown autoradiographically.

The SI area of one side also sends a commissural projection to the contralateral second somatosensory area (SII; figs. 13, 14). The laminar distribution of this projection is quite similar to that in SI, except that layer IV seems to receive a relatively heavy input. In SII, terminals seem to consistently spare layer II. This latter aspect of the pattern is sometimes encountered in SI and seems to be common in cortical areas posterior to SI.

In SI and SII, each commissural bundle can be seen to give rise to a discrete terminal field spreading quite widely in the deeper aspect of layer I, and sometimes fusing there with the terminal fields of its neighbors (fig. 15). The bulk of its terminals, however, lie in a narrower zone in layer III and in layers V and VI. As viewed from above (i.e. in sections tangential to the cortex) the projection fields, therefore, appear as patches or "columns" which may be fused or discrete, depending on the depth of the section, the size of the column and its proximity to its neighbors.

Cells of origin and termination of commissural fibers

The localization of the cells of origin of the commissural projection is similar to the laminar distribution of the commissural terminals. The commissurally projecting cells, as demonstrated by the retrograde transport of HRP from an injection site in the contralateral hemisphere, lie in the layers receiving the bulk of the commissural fiber terminals and are also clustered in discrete patches. The HRP-positive cells found in SI after an injection of the enzyme in the contralateral SI, are concentrated throughout layers III and V, although many can still be found in layer II and a few appear in layer IV and in the upper portion of layer VI (fig. 17). Most of the labeled cells, including the few in layer IV, are small-to-medium-sized pyramidal cells (figs. 19, 20). All lie in small groups outside or between the major cellular aggregates of SI. The patchy localization of the commissurally projecting cells is shown by aggregates of HRP-positive cells separated by gaps of variable size in which few or no cells are labeled. The patches can best be seen in the supra-granular layers. In the deep layers, especially layer V, occasional labeled cells can be seen between clusters of labeled cells and may even appear far (2–3 mm) from the main zone of retrograde labeling.

Control experiments for possible endogenous peroxidase activity in cortical cells were negative. No cortical cells were HRP-positive after the injection of 1 μl of HRP into the contralateral thalamus and none were found in the neocortex of animals not injected with HRP.

The correspondence between the localization of the cells of origin and the terminal distribution of the commissural fibers can be demonstrated by the pattern of labeling seen after an injection containing both HRP and 3H-amino acids made in the SI cortex of one side (fig. 21). This injection led to retrograde labeling of commissurally projecting cells and anterograde labeling of bundles of commissural fibers in the opposite SI. The vast majority of the HRP-positive neurons lie beneath the labeled commissural terminal bundles. Thus, it appears that the patches of commissurally connected cortex are reciprocally linked with the exactly homotopic areas of the other hemisphere.

Preliminary experiments with 3H-adenosine show a pattern of connection (fig. 22) similar to that observed with the other methods. 3H-adenosine has been reported to lead to anterograde, transneuronal labeling of cells receiving terminals from fibers emanating from neurons at the site of injection (Schubert and Kreutzberg, '74, '75). Our observations (Wise and Jones, '76) are consistent with that view, though the possibility of transport in the retrograde direction is not ruled out. After injection of 3H-adenosine into SI of one side, relatively heavy deposits of silver grains
(fig. 22) are found overlying a considerable number of small to medium sized pyramidal cells in the deep half of layer III (fig. 23) and throughout layer V (fig. 24) in the contralateral SI, with a few other cells labeled in layers II, IV, and upper VI. Therefore, as well as giving rise to the commissural fibers, these pyramidal cells may be the major recipients of the commissural fiber projection. In the same brain, labeling of cell somata was observed in the reticular, intralaminar and ventrobasal complexes of the thalamus and in the ventral pontine nuclei. Labeling of the reticular and pontine nuclei which do not project to the cortex would tend to confirm that at least some of the transport of adenosine was anterograde and transneuronal.

Development of the commissural pathway

The development of the commissural system is completed only during the first postnatal week. This aspect of the study was conducted with the autoradiographic method. The injections of isotope are large, but in all cases, in order to control for the possibility that negative results might have been due to: (a) placement of the injection in a region not commissurally connected, or (b) insufficient survival times, the topographic projections upon the ventrobasal complex of the thalamus and upon the ventral pontine nuclei were examined in each brain. The age of the animals is given as the age at time of death since it is considered that the survival times used were sufficiently long for labeled material to have reached the growing axon tips. Thus, the pattern of labeling seen in the autoradiographs of the cortex is a reflection of the state of growth of the commissural fibers at the time of fixation.

All animals which were sacrificed at one day of age (defined as 12–36 hours old), including one animal 15 hours old that had been injected at birth, show intense labeling of a large number of fibers in the rostral part of the corpus callosum (fig. 31) and lighter labeling in the white matter beneath the presumptive SI cortex (figs. 3, 25, 26). However, no labeled material invades the cortex itself.

Not until the third postnatal day are appreciable concentrations of transported label seen over the deepest layer of the cortex, but the density is considerably less than over the underlying white matter. In animals injected on the first or second and killed on the third postnatal day, the grain density over the deepest layer has reached about 1.5 to 2.0 times the background (figs. 3, 27, 28). This deep layer is assumed to represent layer VI of the adult cortex. The presumptive granular and supragranular layers at this stage are presented by a homogeneous mass of densely staining cells (figs. 26, 28) that represent the remainder of the cortical plate. Grain counts over this were within the range of background radiation (fig. 3). The development of the remaining part of the cortical plate into a clearly differentiated internal granular layer, followed by layers II and III takes place over the next two to three days and appears complete by the fifth day of life.

The grain distribution pattern does not change greatly until the fifth and sixth postnatal days. By the fifth postnatal day, the grain density over the infragranular layers is as much as six times background and only a little less than that over the underlying white matter (figs. 3, 29). Here, many linear chains of grains represent the labeled commissural fibers. The supragranular layers, though now individually recognizable, still demonstrate little or no axonal or terminal labeling. By the sixth postnatal day, the axonal labeling over the supragranular layers has reached 2–3 times background as the fibers continue to invade the cortex, but the labeling remains less dense than that over the deep layers. By day seven, however, the superficial layers are labeled as heavily or more heavily than the deep layers and the adult laminar pattern of distribution of the commissural terminals is clearly evident (figs. 3, 30). By the eighth and ninth day and thereafter, the laminar organization of the commissural projection is almost indistinguishable from that of the adult.

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Fig. 3 Grain counts demonstrating the growth of commissural fibers into the presumptive SI cortex in neonatal rats in which (3H) amino acids were injected into the contralateral cortex. Age given indicates age at death. CP indicates remaining, immature part of cortical plate. Note that few or no labeled commissural fibers can be demonstrated in the cortex until the fifth day and that the adult pattern of laminar distribution cannot be demonstrated until the seventh day. Scales on graphs vary.
The patchiness in the distribution of commissural fibers seen in the adult is less evident in those animals in which labeled fibers are first seen growing into the maturing cortex (days 3–4). By the fifth postnatal day, however, when the fibers have penetrated well into the infragranular layers and are beginning to enter the supragranular layers, clusters become evident. By the seventh day, narrow column-like bundles separated by spaces devoid of labeling are clearly visible (fig. 30).

The commissural projection from SI to the opposite SII develops in parallel with that joining the SI areas of the two hemispheres and labeled axons may be seen growing into the SII area over much the same time scale as those invading SI.

Injections of HRP into the SI cortex and underlying white matter of 2-and 3-day-old animals lead to heavy retrograde cellular labeling in the contralateral cortex. It is assumed that these cells are labeled as the result of their growing commissural axons taking up the HRP in the contralateral white matter. All labeled cells lie deep to the compacted cell mass of the remainder of the cortical plate and in these deep layers appear in a clear bilaminar distribution pattern (figs. 32, 33). The labeling is extremely heavy and neurons throughout the whole region of cortex contralateral to the injection site are labeled. There is no evidence of the disjunctive, patchy distribution observed in the adult.

DISCUSSION

Topographic and general organization

The observation that SI projects to both the contralateral SI and SII in the rat agrees with findings in other species (Jones and Powell, '68, '69; Pandya and Vignolo, '68). The lack of a commissural connection between distal limb representations was also to be expected on the basis of similar findings in monkeys (Pandya and Vignolo, '68; Jones and Powell, '69; Jones et al., '75; Shanks et al., '75), cats (Ebner and Myers, '65; Jones and Powell, '68) and other species (Ebner and Myers, '65; Heath and Jones, '71). The lack of commissural connectivity in the vibrissal representation is somewhat surprising since the trigeminal representation is densely connected in other species. However, this finding has also been reported by others in rats (Jacobson, '70, Ryugo and Killackey, '75) and mice (Yorke and Caviness, '75). Therefore, from the point of view of commissural connectivity, perhaps the vibrissae of rodents may be regarded as laterialized sensory organs (Yorke and Caviness, '75) comparable to the hands and feet of other forms. Otherwise, the organization of the commissural system in the rat appears to conform to a general mammalian pattern in that the midline areas of the body representation are heavily connected through the corpus callosum, though, even in these proximal limb, head and trunk regions, the callosal fibers appear to avoid, at least in their deeper ramifications, the layer IV aggregates that form the heart of the representation of the body form.

Columnar organization

Perhaps one of the most striking features of the commissural projection pattern in the rat is its disjunctive nature (see also Heimer et al., '67). The commissural fibers enter the cortex in column-like bundles that may remain discrete, or merge to form larger bundles. Bundles pass along the edges of or between the patches of layer IV granule cells that each contain the representation of the various portions of the body surface (Welker, '74, '76) but tend to spare the patches themselves. It is assumed that the disjunctive nature of the commissural projection in the albino rat is not to any large extent conditioned by the albino trait which leads to abnormalities in the decussation of optic nerve fibers at the optic chiasm (Lund, '65; Guillery and Kaas, '71; Hubel and Wiesel, '71, Sanderson et al., '74). However, since the albino trait seems to lead to a certain diffuseness in the commissural system of the visual cortex in the Siamese cat (Shatz, '74), the present observations clearly should be confirmed in the wild type rat.

The topography of the commissural projection, as indicated by anterograde tracing techniques, especially when combined in the same experiment, is such that, in SI, commissural fibers appear to arise from and terminate upon exactly homotopic groupings of cells. The cells of origin of the commissural projection, thus, also form column-like groupings situated between and around the aggregates of granule cells in SI. Cells lying between the commissural-
ly connected zones in the highly granular regions, therefore, must have different patterns of connectivity.

These large patches of granule cells in layer IV presumably receive their thalamic input from the ventrobasal complex (Killackey, '73) but it is as yet uncertain whether the intervening and surrounding less granular zones also receive thalamic fibers. According to Welker ('76), these zones are not activated by somatic sensory stimuli so that the possibility exists that in the rat there is a segregation of thalamic and commissural inputs. It is unlikely, however, that such a segregation is an absolute one, for the terminal ramifications of many commissural fiber bundles spread quite widely in the supra-granular layers and invade those parts that overlap the granular zones.

The long, narrow, commissurally connected patch lying along the representation of the back and the dorsum of the head (figs. 1, 4) but posterior to the granular boundary that represents the customarily defined border of SI, seems to correspond precisely with the area of the cortex which does receive a thalamic input emanating from a part of the ventral nuclear complex of the thalamus in which neurons have bilateral peripheral receptive fields (Emmers, '65; Donaldson et al., '75). In this case, the commissurally connected area may contain a bilateral representation. Therefore, instead of a segregation of thalamic and commissural inputs (Ryugo and Killackey, '75), an alternative principle of organization in the rat somatic sensory cortex may be that the commissural input to areas between and outside the granular aggregates is distributed to regions that represent midline portions of the body surface and receive bilateral peripheral inputs. The thalamic connectivity of these areas clearly needs to be examined further.

The somatic sensory cortex of the monkey (Powell and Mountcastle, '59; Whitsett and Werner, '68; Dreyer et al., '75) and cat (Mountcastle, '57) is organized into narrow columns in which the cells show similar submodality and receptive field properties in response to peripheral stimuli. These columns, which would appear to be based in the first instance upon the thalamic input, are thought to be approximately cylindrical in shape with a diameter of about 500 µm and lie perpendicular to the surface of the cortex, spanning all layers. As a recording electrode is moved from one column into an adjacent one, there is usually an abrupt shift in the receptive field or submodality properties of the single units. This characteristic of the somatic sensory cortex of primates has recently been demonstrated also in the SI area of both adult and seven day old rats (Armstrong-Jones, '75).

It has been found in monkeys (Jones et al., '75) that the nerve fibers constituting both the commissural and ipsilateral corticocortical projections to and from the SI cortex are arranged in column-like arrays that are often similar in size to the columns that can be defined physiologically. A parallel pattern can obviously be seen in the rat commissural system, as reported here, and preliminary data suggest a similar arrangement in the opossum and cat (unpublished observations). The relationship of these patches of commissurally related cortex to the physiologically defined columns remains, however, uncertain in all species and it will be necessary to determine to what extent columns defined by the thalamic input overlap with the commissural columns.

Laminar organization

The present investigation has established with the HRP method that the commissural projection of the somatic sensory cortex arises from pyramidal cells in all layers but predominantly from layers III and V. These results agree with the report of Jacobson and Trojanowski ('74) in several cortical areas and with the interpretations made by Lorente de Nó ('49) in his Golgi studies. We have also shown by the autoradiographic and Wiitanen techniques that the commissural fibers terminate most heavily in the deep part of layer I and in layers III, upper V, and part of VI. The laminar distribution of the terminal ramifications of the commissural fiber bundles is such that they appear to conform to the pattern of dendritic organization of the pyramidal cells, especially those of layers III and V. Such cells, as seen in Golgi preparations have basal dendritic sprays in the layer containing the soma and their apical dendrites end in a substantial dendritic spray that commences in the up-
per part of layer III and terminates in layer I. The zones of maximal dendritic branching and widest spread are, therefore, the zones in which the concentrations of commissural terminals are densest and most widely spread. This, together with the preliminary data from the experiments using the putatively transneuronal flow (Schubert and Kreutzberg, '74), of $^3$H-adenosine, would suggest that, as well as giving rise to the majority of commissural fibers, the medium-sized pyramidal cells of layers III and V may be major recipients of the commissural projection.

The general pattern of laminar organization defined in the present study is similar to that reported by others in the somatic and visual cortex of the rat (Jacobson, '70; Lund and Lund, '70) and mouse (Yorke and Caviness, '75; see also Lorente de Nó, '49) and is also found in the opossum (unpublished observations). But the primate commissural system differs considerably from this (Jacobson and Marcus, '70; Jones et al., '75). In SI of the rhesus and squirrel monkey there is a heavy commissural input to layer IV as well as to the supragranular layers, but not to the infragranular layers (Jones et al., '75). This observation is curious since in most respects the organization of the rat and monkey commissural systems are rather similar, and moreover, the laminar distribution of the cells projecting to subcortical sites also seems to be similar in the two species (Lund et al., '75; Gilbert and Kelly, '75; Wise, '75). However, the laminar distribution of the cells of origin of the commissural pathway also differs in the rat and monkey. In the monkey SI, the HRP-positive cells seen after injections of the homotopic cortex are strictly confined to the large pyramidal cells of layer IIIB, (Jones et al., '75) although a few more deeply placed labeled cells are found in the visual (Wong-Riley, '74) and frontal (Jacobson and Trojanowski, '74) cortex. In the cat, the distribution of the cells of origin of the callosal projection of the suprasylvian gyrus (Maciewicz, '74) resembles the pattern in the monkey but the distribution of commissurally projecting cells in the somatic cortex of the cat is more similar to the rat (unpublished observations).

It is possible that the more rigid segregation of the cells of origin and the concomitant restriction of the terminal distribution of commissural fibers in the monkey, as compared with the rat, results from different patterns of migration during development. In the monkey, the cells of a particular cortical layer are generated from the neuroepithelium over a much narrower range of time than in the rat (Berry and Rogers, '65; Caviness and Sidman, '73; Rakic, '74). Cells migrating into the cortical plate at any one time may, then, come to rest in any one of several definitive cortical layers in the rat, but tend to reach only one, or a restricted part of one layer in the monkey. Hence, if cells are specified from their time of origin to form and receive certain connections such as the commissural, these cells and the fiber terminals that they receive will have a much more restricted distribution in the monkey than in the rat.

Development

Though many commissural fibers are present in the white matter beneath the developing cortex by one day after birth, they do not invade the cortex until the third postnatal day at the earliest and they have not established their final disposition until the end of the first week. It appears that the cortex must mature beyond the point it has reached at birth before the commissural fibers grow into it and establish synaptic connections. The commissural fibers reach their layers of major termination (the supragranular layers) only after these layers have become fully separated from the remaining part of the fetal cortical plate. This would imply that the cells of these layers must mature in some manner before the arrival of the commissural fibers, rather than pari passu with them.

Preliminary Golgi studies of our own indicate that by the time of arrival of the commissural fibers in the supragranular layers, the pyramidal cells of these layers have largely attained their definitive forms as seen in the adult SI. In the period between birth and the invasion of the cortex by the commissural fibers, cellular maturation, as demonstrated by the Golgi technique, is reflected in a progressive elaboration of the dendritic processes of initially bipolar cells into pyramidal and other forms.
The state of maturation of the dendritic processes of the cortical cells appears to be unrelated to the outgrowth of an axonal process. Cajal ('11), for example, shows that the axons of immature pyramidal cells may grow for long distances into the white matter and even give rise to numerous collateral branches before the establishment of a substantial dendritic tree. The present results, showing the early growth across the corpus callosum of large numbers of axons derived from immature neurons are clearly in keeping with these observations and with those of Seggie and Berry ('72) who found that a transcallosal evoked response could not be elicited in the neonatal rat until a few days after birth. The early outgrowth of axons in comparison with the maturation of the dendrites is also a feature of developing Purkinje and basket cells in the cerebellum (Cajal, '11; Mugnaini, '69; Rakic, '72).

A pattern of development similar to that of the commissural system has been observed in the thalamo-cortical fiber system in the visual (Lund, '75) and somatic sensory (unpublished observations) cortex of neonatal rats, the growing thalamic fibers only entering the cortex at a relatively late stage. Therefore, in the cortex, cellular development proceeds independent of at least two groups of afferent fibers and the acquisition of the mature cell form is unlikely to be determined by an interaction between the maturing cell and its arriving afferent fibers. It has frequently been suggested that afferent connections play a major role in determining the characteristic morphology of neurons in the cerebral cortex (eg. Marin-Padilla, '71) and in many other sites. It is clear from work on the reaggregation of developing cerebral and cerebellar cortical cells in tissue culture (DeLong and Sidman, '70; Wolf and Dubois-Dalcq, '70) that the gross form, the alignment and laminar distribution of cells in these cortical structures can develop normally in the absence of all extrinsic afferent fibers. However, the possibility remains that in the cerebral cortex, as in the cerebellum, intrinsic connections and possibly certain extrinsic afferents may play some role in the finer modelling of the cells. In the cerebellum, for example, the presence of parallel fibers seems to be necessary for the final growth of Purkinje cell spiny branchlets (Altman, '75) and spines (Hámori, '75), for the maturation of the dendrites of basket and stellate cells (Rakic, '72) and for the definitive orientation of the Purkinje cells (Rakic and Sidman, '73; Altman, '75; Sotelo, '75). It will now be necessary to examine the maturation of cerebral cortical cells at the electron microscopic level to determine the sequence of synapse formation made by the commissural and thalamocortical systems upon the recipient cells and to decide whether any interactions comparable to those reported in the cerebellar cortex may also occur there.

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PLATE 1

EXPLANATION OF FIGURES

4 Darkfield photomicrograph of a 30 μ thick Wütanen-stained parasagittal section from the flattened hemisphere of a rat in which the corpus callosum was sectioned five days before killing the animal. Bright patches are vertical bundles of degenerating axons and terminals lying in the vicinity of layer IV. Note how the body representation in SI is almost completely outlined by commissural bundles. Portions of the body representation are indicated thus: F, face and jaws; FL, forelimb; H, head; HL, hindlimb; T, trunk (cf. fig. 1 and figs. 5 and 6 below). The distribution of commissural fibers in many other parts of the cortex is also patchy eg. SII. Note also the lateral border of the striate area (17). × 10.

5, 6 Brightfield photomicrographs of 30 μ thick thionin stained sections from the same brain as figure 4 and respectively lying immediately deep (fig. 5) and superficial (fig. 6) to the section shown in figure 4. These show the disjunctive aggregations of granule cells which make up layer IV of SI. Arrows in figures 4–6 indicate the same blood vessels. Commissural fiber bundles lie at the edges of the granular aggregates but rarely invade them. × 10.
PLATE 2

EXPLANATION OF FIGURES

7, 8 Darkfield (fig. 7) and brightfield (fig. 8) photomicrographs from the same portion of an autoradiographic section cut in the frontal plane. In this animal, (3H) leucine and proline were injected into a large part of the contralateral SI (cf. fig. 10 below). Note the column-like nature of the transported label and the relative decrease in terminal density in layer IV. Since the column-like bundle ascends along the edge of the granule cell aggregates, layer IV does not appear dense in this section. Arrow indicates approximate position of labeled bundle. Thionin counterstain, × 70.

9 Darkfield photomicrograph of a Wiitanen-stained parasagittal section from a flattened hemisphere comparable to that shown in figure 4. Note the variation in size and density of the column-like bundles of commissural fibers and terminals, × 45.

10 A representative example of an injection site in SI. Most large injections of isotopically labeled compounds or of HRP were of this size. In this case the injection consisted of both HRP and tritiated amino acids (fig. 21). Autoradiograph, thionin counterstain, × 10.
EXPLANATION OF FIGURES

11, 12  Darkfield and brightfield photomicrographs from immediately adjacent Wiitanen and thionin-stained sections showing a bundle of degenerating commissural fibers ascending through a relatively agranular part of SI adjacent to one of the granule cell aggregates (large arrow). Small arrows indicate the same blood vessel. × 140.

13, 14  Darkfield and brightfield photomicrographs from alternate sections stained with the Wiitanen method and with thionin showing a column-like bundle of degenerating commissural fiber terminals in SII. Figure 13 is taken from a region approximately corresponding to that indicated in figure 14. Figure 13, × 250; figure 14, × 50.
PLATE 4

EXPLANATION OF FIGURES

15, 16  Darkfield and brightfield photomicrographs of alternate sections cut in the horizontal plane and stained respectively by the Wiitanen method and with thionin. The photomicrographs are at the same magnification; arrows indicate the same blood vessels. Two bundles of degenerating commissural fibers and terminals ascend, mainly between or along the edge of the granule cell aggregates of layer IV ("barrel field" to the left). There is a paucity of degeneration in layer IV but major concentrations are seen in the supra- and infra-granular layers. In the supragranular layers, the two adjacent "columns" tend to merge above the granule cell aggregates. × 50.
RAT COMMISSURAL SYSTEM
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PLATE 4

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PLATE 5

EXPLANATION OF FIGURES

17, 18  Darkfield and brightfield photomicrographs from the same section showing retrograde HRP labeling of cells in SI following an injection of the enzyme in the contralateral SI. The cells are pyramidal cells and though a few are found in all cellular layers, they are concentrated in layers III and V. Figure 17 is from the region indicated in figure 18. This is in a region outside the granular aggregates so layer IV is relatively indistinct. Thionin counterstain; figure 17, × 220; figure 18, × 50.

19, 20  Darkfield photomicrographs of pyramidal cells retrogradely labeled with HRP in layers III (fig. 19) and V (fig. 20) of SI after an injection into the contralateral SI. × 1,200.
PLATE 6
EXPLANATION OF FIGURES

21 Darkfield photomicrograph from an autoradiographic section through a part of SI in a brain in which both HRP and (3H) leucine and proline were injected at the same site in the contralateral SI (fig. 10). The retrogradely labeled cells of origin of the commissural fibers projecting to the injection site with very few exceptions lie within the column-like bundle of anterograde labeling which represents the terminal ramifications of commissural fibers emanating from cells at the injection site. × 85.

22 Darkfield photomicrograph showing presumed anterograde, trans-neuronal labeling of cells mainly in layers III and V of SI following an injection of (3H) adenosine in the contralateral SI. × 150.

23, 24 Brightfield photomicrographs of labeled cells from layers III (fig. 23) and V (fig. 24) from the same section as figure 22. Note the high concentrations of grains which overlie certain cells, compared with the relatively light labeling of the surrounding neuropil. Thionin counterstain, × 350.
PLATE 7

EXPLANATION OF FIGURES

25, 26 Darkfield and brightfield photomicrographs from the presumed SI region of a 1-day-old rat in which $^{3}$H) leucine and proline had been injected into the contralateral cortex at birth. Labeled commissural fibers have reached the white matter (WM) but have not invaded the immature cortex. Thionin counterstain, $\times$ 260.

27, 28 A comparable experiment to that illustrated in figures 25 and 26 but in a 3-day-old animal. Though the cortex is thicker and its deeper layers distinct, the labeled commissural fibers remain mainly in the underlying white matter. Thionin counterstain, $\times$ 260.

29 A comparable experiment in a 5-day-old animal. The labeling of commissural fibers in the white matter is intense and labeled fibers have invaded the cortex diffusely but have not yet reached the superficial layers. $\times$ 20.

30 A comparable experiment in a 7-day-old animal. Labeled commissural fibers have reached layer I and the column-like nature of the commissural projection has become apparent. $\times$ 190.
PLATE 8

EXPLANATION OF FIGURES

31 Darkfield photomicrograph from an autoradiograph showing heavily labeled commissural fibers traversing the anterior part of the corpus callosum (CC) and entering the hemisphere (to the right) contralateral to an injection site in the presumed SI cortex; one day old animal sacrificed 15 hours after birth; LV, lateral ventricle. × 260.

32, 33 Brightfield and darkfield photomicrographs from the same section showing retrograde HRP labeling of many neurons mainly in layers III and V of the presumptive SI in a 2-day-old animal following injection of the enzyme into the white matter underlying the contralateral SI. There is considerable anterograde fiber labeling in the white matter. Thionin counterstain, × 200.