ABSTRACT  Autoradiographic, axonal degeneration, and horseradish peroxidase fiber tracing methods were employed to investigate the organization, development and potential plasticity of the thalamocortical projection to the somatic sensory cortex of the rat. In the adult animal, thalamocortical terminals are concentrated primarily in layers I and IV and in the upper part of layer VI. Fibers terminating in layers IV and VI arise from a different thalamic region than those terminating in layer I. Discrete clusters of fibers and terminals 250-450 µm wide are distributed only to the parts of the SI cortex containing dense aggregates of layer IV granule cells and not to the intervening, less granular and commissurally connected zones.

At birth, thalamocortical fibers have invaded the deep part of the developing SI cortex and are concentrated in the upper part of layer VI. Between the age of two and three days, an additional concentration of fibers appears in the part of the cortical plate which will become layer IV. Layer IV is clearly recognizable by three days of age and the dense granule cell aggregates appear in it no more than one day later. The ingrowth of commissural fibers (Wise and Jones, '76) lags behind that of thalamic fibers. The mature commissural fiber pattern is not established until the age of seven days.

After removal of the developing thalamocortical system by thalamotomy in newborn rats, subsequent investigation of the commissural system in the adult showed that no commissural fibers or terminals had invaded either laminae or zones of the cortex deprived of thalamic input. Similarly, commissurotomy at birth was not followed by sprouting of thalamic fibers into zones or laminae deprived of commissural connections. The connectional specificity observed in these neocortical fiber systems contrasts markedly with the plasticity of connections reported in allocortical systems.

Removal of thalamocortical afferents before they attain their definitive distribution does not radically effect the overall development of the dense granule cell aggregates in layer IV. Within the aggregates, however, subsidiary features such as the "barrels" fail to appear. This finding suggests that certain elements of cortical architecture such as the dense granule cell aggregates are independent of thalamic afferents while others, such as the barrels, result from the interaction of the developing thalamocortical fibers and/or terminals with maturing neurons.

Thalamocortical fibers projecting into the first somatic sensory cortex (SI) of the rat arise from the ventrobasal complex (VB) and the central lateral nucleus (Jones and Leavitt, '74) and terminate in the cortex in a somatotopic pattern (Emmers, '65; Welker, '76; Saporta and Kruger, '77). The SI cortex of the rat consists of areas containing dense aggregations of granule cells in layer IV, with intervening and surrounding less granular areas. Within many of the larger aggregates, subsidiary ("barrel") formations (Welker, '76) are

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seen. Only the granular areas are activated by peripheral somesthetic stimuli in anesthetized animals (Welker, '76); the less granular areas contain the commissural and other corticocortical fibers (Ryugo and Killackey, '75; Wise and Jones, '76). Previous studies have established the time course of development and the laminar, topographic, and columnar organization of the commissural system of the rat SI cortex (Wise and Jones, '76). The present investigations were undertaken: (1) to obtain similar information concerning the thalamocortical system, (2) to investigate the spatial relationships of the commissural and thalamocortical systems in the normal rat SI cortex, (3) following the demonstration of considerable plasticity in the pattern of connectivity of certain afferent fibers to the allocortex (Westrum, '75; Lynch and Cotman, '76; Zimmer, '76) to evaluate the degree of connective plasticity or specificity of the neocortical connections; (4) to determine the influence of the developing thalamocortical system upon certain architectural features of the SI cortex. Some of these data have been previously presented in preliminary or abstract form (Wise, '75, '76, '77).

MATERIALS AND METHODS

A total of 123 albino rats of the Wistar strain were used in these experiments. A variety of experiments were performed.

Organization of thalamocortical fibers

In eight adult rats, injections of radioactive amino acids were made stereotaxically into the ventrobasal complex. Each injection consisted of 0.1-0.2 μl of a 50 μCi/μl solution of 5-[3H]-1-proline (specific activity 17 Ci/m mole) and 4.5-[3H]-1-leucine (specific activity 46 Ci/m mole) and was made through a 31-gauge needle attached to a 1-μl syringe or with air pressure through glass micropipettes having tip diameters of 25-50 μm. After a 1- or 2-day survival period the animals were perfused through the heart with 10%-buffered formalin. The relevant hemispheres from four of the brains were then flattened under pressure (Welker, '76; Wise and Jones, '76), sectioned on a freezing microtome at 30 μm, mounted on glass slides and dipped in Kodak NTB-2 emulsion for autoradiography (Cowan et al., '72). The other brains were embedded in Paraplast, sectioned at 20 μm in the frontal plane and also prepared for autoradiography. After an exposure period of two weeks, the autoradiographs were developed in Kodak D-19 at 17°C, fixed in Kodak Ektaflo and stained through the emulsion with 0.25% thionin.

The spatial relation of thalamocortical and commissural fibers

In two rats, the ventrobasal complex was lesioned stereotaxically from behind. Immediately thereafter, the corpus callosum was sectioned. The animals were killed after five days by perfusion with formalin. Thirty-micron thick sections were later cut on a freezing microtome and alternate sections were stained by the Wiitanen ('69) method for degenerating fibers and terminals and with thionin. The pattern of thalamocortical degeneration could then be compared to the pattern of granule cell aggregates in layer IV of SI (Welker, '76) and to the distribution of commissural fibers (Wise and Jones, '76).

Development of thalamocortical and corticothalamic fibers

The development of thalamocortical fibers was investigated by autoradiography in 56 rats of age zero to eight days. In these animals 0.05-0.1 μl injections of 25-50 μCi/μl [3H]-proline and leucine were made 1.5 mm anterior and 1.5 mm lateral to lambda and 3.7 mm deep to the cortical surface. The development of corticothalamic fibers was investigated in 16 rats, zero to eight days old and adult, in which 0.05-0.1 μl of the same mixture of proline and leucine was injected into the SI cortex. After a 1-day survival the animals were perfused through the heart with 10% buffered formol saline or with a solution for formol (10%)-acetic acid (5%) in 80% ethanol. All brains were embedded in Paraplast and prepared for autoradiography in the manner described above.

A further 18, 1- or 2-day-old animals were injected with 0.05-0.1 μl of a 500 μg/μl solution of horseradish peroxidase (Type VI, Sigma). After a 1-day survival period they were perfused with 1.0-2.0% paraformaldehyde and 1.5-2.5% glutaraldehyde in 0.1 M phosphate buffer. Fixative solutions containing 1% paraformaldehyde and 2.5% glutaraldehyde were found to yield optimal results. These brains were removed, soaked overnight in 30% sucrose in phosphate buffer, then sectioned at 50-100 μm on a freezing microtome, incubated in 0.05% 3,3' diaminobenzidine tetrahydrochloride containing 0.01% hydrogen peroxide (LaVail et al., '73) and counterstained with 0.25% thionin.
Fig. 1 Grain density counts over traverses of the SI cortex after injections of [3H]-proline and [3H]-leucine in VB and a survival of one day. Top: the pattern of labeling of thalamocortical fibers and terminals in a normal adult animal. Bottom: the grain distribution after a VB injection in an adult rat whose corpus callosum had been sectioned at birth (fig. 19). Note that the laminar distribution of grains is virtually identical in the normal and commissurotomized animals. Stipple, background labeling.
Effects of neonatal lesions on afferent fiber systems and on the SI cortex

In nine rats, a lesion of SI was made at one day of age, by removal of the pia mater. In three other 1-day-old rats, the corpus callosum was sectioned. After the animals had reached maturity (>4 months), an injection of \(^{13}\)H-leucine and proline was made into the thalamic ventrobasal complex (on the side opposite the cortical lesion or opposite the hemisphere retracted during the commissurotomy). Thereafter, the distribution of thalamocortical fibers and terminals was identified by autoradiography as described above.

In seven 1-day-old rats the thalamus was damaged unilaterally by passing a 25-gauge needle anteriorly in the horizontal plane through the superior colliculus. In five of these rats, after they reached maturity, a single injection of 1 \(\mu\)l of \(^{3}\)H-proline and leucine was made into the SI cortex on the side opposite the lesion. After a 2-day survival, the distribution of commissural fibers was studied autoradiographically in sections cut in the frontal plane at 20 \(\mu\)m. Brains from the other two thalamotomized rats were sectioned on a freezing microtome at 30 \(\mu\)m.

RESULTS

The first somatic sensory cortex (SI) of the rat consists of a molecular layer (layer I) and five cellular layers. Of these, the supragranular layers (layers II and III) often appear fused. Layers V and VI are the main source of efferent fibers (Wise and Jones, '77) and contain the largest pyramidal cells of the SI cortex. These are situated in the deep part of layer V. The granular layer, layer IV, consists of a number of densely packed aggregations of granule cells which include the barrel fields (Welker and Woolsey, '74) and which each contain the representation of a specific body part (Welker, '76). These aggregations are surrounded by zones of cortex in which layer IV is much less distinct. The laminar organization of the second somatic sensory cortex (SII) is similar to that of SI except that the infragranular layers are more prominent and the internal granular layer is nowhere as distinct as in the granule cell aggregates of SI.

Organization of the thalamocortical fibers

Injections of tritiated amino acids which heavily label cells in the ventrobasal complex (VB) lead to autoradiographic labeling, indicated by an increase in the density of silver grains over background levels, in both the SI and SII cortex. The laminar organization of thalamocortical fibers and terminals is identical in both areas.

After injections confined to VB, the labeling of the SI and SII cortex is most heavily concentrated at two distinct depths within the cortex (fig. 1). The major peak in grain density (fig. 1) is found over the internal granular layer. This band of labeling typically extends for up to 100 \(\mu\)m superficial to the upper boundary of layer IV and into layer III. Beneath this band of labeling a separate, smaller peak in grain density is found at the border of layers V and VI. Most of the grains are in the upper third of layer VI (fig. 1) but there is an extension into layer V which is most clearly seen in neonatal animals. The layers with the peaks in grain density probably represent those in which the majority of thalamocortical fibers terminate. Sparser accumulations of silver grains occupy large parts of the infragranular layers but these are interpreted as representing labeled thalamocortical fibers. Identical observations have been made using degeneration methods after lesions of the thalamic ventrobasal complex (figs. 2, 3).

The thalamocortical fibers emanating from VB are not distributed homogeneously throughout the SI cortex. First, there is a spatial organization such that only the parts of SI which contain the aggregations of granule cells (including the barrel fields of Welker and Woolsey, '74) receive thalamocortical fibers and terminals. The intervening and surrounding less granular zones generally have only background levels of labeling as seen in autoradiographic material (figs. 14, 15). Second, the thalamocortical fibers entering a large granule cell aggregate are organized in disjunctive, column-like clusters, each 250-450 \(\mu\)m in width. In parts of the ventrolateral aspect of SI these column-like clusters abut each other directly and the density of labeling is so high that each individual cluster can only be distinguished from its neighbor with difficulty. In the barrel fields, however, and also in the dorsomedial parts of SI, the distance between each of the thalamocortical fibers and terminal clusters is greater and the column-like bundles are discrete and separate (figs. 2, 3). Where the granule cell aggregates have within them distinct “barrels” (Welker and
Woolsey, '74; Welker, '76), with a central zone of low neuronal density and a peripheral zone of high neuronal density, the thalamocortical terminals are concentrated in the central zone (cf. Killackey, '73; Killackey and Leshin, '75; Caviness et al., '76). The column-like organization of thalamocortical fibers and terminals is most obvious in material stained for degeneration by the Wiitanen ('69) method after lesions of VB (figs. 2-5). With this method, the distinction between fibers and terminals is greater than in the autoradiographic material and the areas of high thalamocortical terminal concentration can be clearly observed.

The labeling of the SI thalamocortical system by autoradiography or degeneration shows that the thalamocortical terminal ramifications in both layers IV and VI conform to the column-like pattern and the labeling in the two layers is in register, i.e., a zone of increased density in layer VI lies immediately deep to one in layer IV (figs. 2, 3).

After injections of $^3$H-amino acids, which also spread to involve parts of the thalamus medial to VB, there is heavy labeling of the superficial half of the molecular layer of SI and SII. This band of labeling is never heavy when an injection is confined to VB (fig. 1). Labeling of the outer part of layer I is often observed in the absence of large peaks in grain density in the deeper, cellular layers. It appears therefore, that the thalamocortical fibers which are found in layer I arise from a part of the thalamus distinct from those which terminate in the other layers. No further identification of the source of these fibers could be made. Their developmental history will be considered with those arising from VB.

**Segregation of thalamocortical and commissural fibers**

It has already been demonstrated (Wise and Jones, '76) that commissural fibers entering the rat SI are distributed in a series of column-
Figs. 4, 5. Higher power dark- and brightfield photomicrographs of adjacent sections from the same brain from which figures 2 and 3 were taken. The same blood vessels are marked (X). The Wiitanen stained section (fig. 4) is slightly shrunken relative to the thionin stained section (fig. 5). Nevertheless, the three thalamocortical terminal clusters can be seen to be concentrated within the dense aggregates of layer IV granule cells. Figure 4 also shows part of the deeper, infragranular concentration of thalamocortical terminals. \( \times 60. \)

like bundles primarily to the relatively agranular zones of cortex lying between and around the granular cell aggregates. Within the column-like bundles, terminal ramifications are concentrated in two major zones: in layers I-III and V-VI. This columnar and laminar distribution has been confirmed in the present set of experiments. In addition, the experi-
ments in which commissural and thalamocortical fibers were labeled in the same experiment clearly indicate a segregation of the two fiber systems. Figure 6 shows clearly separate bundles of degenerating commissural and thalamocortical fibers that give rise to dense clusters of degenerating terminal ramifications that do not overlap to any extent.

**Development of the thalamocortical fibers**

At birth the cortical plate consists of two parts, a superficial, densely packed zone of highly immature and probably to some extent still migrating cells, and a deeper, less densely packed, and much thicker part in which layers V and VI are already distinguishable (the "subplate" of Rice and Van der Loos, '77). Unpublished experiments employing the retrograde transport of HRP in 1-day-old rats have demonstrated that the corticofugal fibers emanating from the SI cortex and projecting to brainstem sites and the spinal cord originate from neuronal somata confined to layer V (S. P. Wise, P. G. H. Clarke and E. G. Jones, unpublished data), as they do in adult rats (Wise and Jones, '77). This finding supports the designation of this lamina in the neonatal cortical plate, as layer V. The superficial, densely packed zone will be referred to as the upper cortical plate. Superficial to the upper cortical plate is the marginal zone or immature molecular layer. Deep to the cortical plate, as a whole, is the intermediate zone which consists mostly at this stage of the immature subcortical white matter.

For the purposes of describing fiber development, rats in their first postnatal day are termed 0 day old and those in their second day 1 day old. The recorded age of the animal, unless otherwise specified, is the age at perfusion. In all cases, preliminary data on the time course of thalamocortical fiber development based on age measurements starting simply at birth were verified with animals of known gestational age (starting at 21-22 days postcoitum). No differences in the pattern of thalamocortical development are observed between SI and SII, but the descriptions and illustrations will be of the development of these fibers in SI.

In rats in which VB and adjacent thalamic nuclei were injected at zero and 1 day old and which were killed within 12 to 24 hours, the pattern of thalamocortical labeling in SI is quite unlike that in the adult. Injections of the thalamus in these animals lead to a concentration of transported label over the outer part of layer VI and over the outer part of layer I but labeling over the intervening upper cortical plate does not greatly exceed background levels (figs. 7, 8, 16). The labeling of the subcortical white matter is very heavy relative to that of the gray matter (figs. 7, 8, 16), suggesting the presence of fibers which have not penetrated the cortex.

In 1- to 2-day-old rats killed 24 hours after injecting horseradish peroxidase (HRP) into the presumptive SI cortex and underlying white matter, there is heavy retrograde labeling of cells in the ipsilateral ventrobasal complex as well as in the central lateral nu-
Fig. 7, 8  Dark- and brightfield photomicrographs from a 20-μm thick autoradiographic section taken from an animal which was perfused at one day of age, 18 hours after an injection of \(^{3}H\)-proline and \(^{3}H\)-leucine into the thalamus. Accumulated labeling can be seen to be confined to the subcortical white matter and to layer VI of the developing cortex. Layer V is present but no granular or supragranular layers can be distinguished at this age (fig. 8). Arrow marks the pial surface (see fig. 16). Thionin counterstain. \(× 70\).
RAT THALAMOCORTICAL DEVELOPMENT

Fig. 9 Darkfield and brightfield (inset) photomicrographs from the same part of a 50-μm frontal section through the thalamus. The darkfield photomicrographs show neurons labeled by retrograde axonal transport of horseradish peroxidase. The animal was two days old when perfused, 24 hours after an injection of the enzyme into the presumptive SI cortex. Note the large number of retrogradely labeled thalamocortical neurons in VB and the central lateral nucleus (CL). The large number of labeled thalamic cell somata suggests that even at two days of age, most of the thalamocortical cells have fibers in the cortex or subcortical white matter. Note also the clustering of neurons in the arcuate nucleus of VB which projects to the barrel fields of SI. Thionin counter-stain. Figure 9, × 65; inset × 25.

Nucleus, both of which are recognizable at this age. Virtually every neuron in VB is found to be heavily labeled with granules of HRP reaction product (fig. 9) and clustered aggregations of cells are clearly seen in the arcuate nucleus (fig. 9). A similar pattern of labeling is observed in all animals injected with HRP at subsequent ages including adults.

Injections of [3H]-amino acids made into the thalamus late on the third day of postnatal life (2-day-old animals) and at the age of three days lead to a more adult like pattern of labeling in the cortex. In these cases, label in the presumptive SI is concentrated in three zones: in the upper part of layer VI with extension into the deep part of layer V; in the outer part of layer I; in the deepest aspect of the upper cortical plate (figs. 10, 11, 16). Since this part of the cortical plate contains, at this age, the cells which form layer IV in the mature animal (Berry and Rogers, '65; Rice and Van der Loos, '77), the thalamocortical fiber distribution in these 2- to 3-day-old animals has attained a pattern similar to that of the mature rat. There is little evidence, at this stage of development, for a disjunctive clustering of the thalamocortical fibers. In the subcortical white matter, labeling is heavy but is not much more intense than the labeling in the cortex; in contrast to the high ratio of subcortical to cortical labeling observed in younger animals. There is however, considerable labeling of the deep layers of the cortex which suggests the continued presence of fibers which have yet to reach their ultimate destination in more superficial parts of the cortex (fig. 16).

None of the dense aggregates of layer IV cells are readily distinguishable at three days of age. Layer IV itself is only barely discernible from the rest of the upper cortical plate at this stage. Between the ages of three and four days, however, the deepest part of the upper cortical plate matures into a clearly recognizable layer IV (discussion and fig. 13) and shortly thereafter, the aggregates of cells in
layer IV become separated from each other to form the pattern of aggregates (some of which contain barrels) seen in the adult (figs. 3, 5, 20).

The laminar distribution of thalamocortical fibers at four days old is virtually identical to that of the adult. After injections of [3H]-amino acids which involve VB at this age, the heaviest concentration of labeling is over layer IV, extending into the deepest part of a now recognizable layer III. Additional peaks of labeling occur over the outer part of layer I, and over the upper part of layer VI, extending into layer V in the zone beneath the layer of large pyramidal cells (figs. 12, 13). By the age of four days, the labeled thalamocortical fibers are clearly distributed only to the granule cell aggregates and now form disjunctive, column-like masses approximately 100-400 μm in width. The labeling of the cortex at short survival periods is now greater than that of the white matter and this pattern is found in adulthood.

At ages later than four days, the cortex, particularly the supragranular layers, continues to mature and the upper cortical plate becomes an obvious layer II. By six to seven days of age, the supragranular layers have begun to achieve an adult-like architecture, though initially they are still much thinner than in the adult. The distribution of thalamocortical fibers, as demonstrated by the density of labeling following injections of [3H]-amino acids into the thalamus, does not greatly change after this time (figs. 14-16). As noted above, however, it becomes possible to distinguish in the larger brain the differential thalamic projection to layer I from that to layers IV and VI. Moreover, the proportion of labeling in layer IV increases (fig. 16) during the first postnatal week.
Development of corticothalamic fibers

Injections of $[^3]H$-amino acids into the cortex of 0-day-old animals perfused at one day of age, show that fibers, at this early stage, have reached the ventrobasal (fig. 17), posterior, reticular, central lateral and other nuclear complexes in the thalamus. In comparison with similar experiments conducted in adult animals, the density of labeled corticothalamic fiber ramifications in the thalamic neuropil of the neonate does not appear to be as great. Conversely, a relatively larger proportion of labeling is observed over fiber bundles within the external medullary lamina and in the ventrobasal complex than in the adult. The distribution of the corticothalamic labeling changes little over the course of post-natal development and the relative proportions of fiber and neuropil labeling assume their adult pattern within the first two or three days. A more complete description of the distribution of corticothalamic fibers emanating from the SI of the mature rat has been published elsewhere (Wise and Jones, '77).

Influence of thalamocortical and commissural fibers on cortical cytoarchitecture

In the brains of adult animals, thalamotomized at birth, some aspects of cortical cytoarchitecture in SI, such as the dense, granule cell aggregates are easily seen, especially in the 30-$\mu$m thick sections.

In a typical case most of the ventrobasal
complex and many other thalamic nuclei are clearly absent on the operated side. In the case illustrated (fig. 18), only the most dorsolateral aspect of VB, a large part of the lateral posterior nucleus, and a few of the midline nuclei, are present on the affected side, while the contralateral thalamus is largely unaffected. Incidental damage includes significant destruction of the striatum, dilation of the lateral ventricle with displacement of the hippocampal formation, and a nearly complete section of the internal capsule. The latter is reflected in a considerable reduction in the size of the cerebral peduncle and pyramidal tract on the side ipsilateral to the lesion (fig. 21). In the cortex there is shrinkage of the layer V pyramidal cells in all areas, particularly the sensory and motor. In those areas of SI topographically related to the lesioned part of VB, the total combined thickness of the supergranular and granular layers is significantly reduced (13%; p < 0.001, Kolmogorov-Smirnov test) and the layers appear thinner and more palely staining upon qualitative examination (figs. 20, 21).

The aggregates of layer IV granule cells on the lesioned side of the brain, do not appear as dense as on the contralateral (normal) side. However, they are roughly equivalent in position to the normal, their horizontal extent is the same and they are separated from each other by relatively less granular cortex with about the same spacing as normal. Subsidiary cytoarchitectural features of the layer IV aggregates, on the lesioned side, particularly the barrels with clear centers, are not seen, though they are obvious in the control hemisphere (figs. 20, 21). In the case illustrated, a
few barrels are observed in the caudal aspects of SI on the lesioned side, in a part of the cortex where the reduction of thickness in layer IV is also minimal. The preservation of this part of SI is considered to be due to the maintenance of some normal thalamic connections with the intact dorsolateral part of VB (Davidson, '65; Emmers, '65).

In other cases with thalamic lesions in which all or part of VB had been destroyed at one day of age, the same observations were made. In each case, the part of SI topographically related to the part of VB removed has attenuated; granule cell aggregates are present but no barrels appear within them. In all cases, the contralateral, control hemisphere shows dense granule cell aggregates of normal thickness and with a normal complement of barrels.

In complementary experiments, the corpus callosum was sectioned at zero or one day of age and the cortex subsequently examined at maturity (fig. 19). In these cases there is incidental damage to the cortex of one and sometimes both sides, but in all cases the dense, granule cell aggregates and the barrels within those that normally contain them, appear unaffected, in spite of the failure of the com-
Fig. 17 Darkfield and brightfield (inset) photomicrographs taken from a 20-μm thick frontal section of the thalamus of a rat perfused at 1 day old 12 hours after an injection of [3H]-amino acids in the presumptive SI cortex. This experiment demonstrates that before the thalamocortical fibers have established a mature organizational pattern (fig. 16), corticothalamic fibers, as demonstrated by transported label, are present in the ventrobasal complex. Thionin counterstain. Figure 17, × 70; inset, × 25.

missural fibers to penetrate the intervening less granular areas.

Connectional specificity of thalamocortical and commissural fibers

Several experiments were performed to evaluate the potential of either commissural or thalamocortical fibers to form aberrant connections following the destruction of the other set of fibers early in development.

In mature animals in which the commissural system had been removed by hemidecortication or commissurotomy (fig. 19) at zero or one day of age, the distribution of thalamocortical fibers was demonstrated by injection of [3H]-proline and [3H]-leucine into VB. In all of these cases, the distribution of thalamocortical fibers in SI and SII was identical to that seen in normal, mature animals (fig. 1). After injections of VB and adjacent nuclei, the labeling is concentrated in the outer part of layer VI extending into layer V, in layer IV extending into layer III, and in the superficial part of layer I. The labeling of thalamocortical fibers remains restricted to those areas of cortex containing the dense aggregates of granule cells, as in normal animals, even though the commissural fibers have never entered the cortex and are absent from the adjacent, less granular zones of SI.

In parallel experiments, a thalamotomy at one day of age (fig. 18) was followed by investigation of the distribution of commissural fibers and terminals in the mature animal. Here, also no discrepancy could be detected between the pattern of organization of the commissural fibers in comparison with normal rats. The commissural fibers, in the experimental cases in which VB and other thalamic nuclei had been destroyed terminate in discrete patches in the relatively agranular parts of the SI cortex, between and surrounding the dense, granule cell aggregates. The patches are 200-300 μm in width and labeled fibers and terminals are concentrated primarily in the supragranular layers (fig. 22).
Fig. 18  Photomicrograph of a 30-μm thick frontal section of the brain of an adult rat that sustained a unilateral thalamotomy (right) at birth. Only a small portion of the lateral thalamic complex remains intact on the lesioned side. Frozen section, thionin stain. ×10.

Fig. 19  Photomicrograph of a 20 μm thick, frontal section of the brain of an adult rat whose corpus callosum had been sectioned at birth. The right hemisphere has been damaged by retraction during surgery. Injections of tritiated amino acids in the thalamus of the undamaged side reveal no abnormalities of the thalamocortical fiber distribution (fig. 1). Paraffin section, thionin stain. ×10.

DISCUSSION

Laminar organization

The terminal ramifications of thalamocortical fibers in the somatic sensory cortex of the rat are concentrated at three depths: (i) at the junction of layers V and VI, mostly in layer VI, (ii) in layer IV with an extension into the deepest part of layer III; (iii) in the superficial part of layer I. These findings are comparable to those that have recently been reported with autoradiographic and electron microscopic studies in other species and in other areas of the rat cortex (Dräger, '74; Strick and Sterling, '74; Rosenquist et al., '74; Benevento and Rezak, '75; Jones, '75a; Ribak and Peters, '75;
Figs. 20, 21 Photomicrographs taken from the two sides of a frontal section of the brain of an adult rat which had sustained a thalamotomy at birth (fig. 18). The SI cortex on the normal side (fig. 20) shows some of the dense aggregates of granule cells in layer IV. The edges of these aggregates are marked by thin arrows. Within one of the aggregates, "barrels" can be seen (open arrows). The SI cortex from the lesioned side (fig. 21) is significantly reduced in thickness, and the cells of the infragranular layers are markedly shrunken but the aggregations of layer IV granule cells, the edges of which are marked by thin arrows, remain. However, no barrels are present in the lower aggregate corresponding to that shown in figure 20. Thirty-micron thick frozen sections, thionin stain. × 40.

Robson and Hall, '75; Wise, '75; Jones and Burton, '76; LeVay and Gilbert, '76; Peters and Feldman, '76; Rakic, '76, '77). The observation that a thalamic projection to the superficial part of layer I arises from a nucleus of the thalamus other than VB, accords well with previous investigations of thalamic inputs to this layer (Benevento and Rezak, '75; Jones, '75a; Jones and Burton, '76). The identification of the cells of origin of this projection remains uncertain. The likely origin seems to be in the intralaminar nuclei (Jones and Leavitt, '74) but other nuclei such as the ventromedial complex (Herkenham, '76) are also possible.

Topographic and spatial organization

The terminal ramifications of thalamocortical fibers in the mature rat SI are restricted to those zones of cortex which contain the dense granule cell aggregates described by Welker ('76) and Welker and Woolsey ('74). This find-
ing has been reported previously in abstract form by Ryugo and Killackey ('75; see also Killackey and Belford, '76). The commissural fibers do not penetrate the granule cell aggregates (Ryugo and Killackey, '75; Wise and Jones, '76), and as shown in the present study, there appears to be little or no spatial overlap between the two afferent systems. In the rat the relatively agranular zones also contain the cells that give rise to the commissural fibers (Wise and Jones, '76; see also York and Caviness, '75, in the mouse). Therefore, there appears to be a complete segregation of thalamic and commissurally related cortex. Whether the relatively agranular areas of SI between and surrounding the granule cell aggregates are truly "thalamic" or receive thalamic input from some as yet unidentified thalamic nucleus is not known but according to Welker ('76), the less granular zones between the aggregates cannot be activated by stimulation of the periphery in deeply anesthetized animals. No similar "thalamic" areas seem to be present in the SI cortex of cats or monkeys. In these species, the distribution of bundles of commissural fibers is also disjunctive but where commissural bundles are present they overlap bundles of thalamocortical fibers to a considerable extent (Jones et al., '75; Wise and Jones, unpublished observations).

**Columnar organization**

In the sensory areas of the cortex, the similarities in receptive field and submodality properties of cells in vertically oriented columns extending through all layers are thought to be based in the first instance upon the thalamocortical input (Mountcastle, '57; Powell and Mountcastle, '59; Hubel and Wiesel, '62, '72, '74a,b; Abeles and Goldstein, '70; Wiesel et al., '74; Jones, '75a,b; LeVay et al., '75). Columns of this type have been found to have a discrete cylindrical or slab-like organization. They measure about 500 µm in width in primates and are somewhat narrower in rats (Armstrong-James, '75; Wise and Jones, '76) though the total width of the terminal arborization of single thalamocortical fibers as demonstrated by the Golgi technique is similar in both rodents (Lorente de Nö, '49) and monkeys (Jones, '75a). In the present study, it has been possible to demonstrate the same type of column-like organization with axonal degeneration and autoradiographic techniques. The 250-450 µm wide clusters of thalamocortical ramifications in layer IV, seem to represent the terminations of bundles containing many fibers. As in the layer IV clusters, in the infragranular layers there is a similar but less dense, secondary patch of apparent thalamocortical terminations. This cluster has similar dimensions and is in register with a cluster in the overlying layer IV.

The column-like clusters of thalamocortical fibers with their bilaminar termination are found in relation to each of the large aggregations of granule cells that make up layer IV. They are most discrete and most clearly separated from one another in the aggregations which represent the mystacial and other facial vibrissae (see also Killackey and Leshin, '75; Caviness et al., '76) and in the dorsomedial cortex which represents limbs and trunk. Each cluster is interpreted as forming the basis of an electrophysiologically defined column (see also Axelrad et al., '76; Durham and Woolsey, '77).

**Development**

At about the time of birth, thalamocortical fibers have penetrated the somatic sensory cortex and accumulate in the upper part of layer VI. At this time, corticothalamic fibers which in the adult arise from layers V and VI (Wise and Jones, '77) have already entered the ventrobasal complex (see also Wise et al., '77 in the cat). At present, it is uncertain whether the peak of thalamocortical labeling seen in layer VI in our experiments at these earliest stages represent fibers terminating there, or growing fibers which ultimately will terminate in more superficial laminae. The heavy concentration of label in the white matter beneath SI suggests the presence of many growing fibers that have not yet entered the cortex. Although the retrograde labeling experiments would imply that the axons of all the thalamocortical relay neurons in VB have already reached this general region by the time of birth, no evidence has been obtained in the present study to show whether they accumulate there over a long period of time before invading the cortex. A relatively protracted "waiting period" of this type has been demonstrated in thalamocortical fiber development in the striate cortex of the rat (Lund and Mustari, '77) and monkey (Rakic, '76, '77), in the development of the commissural system of the rat SI cortex (Wise and Jones, '76); in the development of thalamocortical fibers of the cat sensory motor cortex (Wise et al., '77); and in the development of retinotectal fibers in
the chick (Crossland et al., '74). It is likely therefore, that such a stage occurs in the rat SI thalamocortical system, and that it does so prenatally.

The thalamocortical fibers grow into the upper cortical plate between the ages of two and three postnatal days. This timing seems to coincide with the first appearance of cortical evoked potentials in response to peripheral or thalamic stimulation (Verley and Axelrad, '75) and with the appearance of histochemically demonstrable succinate dehydrogenase activity (Killackey and Belford, '76). It is not yet known, however, whether synapses are already formed at this stage. In the visual cortex of the rat (Lund and Mustari, '77), synapses start to form as soon as the thalamic fibers begin to enter the cortex.

The invading thalamocortical fibers seem to accumulate only in the deepest part of the upper cortical plate and at no stage are they distributed uniformly throughout the upper cortical plate. Berry and Rogers ('65) have shown that neurons “born” on the eighteenth day of gestation are segregated in the deepest aspect of the upper cortical plate by two to three days of age (24 days post-conception). Here, they will form layer IV of the mature cortex. This suggests that the cells upon which the arriving thalamocortical fibers will terminate aggregate first in layer IV and that the fibers grow directly to meet them. There is no clear evidence of an initial diffuseness of laminar organization followed by reorganization, even though the deep part of the upper cortical plate does not become obvious as layer IV until about a day after the arrival of the fibers. Thereafter, layer IV becomes discontinuous and forms the dense, granule cell aggregates typical of the adult internal granular layer (at 4 days old).

The column-like pattern of thalamocortical fiber distribution is apparently established very early in development for it can be clearly observed in 4-day-old animals. The early development of a columnar pattern is comparable to the development of the precursors of ocular dominance columns in the monkey striate cortex (Rakic, '76, '77) before birth and possibly to the disjunctive patches of corticocortical fibers seen in the frontal cortex of immature monkeys (Goldman and Nauta, '77). Physiological studies have shown that the columnar properties of the rat SI cortex are established by six days of age, but no data are available on younger animals (Armstrong-James, '75). It is interesting that the small aggregations of VB cells demonstrated with the HRP method, (similar to the “barreloids” of Van der Loos, '76) and which may form the basis of the clustering of the thalamocortical fibers, are present at or shortly after birth (fig. 9). This finding would seem to imply that the secondary aggregation of the fibers in the cortex could be to some extent affected by extrinsic factors such as bundling of incoming fibers, and not simply by factors within the maturing cortex itself.

Influence of afferent fiber systems on cortical cytoarchitecture

Removal of the thalamic afferents to the cortex before they penetrate into the developing granular layer prevents the formation of the barrels, but not of the layer IV granule cell aggregates in which the barrels normally lie. The aggregates, though reduced in thickness, acquire in the absence of thalamic afferents, a similar horizontal extent to that seen in the normal rat. These findings are consistent with the hypothesis that the thalamocortical fibers and terminals interact during development with the immature neurons of the cortex, particularly those of layer IV, and are necessary for the formation of the definitive barrels. The findings of several other studies are consistent with this view. Subsequent to removal of vibrissae in neonatal mice (Van der Loos and Woolsey, '73; Weller and Johnson, '75) and rats (Killackey et al., '76) the barrels related to those vibrissae fail to appear in their definitive form. The effect of removal of a vibrissa on the ultimate appearance of a barrel is greatest in very young animals and becomes less with age (Woolsey and Wann, '76). On the fifth postnatal day, when the barrels first become evident in the mouse (Rice and Van der Loos, '77), removal of vibrissae is almost ineffective in altering cortical architecture. Thus, the presence of thalamocortical fibers seems to be unnecessary for the formation of basic cortical lamination and cytoarchitecture, as reflected in the large granule cell aggregates, but the establishment of thalamocortical connections is vital for the appearance of those special subsidiary units of cortical cytoarchitecture that are unique to rodents and a few species belonging to other orders.

Connectional specificity

The time course of thalamocortical fiber de-
development in the rat SI cortex shows that these fibers have established an adult-like pattern in the highly immature cortex. They have also established this pattern before the fibers of the commissural system penetrate the cortical plate in large numbers. The commissural fibers enter the cortex at five days of age and only adopt a relatively mature pattern of organization by seven days of age (Wise and Jones, '76; see also Mares et al., '75).

The two fiber systems not only have a different time course of postnatal development but are also distributed largely to different zones and laminae in SI. It was therefore of interest to determine whether the laminar or spatial organization of either of these fiber systems could be modified by removal of the other system early in postnatal life. Somewhat surprisingly, no experimental manipulation resulted in the formation of aberrant connections by either fiber system. The fibers grow specifically into those zones and laminae of the SI cortex in which they are normally found. These neocortical fiber systems are, therefore, clearly different from comparable systems in the allocortex. In the dentate gyrus similar experiments have shown that commissural fibers have the potential to sprout and occupy laminae and synaptic sites denervated by removal of other afferent fibers normally emanating from the entorhinal cortex (Lynch, Mosko, Parks and Cotman, '73; Lynch and Cotman, '75). Similar examples of sprouting of one afferent fiber system into a deafferented zone have been reported for other connections of the hippocampal formation (Lynch and Cotman, '75; Zimmer, '76).

Although it probably does not occur in all systems, neuroplasticity of this type has been demonstrated in many other sites (Bernstein and Goodman, '73; Kerr, '75). Generally, it has been found that developing or immature neuronal systems are more "plastic" and therefore form aberrant connections more readily than mature systems after experimental manipulation (Lynch, Stanfield and Cotman, '73; Lund and Miller, '75; Leong and Lund, '73). We have not been able to obtain evidence that this occurs in the neocortex. Also of relevance to our findings is the evidence that different fiber systems projecting into the same terminal region compete for available synaptic sites in a manner which gives the earliest arriving fibers an advantage (Gottlieb and Cowan, '72). Therefore, the finding that the thalamocortical and commissural systems tend to be dissociated and innervate adjacent patches and different laminae of the SI cortex suggested the possibility that in the absence of competition from one set of fibers the remaining system might grow into the inappropriate zones and/or laminae. This occurred in neither of the two types of experiments carried out.

One explanation for the failure of thalamic fibers to sprout into the agranular zones and the laminae normally occupied by commissural fibers is that the thalamic fibers clearly arrive in advance of the commissural fibers (Wise and Jones, '76). They may, therefore, have irreversibly established their appropriate number of synapses before synaptic sites become available in the zones and laminae that are to become commissurally connected, or may have been prevented from doing so by a third competing system. The possibility that regrowth of the commissural fibers (through some route other than the corpus callosum), prevents thalamocortical fibers sprouting, has been ruled out by autoradiographic experiments on split-brain rats in which no labeled fibers could be traced to the opposite side after injections of SI (unpublished results).

In the case of the later developing commissural system, removal of thalamic fibers before they establish connections, should leave potential thalamocortical synaptic sites vacant, though it is possible that in the two to three day lag between the arrival of the two systems, thalamocortical synaptic sites, not colonized by thalamic terminals may have failed to appear, appeared and disappeared, or become occupied by a third fiber system. The third "competing system" might be derived from an axonal system intrinsic to the cortex. However, the developmental history and even the mature organization of associational fiber systems in the rat SI cortex is scarcely known (but see Ryugo and Killackey, '75).

It is also possible, in the rat, that the afferent fibers are restricted to a radial orientation, and that limitations on their horizontal growth prevents them from invading cortical zones in which they are not normally found. In the cat SI cortex, where, in the commissurally connected parts of the representation, thalamocortical and commissural fiber bundles are not dissociated from one another but overlap considerably (Wise and Jones, unpublished), neonatal removal of the commissural system does not result in any alteration in the
laminar distribution of thalamocortical fibers and terminals (Wise, '76). In this case radial sprouting of thalamic fibers clearly does not occur. This, together with the work on the rat, would seem to imply that the thalamocortical and commissural fiber systems each form connections with specific classes of cells (or specific parts of cells) in a particular layer of the cortex and that they are not able to form aberrant connections on inappropriate cells or in inappropriate parts of cells.

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