Axonal Guidance During Development of the Great Cerebral Commissures: Descriptive and Experimental Studies, in Vivo, on the Role of Preformed Glial Pathways

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Abstract:

Do structures exist within the embryonic central nervous system that guide axons across the midline during development of the great cerebral commissures (corpus callosum, anterior commissure)? With the use of serial section and reconstructive computer graphic techniques we have found that during normal ontogeny of the mouse forebrain and before the arrival of the pioneer fibers of the corpus callosum at the midline, a population of primitive glial cells migrates medially (through the fused walls of the dorsal septum) from the ependymal zones of each hemisphere. At the midline, and well rostral to the lamina terminalis, these cells unite to form a bridgelike structure or "sling" suspended below the longitudinal cerebral fissure. The first callosal axons grow along the surface of this cellular bridge as they travel toward the contralateral side of the brain. The "sling" disappears neonatally. The fibers of the anterior commissure grow within the lamina terminalis along a different type of preformed glial structure. Movement of these axons occurs through an aligned system of glial processes separated by wide extracellular spaces.

Do these transient glial tissues actually provide guidance cues to the commissural axons? Analyses of three situations in which the glial "sling" is genetically or surgically impaired or nonexistent indicate that this structure does, indeed, play an essential role in the development of the corpus callosum. We have analyzed (1) the embryonic stages of a congenitally acallosal mouse mutant (strain BALB/cCF), (2) several pouch stages of a primitive acallosal marsupial, *Didelphys virginiana* (opossum), and (3) animals in which the "sling" had been lesioned surgically through the uterine wall in the normal embryo (strain C57BL/6J).

In the acallosal mouse mutant fusion of the septal midline is delayed by about 72 hours and the "sling" does not form. Although the would-be callosal axons approach the midline on schedule, they do not cross. Instead, the callosal fibers whirl into a pair of large neuromas adjacent to the longitudinal fissure. Similarly, in the opossum, fusion of the medial septal walls and formation of the glial "sling" are also lacking. However, in this species, instead of traveling dorsally, the "callosal" axons turn ventrally and pass contralaterally by way of the anterior commissure pathway. Surgical disunion of the glial "sling" also resulted in acallosal individuals. The callosal pathology in these affected animals mimicked exactly that of the genetically lesioned mutant. Our observations suggest that many different types of oriented glial tissues exist within the embryonic neural anlage. We propose that such tissues have the ability to influence the directionality of axonal movements and, thereby, play a crucial role in establishing orderly fiber projections within the developing central nervous system.

Article:

Within recent years it has become apparent that particular types of radially organized, astrocytic glial cells (e.g., Bergmann glia in cerebellum, Muller cells in retina, radial glia in cortex) may function during early brain development to direct the orderly migrations of neuronal cell bodies away from the ependymal surface (Rakic, '71; Rakic and Sidman, '73; Sidman and Rakic, '73; Sotelo and Changeux, '74; Sidman and Wessels, '75; Levitt et

al., '81; Sommer et al., '81). A larger stumbling block in the path of our understanding the mechanisms that organize neuron networks, in vivo, has been the elucidation of the role, if any, of glia in the guidance of axons. It seems paradoxical that these far-ranging neurites often move in a direction orthogonal to their own migrating somata and, thus, at an orientation different from that of the glial terrain through which they must grow (Ramon y Cajal, '60; Molliver et al., '73; Shoukimas and Hinds, '78).

Our interest in the relationship between developing axons, especially the pioneering fibers, and their glial environment stems from several previous studies of the mechanisms that guide ganglion cell axons in the embryonic retina and optic nerve (Von Szily, '12; Silver and Robb, '79; Silver and Sidman, '80; Silver and Sapiro, '81; Krayanek and Goldberg, '81) and descending fiber tracts of the developing tail cord (Egar and Singer, '72; Singer et al., '79). In these regions (where the movement of axons is mostly unidirectional and confined within the marginal zone of the early neural rudiment) the preaxonal, germinal neuroepithelium (primitive ependyma) generates within it highly localized pathways of matrix-filled, extracellular spaces oriented largely perpendicular to their surrounding membranes but in the same direction and position as that of eventual nerve fiber outgrowth. That the ocular conduits or their contents play a role in guiding axons out of the globe is suggested from studies of an optic nerve aplastic mutant mouse (ocular retardation, or^{J}). In this strain the extracellular spaces are genetically eliminated during preaxonal stages and although in the mutant optic nerve fibers are generated intraocularly, they become entrapped within the eye and eventually degenerate (Silver and Robb, '79).

The question which we now ask is: Do similar or some other, as yet undiscovered, glial structures exist that can direct the growth of an apparently more complicated, bidirectionally moving system of axons? In most mammals, the dorsal corpus callosum and pars posterior of the anterior commissure comprise a continuous and rather massive beltway of cortical commissural axons located in a submarginal position around much of the forebrain (Sidman et al., '71; pgs. 29-35). Callosal axons from the two hemispheres not only have to find their way dorsally to the cerebral midline, they must also pass one another and cross into opposite cortices at levels once occupied by the interhemispheric fissure (a chasm which completely separates the paired telencephalic vesicles during early stages of embryonic development). Axons destined for the anterior commissure turn in a reverse direction to those of the corpus callosum. They first move ventrally through the external capsules and proceed contralaterally within a discrete bundle located immediately above the preoptic recess. Indeed, for these two grandest of forebrain pathways the problem of axonal guidance seems appropriately confounded.

We have adopted five courses of study in our attempt to understand the guidance mechanisms (especially those at the midline) that direct the commissural axons along their stereotyped routes within the brain. First, our analysis began with observations of precisely where and when the pioneering fibers of the various aforementioned pathways emerged. Second, we carefully examined the youngest embryos (i.e., those at preaxonal stages) to determine the nature and configuration of the cells located along the prospective terrain through which the first axons eventually grew. Third, we made surgical intrauterine lesions of the presumptive callosal pathway in the normal mouse embryo (C57BL/6J). Fourth, the developmental stages of a congenitally acallosal mouse mutant (BALB/cCF) were analyzed to see if discrete malformations occurred in the regular development of the precallosal environment in advance of the first morphological signs of the axonal abnormality. This hereditary defect is unusual and of considerable interest because in the acallosal individual the full complement of "callosal" axons is actually retained even though the fibers have failed to cross into opposite hemispheres. Instead, the would-be callosum persists indefinitely as two, immense, longitudinally directed bundles alongside the medial walls of each hemisphere (Probst, '01; King and Keeler, '32; King, '36; Loeser and Alvord, '68; Bossy, '70; Ettlinger et al., '72; Noel, '73; Shapira, '74; Dennis, '76; Gott and Saul, '78). Until now the detailed embryogenesis of this anomaly, which is also present in humans (Warkany, '71), has gone uncharted. Last, and for reasons of gathering comparative observations that might provide further insights into the developmental scenario of the corpus callosum, we examined several early pouch stages of a normally acallosal marsupial, *Didelphys virginiana* (the North American opossum). Preliminary reports of these studies have been presented (Silver, '80, '81).

MATERIALS AND METHODS

Timed pregnant C57BL/6J mice were obtained from the Jackson Labs, Bar Harbor, Maine. Staged fetuses of the inbred strain BALB/cCF (which suffers hereditary agenesis of the corpus callosum; Wahlsten, '74) were collected and fixed by D. Wahlsten, then shipped to Cleveland for analysis. The opossum material was taken from a series of previously prepared slides that were present in the collection of the late Arthur F. Hughes, Anatomy Dept., CWRU. Pregnant mice were isolated for varying lengths of time and then killed by an overdose of pentobarbital (BALBc mothers) or by cervical dislocation (C57 mothers). The embryos were quickly removed from the uterus in phosphate buffer at 37°C and then fixed in a combined 0.5% glutaraldehyde/2.0% formaldehyde solution in the same buffer overnight at 4°C (Yanoff, '73). Some of the normal and lesioned C57BL/6J embryos, the BALB/cCF embryos, and the opossum pouch young were embedded in paraffin, sectioned at 10 μm, and stained with hematoxylin. Some of the lesioned embryos and controls were immersed in 5% acetic acid-5% formaldehyde-70% ethanol fixative for 3 days, embedded in paraffin, sectioned at 10 μm, and silver stained using the protocol of Rager et al. ('79). In other normal and lesioned C57BL/6J embryos the brains were dissected free of their calvaria, sliced into slabs (coronally) embedded in Spurr's plastic and then processed for electron microscopy using standard procedures. Other plastic-embedded material was sectioned *serially* at 1 μm and stained with toluidine blue. Neonatal animals were decapitated with the use of a razor blade; the brains were quickly dissected from their cranial vaults and immersed in the various fixatives.

Techniques for intrauterine lesion of the "sling"

In order to make accurate intrauterine lesions, we first determined by dissection of freshly killed fetuses and also by histology that the "sling" (a glial guidance pathway for the corpus callosum, see Results) lies midway between two easily recognizable surface landmarks: (1) the sinus confluens and (2) the base of the olfactory bulb. Prior to surgery the timed pregnant mothers were anesthetized with ether, turned in the supine position, and pinned by the skin of the feet to a cork board. With the use of a Zeiss operating microscope and with the aid of an American Optical fiber optic illuminator, a small (8-11 mm) midline incision was made through the lower abdominal cavity. Care was taken not to injure the bladder.

Two micro—tissue forceps were then inserted through the incision and by gently grasping the uterine musculature, two or three fetuses were pulled through the opening. A selected fetus was then grasped loosely (between the operator's thumb and forefinger). The embryo was prodded with the blunt end of a micropipette (the other end of which had been pulled into a fine microneedle) until the surface of the head rotated upward. The dural sinuses and olfactory bulb were easily seen through all intervening membranes. After the fetus moved into the proper position the head was held a bit more firmly until the cranium and surrounding amnion were fixed against the uterine wall. The microneedle was then inserted through the uterus and calvarium into the longitudinal cerebral fissure (at a position midway between the two surface landmarks) to a depth of about 1.5 to 2 mm. Using the surface of the cranium as a fulcrum and with a caudally directed arcing motion of the handle, the point of the needle was first brought forward and upward (thereby cutting all structures in its path) and then pulled free of the brain through the original point of entrance. This technique greatly minimized bleeding and loss of amniotic fluid. The fetus was then reinserted into the body cavity, an additional length of uterus was exposed and the operation was repeated until all embryos (or as many as possible) received a lesion. The incision in the mother was closed with surgical silk. The embryos were then allowed to survive for varying lengths of time in utero and after partuition. The mothers and embryos (or neonates) were sacrificed by the procedures described above. We have found that this type of operation is most feasible in the mouse after about embryonic day 14. Operations earlier than this often resulted in a rapid (overnight) resorption of the fetus.

Techniques for three-dimensional reconstruction

For the three-dimensional reconstructions, complete sequences of 10 μm thick, hematoxylin-stained paraffin sections or serial 1 μm thick, toluidine-blue-stained plastic sections, passing coronally through the forebrain were examined or photographed with the use of a Zeiss microscope equipped with planapochromatic objectives. The serial negatives were realigned and rephotographed as sequential frames of high-resolution 35-mm motion picture film by best-fit procedures utilizing a tine reconstruction device designed by Ware and Lopresti ('75) and built by Mekel Engineering Co., Covina, CA. For reassembly and display of stacked contours successive

frames were projected individually on a Vanguard motion analyzer, equipped with a Numonics electronic digitizer. Individual boundaries of the various selected structures were traced manually. A D.E.C. 1134 computer and Megatek Whizzard 7000 graphics terminal were used to analyze and reconstruct the three-dimensional image. Current software routines for our system, originally designed by Dr. Randy Ware and modified extensively by Julius P. Neumann and Gary Goins, allow up to approximately 12,000 vectors to be displayed with any angle of rotation, phantom overlay, hidden line suppression and line thickness weighting to provide depth cueing. Final output is photographed directly from the cathode ray tube.

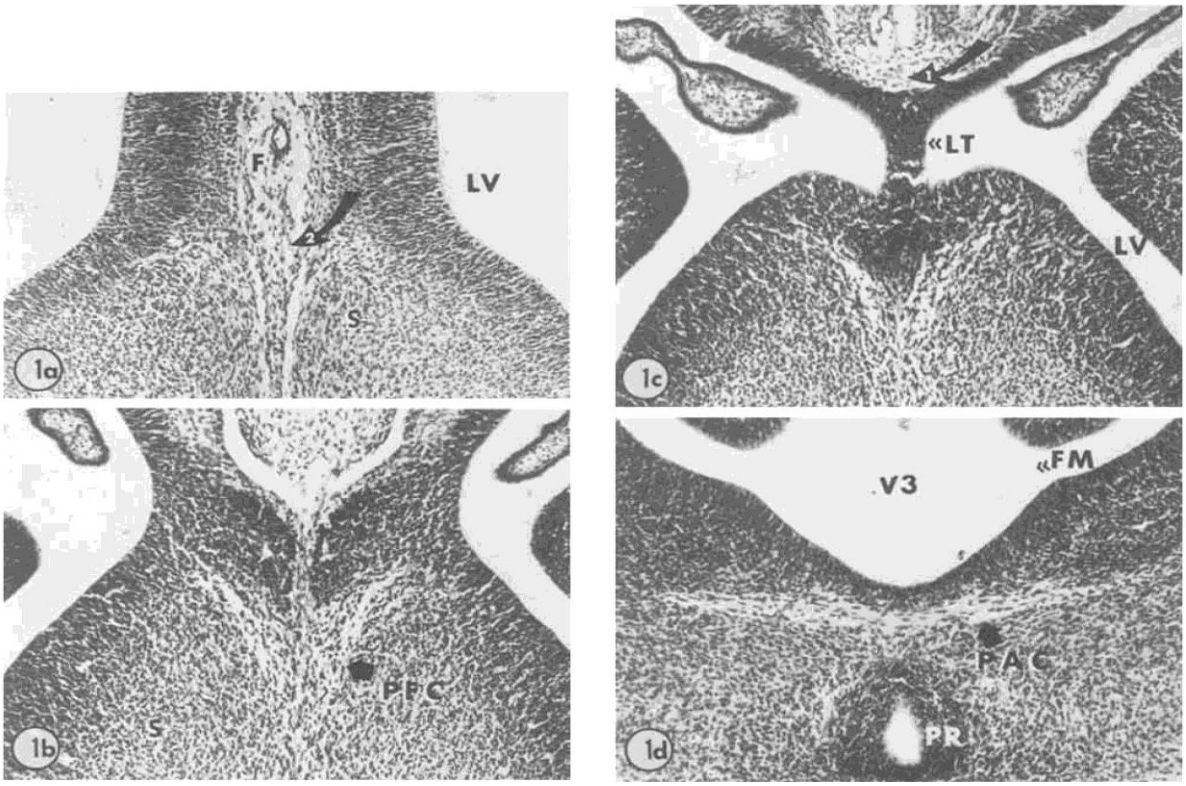


Fig. 1. ***Coronal sections (c) through the lamina terminalis (LT) (b) 50 am rostra! (a), 200 am rostra] and (d) 60 am caudal to the ventricular surface of the lamina terminalis of a 13.5-day mouse embryo (C57BL/6J). Note the cell nuclei sparse zones that represent the preformed pathways for the anterior commissure (PAC in d) and fomix columns (PFC in b and c). Note in a that the medial walls of the presumptive septal region are unfused rostral to the lamina terminalis. Arrows 1 and 2 indicate proposed routes for the pioneer callosal axons (see text). Lateral ventricle, LV; third ventricle, V3; septum, S; interhemispheric fissure, F; foramen of Monro, FM; preoptic recess, Pit. X 160.***

RESULTS

Descriptions of normal development

Embryonic (E) days 13 and 14. The morphology of the early developing mouse forebrain (E13.5-14.0) at levels taken through, rostral and caudal to the lamina terminalis is shown in Figures 1 and 14. Note that we rather rigidly define the lamina terminalis as the most forward wall of ventricle III, directly at its junction with the paired foramina of Monro (Fig. 1c). Even at this primitive, preaxonal stage (the presence of axons was verified by E.M.), there is already evidence in the septum (the area lying below and in front of the lamina terminalis) of two distinct, oriented arrays of glial cell processes forming wide extra-cellular spaces. The most striking features of these regions are that (1) they contain very few cell nuclei, (2) they contain large numbers of degenerating cells, and (3) they occupy midline portions of the presumptive routes of two distinct fiber systems, the anterior commissure (Fig. 1d) and fornix columns (Fig. lb,c). The spatial relationship between these prospective axon tracts and their preformed pathways can be demonstrated with confidence when one compares sections through similar levels at stages after the axons have arrived midsagittally (see below). For the sake of such comparisons the position of the preoptic recess (an evagination of the lower rostral wall of the third ventricle, and considered by some to be part of the lamina terminalis; Johnston, '13) is a particularly useful land-

mark since it forms very early on (E11-12) and comes to lie immediately subjacent to the presumptive anterior commissure (Fig. 1d) (Silver, '77). These two structures then remain together throughout ontogeny. The lamina terminalis proper (as defined here) is also positionally stable (relative to the callosum) and, after it appears (E10), can be easily recognized at any stage pre- or postnatally at the upper rostral end of ventricle III.

Prior to and during the early hours of E 14 the interhemispheric fissure completely separates the paired cerebral vesicles at all levels rostral to the lamina terminalis (Figs. 1a,b, 14). Thus, at these earliest of stages, there is no evidence in the tissue of any continuous interhemispheric route for the pioneer callosal axons except via the lamina terminalis itself.

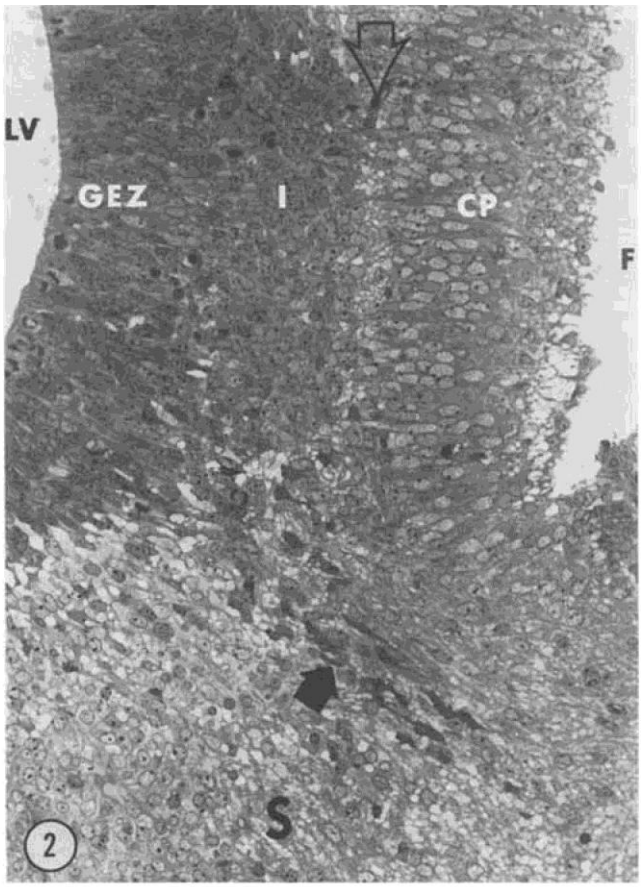


Fig. 2. A coronal section taken about 200 μm rostral to the ventricular surface of the lamina terminalis in a 15.5-day control embryo (C57BL/6J). The medial septal walls (S) have fused. Glial precursors (darkly stained cells, solid arrow) can be seen migrating away from the germinal ependymal zone (GEZ) toward and below the longitudinal cerebral fissure (F). Also note the aligned interface (open arrow) between nuclei of the cortical plate (CP) and intermediate zone (I). Septum, S; lateral ventricle, LV. × 400.

Embryonic days 15 and 16. During day 15 of gestation the previously separated medial walls of the cerebral hemispheres (septa' regions only) begin to fuse, obliterating the lower half of the interhemispheric fissure, but allowing the longitudinal cerebral fissure to remain dorsally (Figs. 2, 14). At this stage septal fusion has progressed for approximately 200 μm rostral to the ventricular surface of the lamina terminalis (Fig. 15). In addition, at the forward point of septal fusion, and adjacent to the bottom of the longitudinal fissure a population of darkly stained cells is apparently in the process of migrating away (medially) from the ependymal zones of each lateral ventricle. The darkly stained cells steadily increase in number and eventually move completely through the septum to take up positions below and lateral to the longitudinal fissure (Fig. 2).

A more massive cell migration, that which leads to the formation of the cortical plate, is also well underway at this stage. (For a more detailed description of cortical plate development *see* Caviness and Rakic, '78; Caviness et al., '81; Tsai et al., '81). Thus, although many cortical neurons have already approached the pial surface, the callosal population (mainly from presumptive cortical layers III and Va, Ivy et al., '79) has not yet sent out

axons toward the midline. Interestingly, our silver-stained material reveals that the earliest cortical fibers turn laterally toward the corpus striatum (also see Sturrock, '82). It is noteworthy that during the establishment of the cortical plate, a distinct and oriented line of separation is maintained between the cell bodies of migrating and differentiating cortical neurons (Fig. 2). The result of this nuclear parcellation is the creation of an aligned interface much like that described by Tsai et al. ('81) in the developing telencephalon of the chick embryo and similarly by Nornes and Das ('72) in the embryonic rat ventral spinal cord. Early on, the movement of all cortical afferent and efferent fibers is largely confined within this zone (see below).

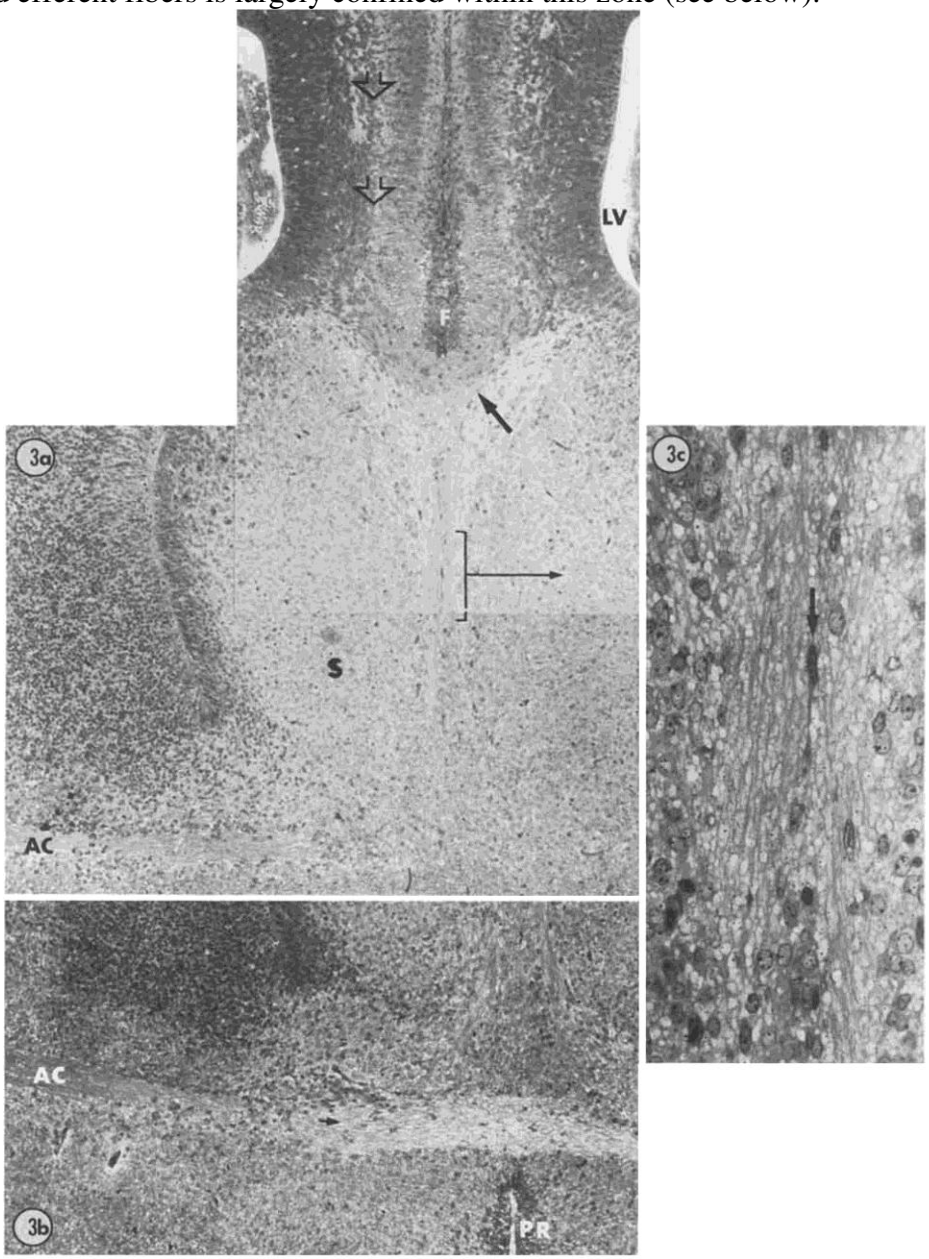


Fig. 3. Coronal sections of a 16.5-day mouse embryo (C57BL/6J) taken 200 μm rostral to the ventricular surface of the lamina terminalis (a) and *through* the region of the preoptic recess (PR) (b). Note in a the dense accretion of glial elements forming a bridgelike structure or "sling" (solid arrow) suspended from the medial walls of each lateral ventricle (LV) and coursing beneath the longitudinal cerebral fissure (F). Note also the production of extracellular space and alignment of darkly stained cells (bracket) in the future region of the fornix (higher magnification in c). Arrow in c points to an aligned cell and indicates direction of axons. Note in b the developing anterior commissure axons (AC) approaching the midline and the tunnellike system of cell processes and extracellular spaces ahead of them (preoptic recess, PR; septum, S). In a the future route of the corpus callosum is indicated by the open arrows. a,b, × 160; c, × 620.

Late on day E 15 and on day E 16, fusion of the septal walls has moved further rostrally so that now approximately 240 μm of midline tissue is united forward of the lamina terminalis (Fig. 15). Pioneer fibers of the anterior commissure have entered the septum. Figure 3b was taken from a rare embryo, in that it was fixed at a stage which has captured the anterior commissure axons adjacent to the midline but still on route to their destinations. It shows clearly, within a single photomicrograph, the preformed, nuclei-free system of tunnellike glial cell processes and extracellular spaces that lie ahead of a growing front of fibers. Since the anterior commissure is horseshoe shaped, many serial coronal sections through the forebrain of this animal contain

laterally located anterior commissure axons (two selected sections, 11 μm apart, are shown in Fig. 3a,b). However, the presumptive guidance pathway *at the midline* is located only above the preoptic recess, the very place where the commissural axons would have eventually crossed into the contralateral hemisphere.

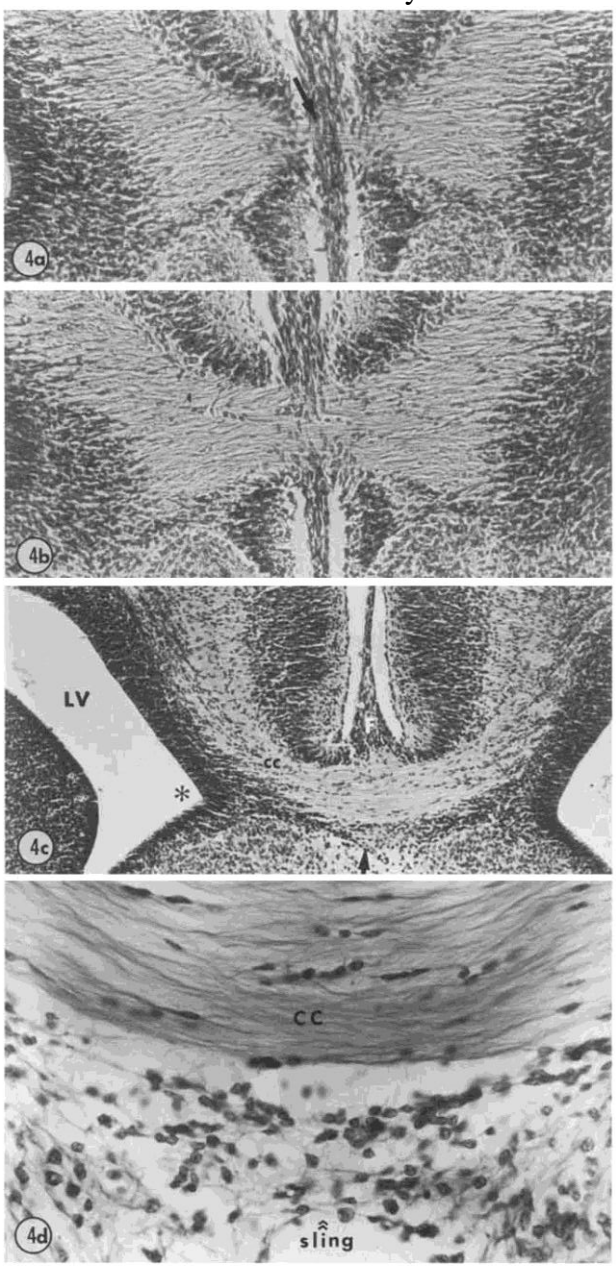


Figure 4

Fig. 4. ***Corona', hematoxylin-stained paraffin sections taken 200 μm rostral (c) 440 μm rostral (b) and 450 μm rostral (a) to the ventricular surface of the lamina terminalis of a 17.5-day C57BL/6J embryo (phase microscopy). Callosal axons (CC) cross the midline at this stage (c). They do so (in c) by traveling upon the surface of the underlying glial "sling." Note the kink in the walls of the lateral ventricles at the site of the "sling" attachment. A higher magnification of the "sling" is shown in d. Figures a and b demonstrate the farthest forward position in the cerebrum that contains callosal axons at the midline. There is no evidence of a dense cellular scaffold below these most rostrally located callosal axons. However, there is a fusion (i.e., close apposition, arrow) of midline pial glial cells immediately in front of the callosum. (This glial fusion has been verified in serial plastic sections.) Lateral ventricle, LV; longitudinal cerebral fissure, F. a—c, × 160; d × 620.***

Immediately above the anterior commissure pathway and extending dorsally through the septum along its original line of fusion is a V-shaped pathway of darkly stained glial cell processes (but few somata), oriented tangentially to that of several fiber systems that are beginning to invade this region (Fig. 3a,c), namely, the

fornix columns (medially) and some of the septal efferent pathways (mediolaterally) (Nauta and Haymaker, '69; Sidman et al., '71; Swanson and Cowan, '77). Although on day E 16 fibers of the hippocampal (fornix) commissure are already present within the region of the lamina terminalis, the callosal axons have still not reached the center line. However, some of the lead fibers of the callosum can now be identified along the banks of the longitudinal fissure within the cingulate gyrus.

By stage E 16 the septal midseam has been dramatically augmented dorsally by the continuing influx of darkly stained cells from the adjacent ependyma. The cells have become more densely packed than at earlier stages and have coalesced tightly to form a slinglike structure, spanning from ventricle to ventricle, and suspended directly below the longitudinal cerebral fissure (Figs. 3a, 14). At this stage, and all subsequent stages, the "sling" lies well forward and separated from the lamina terminalis. At E 16 the "sling" is approximately 100 μm long (rostrocaudal dimension) with its caudal end reaching the front end of the hippocampal commissure (Fig. 15). In turn, the hippocampal commissure occupies about 70 μm of midline tissue between the caudal end of the "sling" and the point where it becomes embedded within the lamina terminalis.

Embryonic day 17. Early on day E 17 the pioneer callosal axons finally reach the midline. On route to the midsagittal plane most fibers travel within the interface of nuclei between the cortical plate and intermediate zone of migrating neurons. The interface contains processes of radial glial cells. However, the movement of callosal axons within the hemispheres is not absolutely constrained within this zone. Indeed, some of the callosal fibers seem to stray from the main contingent and can be found fairly close to the ependymal surface. However, as the callosal axons from the two hemispheres approach the midline they become increasingly more compressed and, while moving in opposite directions, travel contralaterally within a very compact bundle located immediately beneath the longitudinal fissure and *above* (not among) the cells that form the slinglike structure suspended below them (Fig. 4, also see Fig. 11 for location of "pioneer" callosal fibers). Early on, at its ventral and two lateral edges the "sling" melds smoothly with the underlying septum and ependymoglial cells of the paired lateral ventricles. However, at the stage when the callosal axons cross midplane, the ventricular walls at the lateral sites of "sling" attachment develop a definite inward (medially directed) kink, suggesting that the ventricles may be under some sort of tension generated within the "sling" itself (Fig. 4). A three-dimensional computer graphic reconstruction of this callosal bridge (now 200 μm front to back) is shown in Fig. 5.

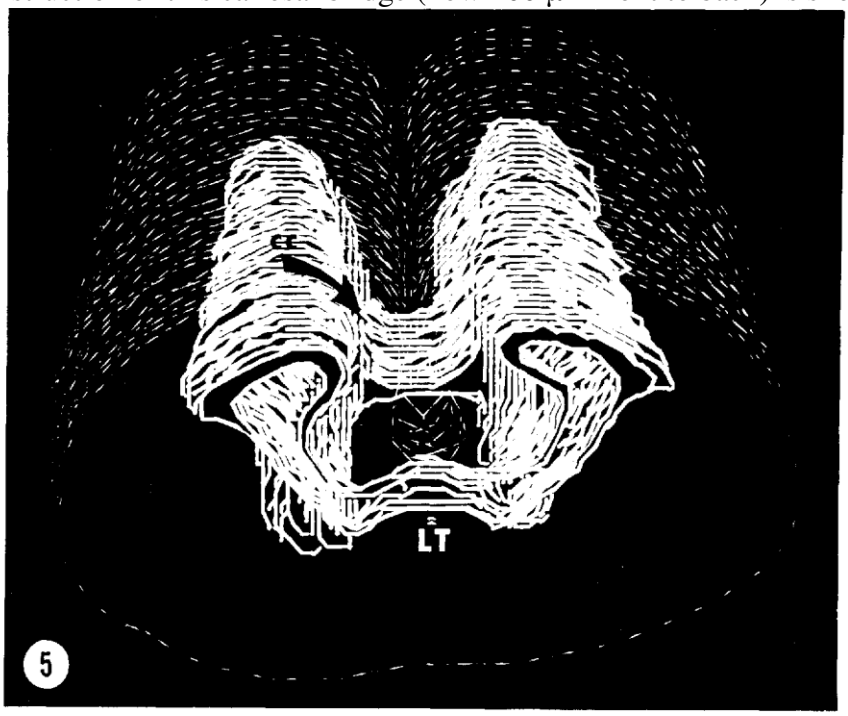


Fig. 5. A computer graphic reconstruction of the "sling" on E 17.5. The reconstruction shows the separation between the "sling" and the lamina terminalis (LT). The structures that can be visualized were generated by tracing around the walls of the ventricles, the outer surface of the ependymal layer, and the pial surface of the brain. (The pial surface is shown as a phantom overlay.) The route of the corpus callosum above the "sling" is indicated by the arrow. The fornix fibers pass rostrally but beneath the "sling."

Fasciculation of later-forming callosal axons

Fusion of the septal midline (a joining of pial glial cells forward of the lamina terminalis; see Streeter, '12) is a continuing process that is coordinated beautifully with the rostrally directed fasciculation of callosal fibers. Thus, the fusion occurs in the fashion of a moving wave that constantly keeps pace with the front of newly arriving axons (Figs. 4, 15). The fusion occurs first immediately ahead of and only later (by about 12 hours) below the point where the callosal axons cross. Therefore, during the fasciculatory phase of callosal growth new fibers always insert themselves between the older fibers and the pial glial cell processes ahead of them. Late on E 17 the most rostral point of septal fusion and lead fibers of the corpus callosum are located about 450 μm forward of the ventricular surface of the lamina terminalis (Fig. 15). It should be stressed, however, that rostral growth of the "sling" does not advance at a rate equal to that of the fusion process nor to that of the orderly accumulation of callosal axons. Thus, late on E 17, although the "pioneering" callosal fibers are located above the "sling," about 50 μm of rostral callosum is not undercoated by a dense cellular bridge (Figs. 4a,b, 15). There are also a small number of callosal axons that cross caudal to the "sling" and appear to blend imperceptibly with the most rostrally located fibers of the hippocampal commissure. Indeed, the earliest wave of callosal fibers crosses rapidly and in rather massive numbers.

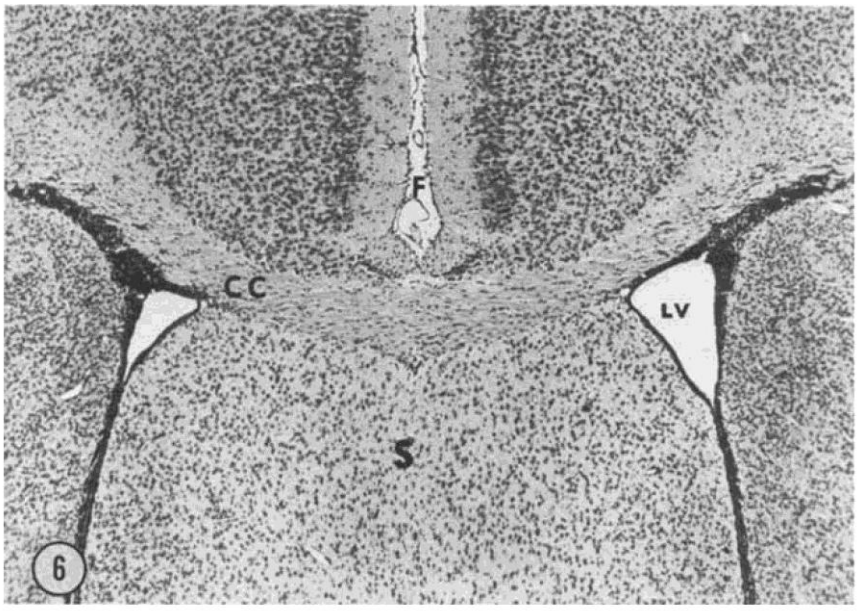


Fig. 6. A coronal, hematoxylin-stained paraffin section of the mouse brain (C57BL/6J) at postnatal day 3. The section is taken 200 μm forward of the lamina terminalis. Note the absence of a dense cellular bridge beneath the callosum. Septum, S; corpus callosum, CC; lateral ventricle, LV; longitudinal cerebral fissure, F. × 160.

Embryonic days 18 and 19 and postnatal stages. On days E 18-19 growth of the corpus callosum progresses quickly in the caudal direction so that callosal axons now appear distinctly within the lamina terminalis, directly above the hippocampal commissure (the configuration described by Abbie, '40). Although the callosum has also enlarged rostrally by the fasciculation of axons (fibers now extend forward, and above the septum, for about 500 μm) the length of the "sling" remains unchanged from previous stages. Thus, the "sling" lies subjacent to a progressively smaller fraction of the callosum as a whole (Fig. 15).

The callosum is still expanding at the end of the first postnatal week. Its length after fixation is about 1.2 mm in the newborn and nearly 2.0 mm at P7 (Suitsu, '20). In the mouse, as well as in other species, callosal growth caudal to the lamina terminalis is a relatively late event. However, just like the more rostrally directed fibers, those that form caudally also cross in a secondary zone of fusion of the medial hemisphere walls (Rakic and Yakovlev, '68).

A most important observation concerning the early postnatal stages of the callosum (about P3 and afterward) is that there is no longer any evidence of a dense tissue lying anywhere along the length of its ventral surface. *Therefore, the "sling" is a transient structure* (Fig. 6).

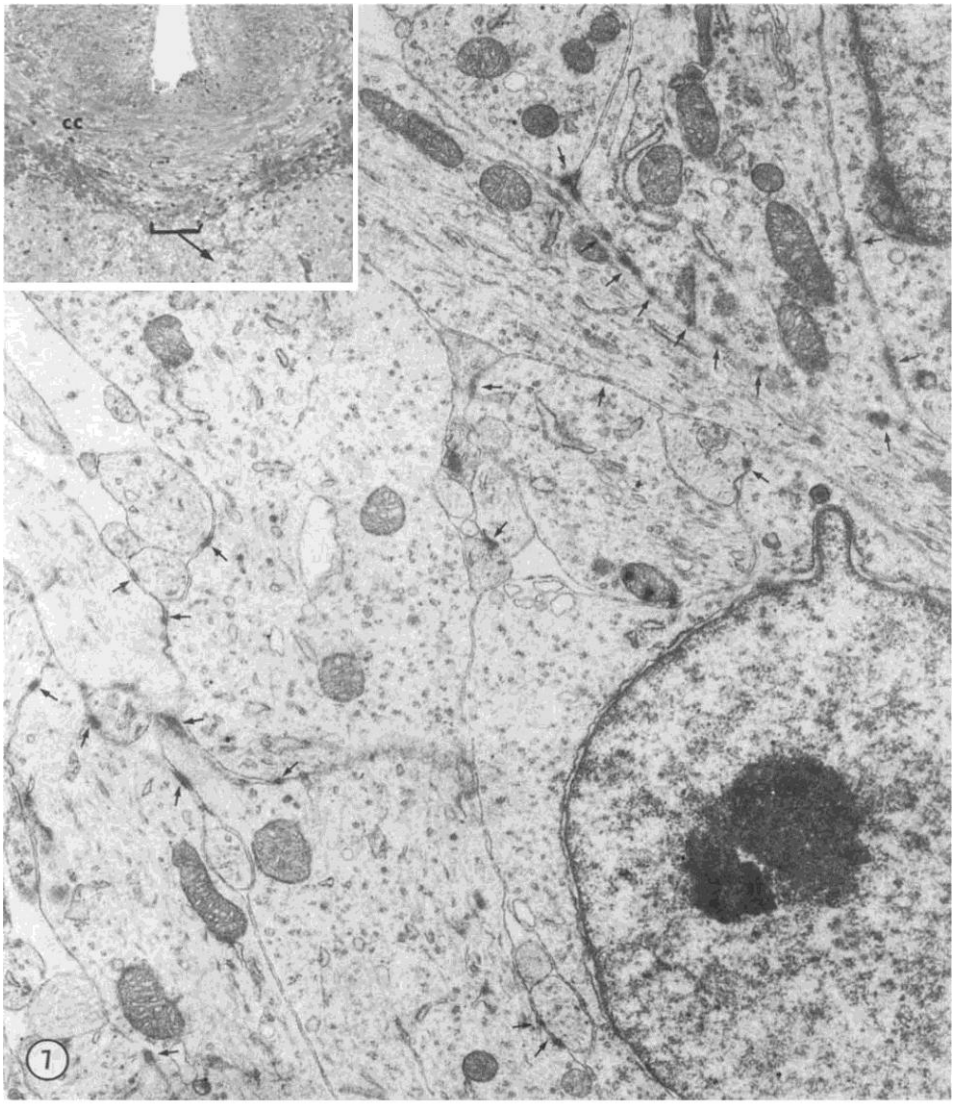


Fig. 7. An electron microscope picture of the "sling" at E 17.5 (the region selected for E.M. is indicated in the light micrograph insert). Puncta adhaerentia are indicated by the arrows. Corpus callosum, CC. × 10,000.

The "sling": Ultrastructural observations

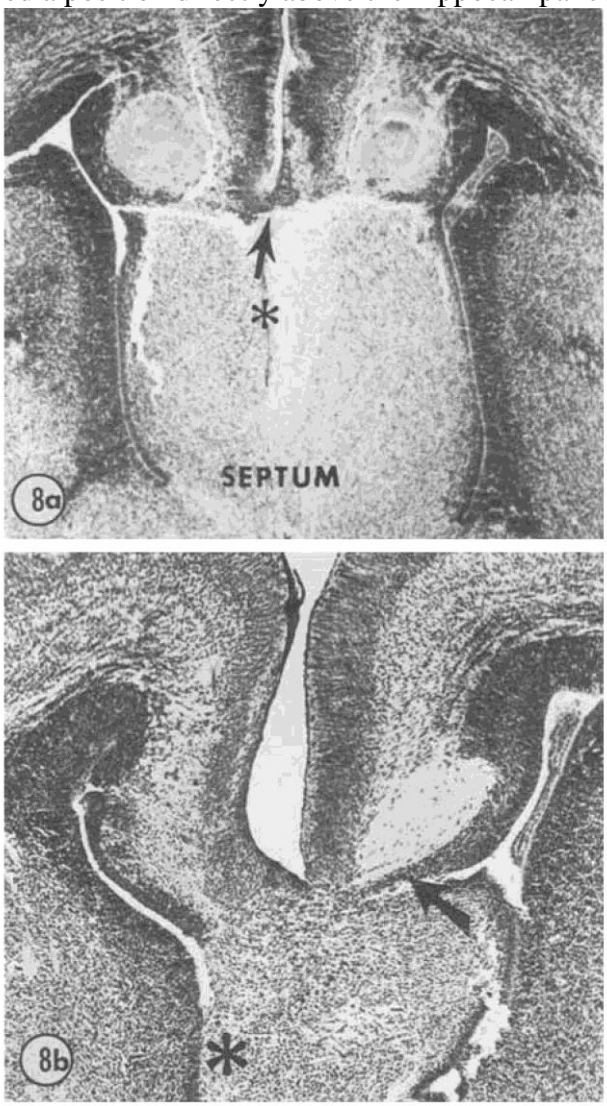
Well before, during, and after the arrival of the pioneer callosal axons at the midline, the individual cells and cell processes of the "sling" become united one-to-another by forming a tangled, interconnecting meshwork configured with an oriented and relatively flat upper surface. Apparently, the "sling" cells are metabolically active. They are packed with clusters of free ribosomes, rough and smooth endoplasmic reticulum, and a variety of other organelles and inclusions (including microtubules, a few filaments, some glycogen granules, a prominent Golgi apparatus, and large numbers of mitochondria). The mitochondria tend to gather near zones of close membrane apposition. Such cellular contacts are particularly prevalent within the "sling" (especially on E 17) and are always associated with tremendous numbers of puncta adhaerentia (a sticky type of junction, based on criteria of Peters et al., '70; Krayanek and Goldberg, ('81). Indeed, it would appear that the many individual cellular elements of the "sling" are, in essence, spot welded together to form a cohesive structure (Fig. 7). Thus, our morphological evidence suggests that the "sling" may be comprised of a population of very primitive cells that very closely resembles the intracallosal glioblast described previously by Paterson et al. ('73) and Sturrock ('76). However, since the subcallosal cells are only temporary residents in this location, it would be premature to characterize them as blast cells for a particular glial subclass.

Affects of intrauterine lesion of the "sling" on callosal development

We have demonstrated that early callosal axons grow along the surface of a preformed cellular scaffold as they travel toward the contralateral side of the brain. However, an important question remains. Does this transient, glial tissue actually provide guidance cues to the commissural axons? In an attempt to answer this question we

have made intrauterine lesions of the "sling" at or near the midline during stages prior to the expected time of arrival of the callosal fibers (E 15—E 16). We then sacrificed the embryos soon (E 17) or well after the callosum would have normally formed (E 18, E 19, P 1, P 7, P 14).

Such mechanical disunions of the "sling" either midsagittally or parasagittally, without touching the lamina terminalis (and hippocampal commissure embedded within the lamina terminalis), consistently resulted in the production of acallosal individuals. Usually, at least a portion of the septum refused within 24 hours following the surgery. Although the would-be callosal fibers approached the midline on schedule, they did not cross. Instead, most whirled into a pair of longitudinally oriented, cigar-shaped neuromas adjacent to the longitudinal fissure. These neuromas were of collossal dimension. In general, they became progressively thicker and longer as increasingly more time was allowed to elapse between the day of lesion embryonically and the moment of sacrifice. In animals killed at the earliest embryonic stages (early E 17) the neuromas were localized well in front of the lamina terminalis (Fig. 8a), whereas in animals killed late in gestation or postnatally, the neuromas often stretched for a millimeter or more, beginning in the rostral forebrain and extending caudally, where they occupied a position directly above the hippocampal commissure (Figs. 9, 10).



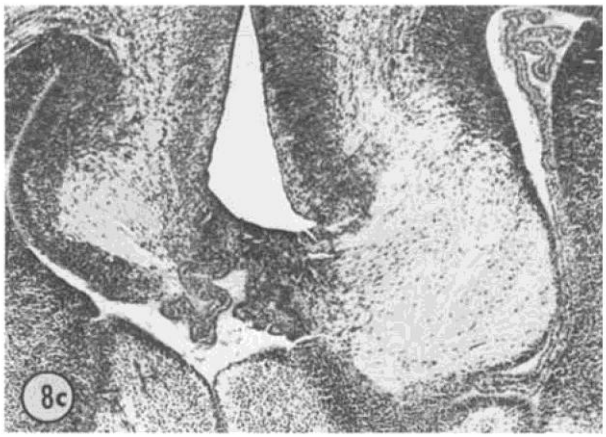


Fig. 8. Coronal, hematoxylin-stained paraffin sections of surgically lesioned C57BL/6J embryos (E 15) and sacrificed on E 17. The embryo in a received a direct midline cut of the "sling" (asterisk). The embryo in b and c (c is slightly caudal to b) received a parasagittal lesion (asterisk). In both animals the callosal axons failed to cross midplane. Instead, the callosal fibers gathered into a pair of neuromas adjacent to the longitudinal cerebral fissure. The remnants of the "sling" are indicated by arrows. a, × 60; b,c × 80.

The embryo shown in Figure 8b,c is particularly instructive because its lesion missed the midline. As the needle passed through the brain it damaged cingulate cortex on one side, and cut through the "sling" along only one of its attachment points with ependyma of the lateral ventricle. Although, in this animal (and in two others like it), the septal midline was relatively unaltered and completely fused, the "sling" was only partially formed, appearing within the cerebral hemisphere only on the unlesioned side. (Apparently, the "sling" does not usually reform into its characteristic geometry once it is damaged). Note that although the septum itself provided a direct tissue continuity between the two cerebral cortices, the callosal axons of *both* hemispheres failed to cross

midplane. Instead, the fibers gathered into a pair of asymmetrically shaped neuromas (one large in the unlesioned hemisphere, one small in the lesioned hemisphere) alongside the longitudinal fissure. Animals with exact midline lesions usually had two large, symmetrically formed neuromas, enclosed laterally and ventrally by glial elements. It should also be noted that unless the needle touched the lamina terminalis, the hippocampal and anterior commissures were always normal in surgically induced acallosal animals (Fig. 10a,b). Although in affected animals there was usually little evidence of a large-scale crossing of callosal axons within the lamina terminalis (Fig. lob), in a few specimens (four of 56) axons did leave the longitudinal bundle in greater numbers to form a small callosum upon the surface of the hippocampal commissure. Control embryos with lesions of the far rostral midline (i.e., E 16 lesions forward of the "sling") were normal.

The acallosal mutation of the mouse and the normally acallosal opossum: Some consequences of the lack of formation of the "sling" on callosal development

The acallosal mouse. Among BALB/cCF inbred mice about 20% of the adult animals show a clear deficiency of the corpus callosum (Wahlsten, '82). Nine litters ranging in age from 16.0 to 18.0 days from conception were compared to C57BL/6J embryos at similar degrees of maturity. Although some of the BALB/cCF embryos appeared to be normal, in others, fusion of the septal midline was markedly delayed. In the period from E 16 to E 17, the longitudinal cerebral fissure was deeper in certain BALB/cCF embryos than in their normal littermates or normal C57BL/ 6J embryos (Fig. 11), and the fissure was often engorged with blood and tumorlike formations (Fig. 12). Although the glial elements of the "sling" are normally united at midline and form a continuous band between the hemispheres in C57BL/6J embryos at E 16 (before callosal fibers arrive), in the abnormal BALB/cCF embryos, these glial elements did not migrate far enough nor in sufficient numbers to form a cohesive structure. The septum did eventually fuse in all BALB/cCF embryos (by E 18).

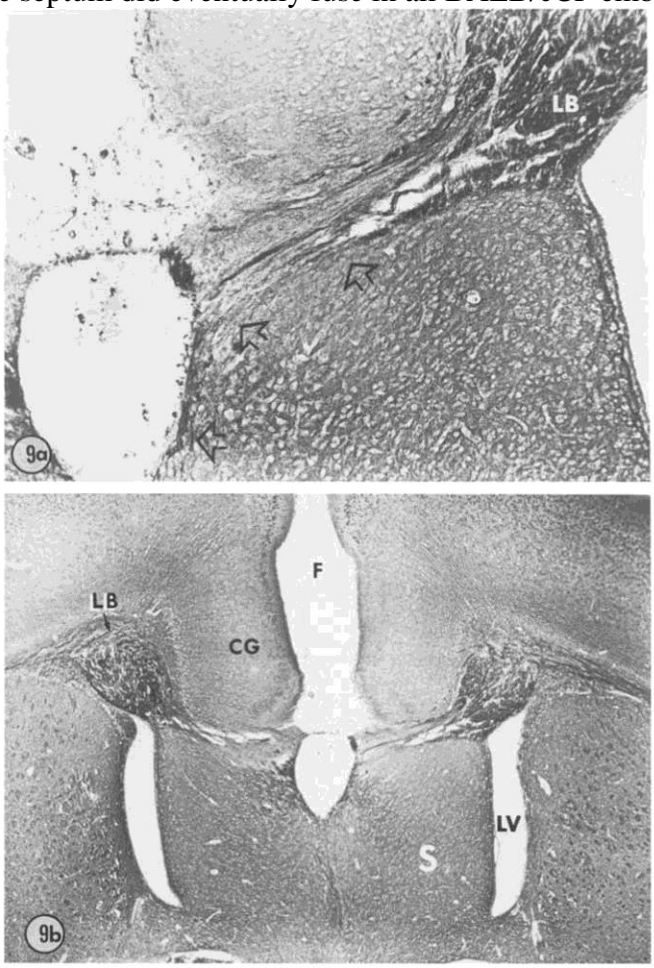


Fig. 9. Coronal, silver-stained sections through the septal region of a surgically lesioned (C57BL/6J) embryo (E 16) and sacrificed on P7. The septum (S) is fused in this animal. In b the paired, longitudinal bundles (LB) of stained axons are located above the lateral ventricles (LV) and adjacent to the cingulate cortex (CG). Note that with increasing development of the cingulate cortex, the callosal neuromas become displaced laterally and away from the longitudinal fissure (F). Note in a (a is higher magnification of b) that some fibers leave the longitudinal bundle to enter the ipsilateral septum (arrows). b, × 60; a, × 160.

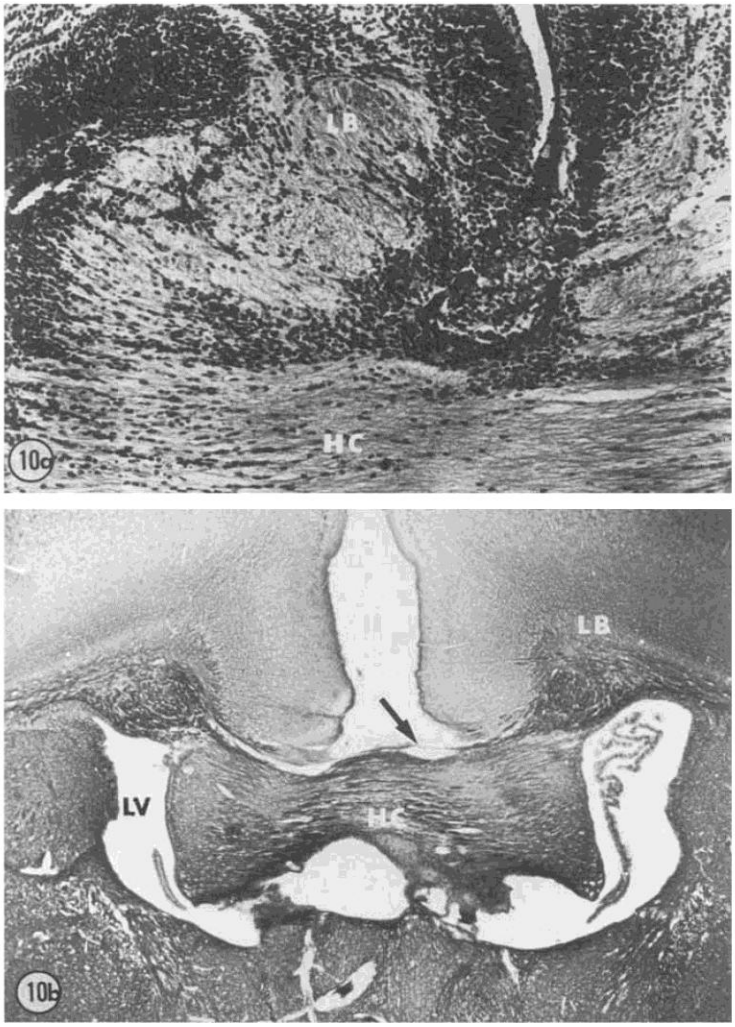


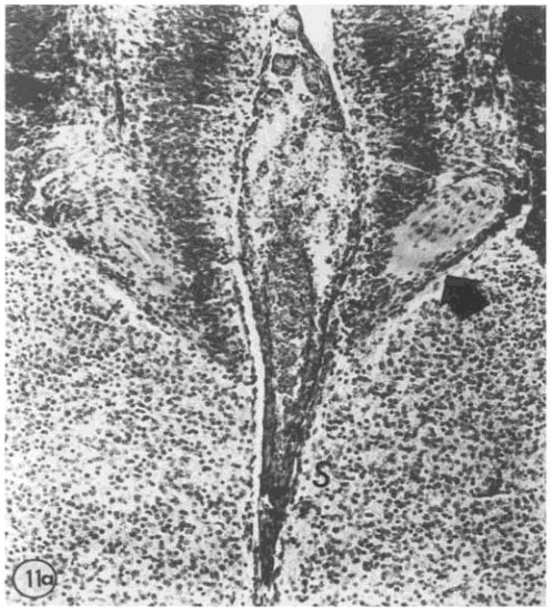
Fig. 10. Coronal sections (a, hematoxylin stain; b, silver stain) through the region of the hippocampal commissure (i.e., lamina terminalis) in C57BL/6J animals that had their "sling" lesioned on day E 16 and were sacrificed on P1 (a) and P7 (b). The section in b is from the same acallosal animal shown in Figure 9. Note in these specimens that with the exception of only a few fascicles of axons (arrow in b) callosal axons do not leave the longitudinal callosal bundle (LB) to grow upon the surface of the hippocampal commissure (HC). However, in a small minority of lesioned animals, callosal axons do use the upper surface of the hippocampal commissure as a pathway. These individuals are born with a partial callosal defect characterized by having Probst's bundles rostrally and small, normal appearing callosi and no Probst's bundles a bit more caudally. Lateral ventricle, LV. b, × 60; a, × 160.

It is noteworthy and rather remarkable that the callosal pathology in older BALB/cCF fetuses (and neonates, unpublished) resembled closely that of C57BL/6J mice with early surgical transection of the "sling" (see previous section). In both instances the most prominent characteristic of the defective brains was the longitudinal neuromatous bundle (i.e., the aberrant callosal bundle of Probst, '01) running in the sagittal plane adjacent to the longitudinal cerebral fissure (Fig. 12). In acallosal embryos and neonates, other forebrain commissures were present (Fig. 11). It should be stressed that the very earliest "callosal" axons to arrive midsagittally in mutant embryos (just as in controls) were not associated with the lamina terminalis. Rather, the pioneer fibers first began to accumulate into Probst's bundles between about 200 and 300 μm forward of the lamina and precisely in the region of the malformed "sling" (Figs. 11, 15).

In addition to the callosal neuromas there were usually two small contingents of ipsilateral fibers that left the longitudinal bundles to join either the corticoseptal and septohypothalamic tracts (rostrally) or fornix columns (caudally) (King, '36; Ivy and Killackey, '81; Lent, in press). Similar projections from Probst's bundles into the septum also developed following surgical (E 16) "sling" lesions. However, these fibers were only distinguishable with silver staining in animals that were killed during the first postnatal week (Fig. 9).

A difficult problem remains to be solved concerning the etiology of the acallosal malformation. It is not known whether the primary defect in the acallosal BALB/cCF embryo is in the glial "sling" cells themselves or in some other process, acting at or near the longitudinal cerebral fissure, which prevents the migration and joining of these cells to form a complete bridge. It is possible, of course, that both the "sling" cells and the cells along their

path of migration are abnormal. Whatever the nature of the primary hereditary defect may be, the result is malformation of the glial "sling" and consequent failure of callosal axons to traverse the hemispheric midline on schedule.



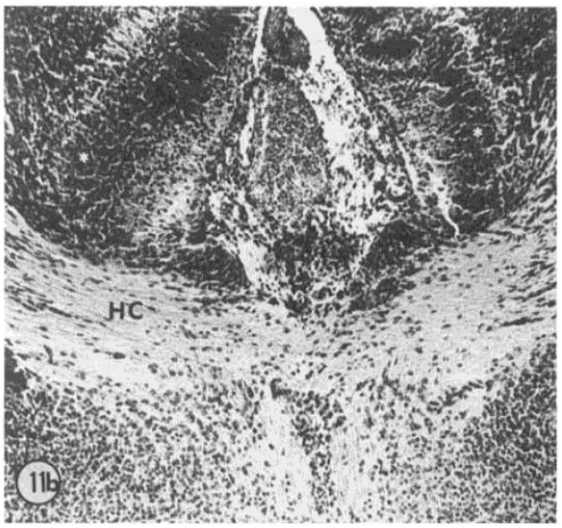


Fig. 11. Coronal hematoxylin-stained sections (b) through the lamina terminalis [and hippocampal commissure (HC)] and (a) 250 μm rostral to the lamina terminalis in an early E 17.0 BALB/cCF mouse. Note the large separation between the septi (S). The very earliest "pioneer" callosal axons have arrived midsagittally. They are clearly visible because they form into Probst's bundles. However, the bundles appear first only in the region of the malformed "sling" (arrow). × 160. Asterisks in (b) indicate position above the HC where *later* forming callosal axons form into Probst's bundles.

The normally acallosal opossum

A striking difference between the forebrains of a variety of primitive mammals (certain monotremes and marsupials) and other more advanced mammals is the lack of a dorsally located corpus callosum. In these "lowest" of forms all commissural axons of the neopallium take an alternate ventral route passing by way of the external capsules and thence through the anterior commissure pathway to the opposite side of the brain. In "higher" mammals the pars posterior of the anterior commissure is actually a mere remnant of the former pathway and only remains to serve cortical commissural axons from the far temporal regions (Kappers et al., '36; Horel and Stelzner, '81). Thus, the dorsal corpus callosum is a phylogenetically novel projection.

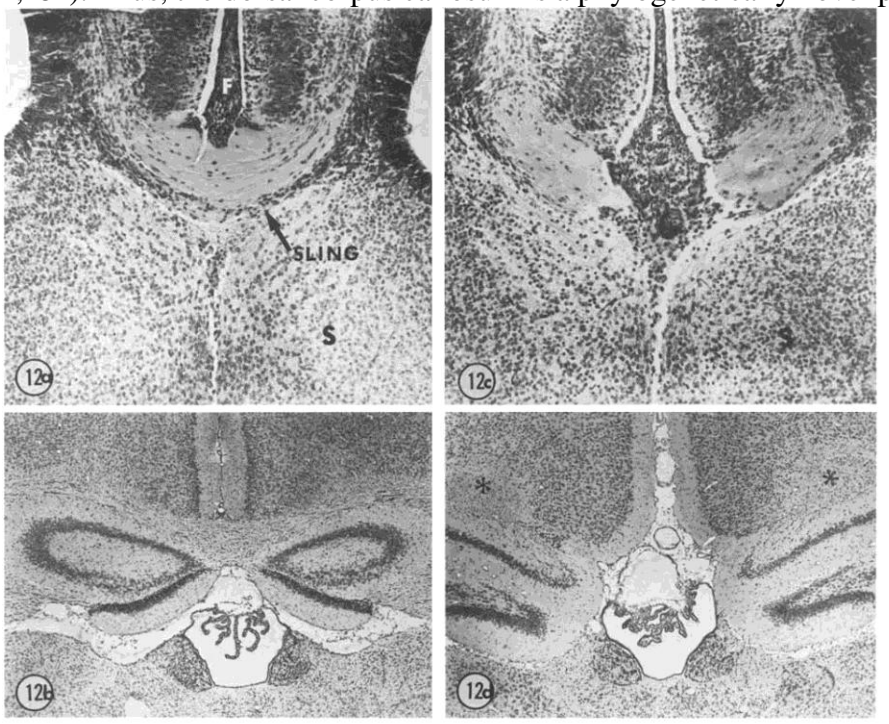


Fig. 12. Comparable coronal sections through the normal (C57BL/6J) mouse brain at P 30 (b) and E 17.5 (a), and through the acallosal mouse brain (BALB/c) at P 30 (d) and E 17.5 (c). In c note the comparatively greater depth of the longitudinal fissure (F) in mutants than in the normal embryo. Note also the obvious absence of crossing fibers of the corpus callosum (but the presence of the Probst's bundles laterally). Note (in fig. c) the tumorlike formation in the longitudinal fissure. In d the regions of Probst's bundles are indicated by asterisks (septum, S). a,c, × 160; b,d, × 60.

Is the glial "sling" also an evolutionarily new structure and, therefore, lacking in the opossum's neural itinerary? Indeed, our histological sections reveal that nothing even resembling a "sling" is ever present in the developing opossum's brain. In addition, there is a complete and permanent lack of septal fusion forward of the lamina terminalis. These most basic of developmental differences, as well as the relative changes in the amount of cerebral cortex and septum in the two species, are demonstrated side-by-side in Figure 13 (opossum day 18 in pouch; vs. mouse E 18 in utero, are stages selected as examples for comparison). Thus, although a major difference exists in the direction of movement of the commissural axons in opossums and mice, it would appear that the sequence of ontogenetic events that occurs at the midline of the opossum forebrain closely parallels that of the very early developing acallosal mutant. In this regard, however, it is intriguing that in surgically induced acallosal hamsters, a few commissural fibers from cortical regions that would normally project contralaterally via the dorsal corpus callosum can bypass Probst's bundles by using the anterior commissure as an alternate pathway (Lent, in press). A more complete discussion of our observations on the opossum will be presented in a forthcoming paper (Katz et al., in preparation) and we have only mentioned the most essential findings here.

DISCUSSION

Although there has been a large interest in the developing forebrain commissures, and particularly the corpus callosum, for well over a century, there is still little agreement as to the way in which these cortical axons reach the contralateral side of the brain. There are two opposing views. The older of the two theories, which was first proposed by Mihalkovics (1877) and supported by His (1889), Langellaan ('08), and more recently by Rakic and Yakovlev ('68), suggested that fibers of the corpus callosum traverse the midplane in a broad zone of fusion formed as a consequence of the close apposition of the medial hemisphere walls (this route is designated as #2 arrow in Fig. 1). However, the most widely accepted theory and that which is still presented in most textbooks of classical embryology (e.g., Hamilton et al., '72) was aggressively proposed by Elliot Smith (1896) and later adopted by many others (Kappers, '08; Streeter, '12; Johnston, '13; Hochstetter, '29), who argued that the corpus callosum and, indeed, all commissural forebrain pathways first crossed the cerebral midline within the substance of the lamina terminalis itself (a region designated as the commissural bed and labeled route #1 arrow on Fig. 1). For the corpus callosum the later addition of fibers would result from a stretching of the lamina forward and backward into the interhemispheric fissure. The results presented here would appear to amend this long-standing controversy concerning the overall development of the great cerebral commissures, and have shed some new light on the role of preformed glial pathways in the guidance of these fiber systems.

Fusion of the septal midline forward of the lamina terminalis is, indeed, an observable event during ontogeny of the mouse forebrain and, as suggested by Mihalkovics (1877) and others of similar opinion, is closely associated spatially and temporally with the development of the corpus callosum. However, the results of our lesion experiments and observations on the acallosal mouse mutant suggest that neither septal fusion nor the presence of an intact lamina terminalis (with hippocampal commissure) are, in themselves, sufficient to allow for the proper commissuration of the cerebrum by callosal axons. It would appear, rather, that the normal fusion of the septal midline is a necessary prerequisite for the regular formation of the glial bridge or "sling" which spans the two cerebral hemispheres prior to callosal development. It is upon the oriented upper surface of this embryonic scaffold and, thereby, physically separated from the fornix and septum below, that the pioneering callosal axons grow and first cross each other as they make their way contralaterally. Indeed, our data suggest that this transient population of cells located midway along the presumptive route of the earliest (and apparently very essential) callosal axons may be equally as important for directing their growth as the cortical targets with which they must eventually synapse. Thus, if this midline tissue alone is permanently damaged genetically or surgically the great majority of all callosal axons fail to pass into opposite hemispheres.

Although in vitro studies have demonstrated repeatedly that axons will grow preferentially on glial substrata (Guillery et al., '70; Luduefia, '73; Sensenbrenner and Mandel, '74; Varon, '75; Letourneau, '75a; Wessels et al., '80; Adler and Varon, '81; Hatten and Liem, '81) and that axons will move with directionality (by contact guidance) when confronted with substrata that are aligned (Harrison, '14, '35; Weiss, '34; Letourneau, '75b; Overton, '79) there has been little attempt to inquire whether any polarization of the glial environment is present along the track of a would-be nerve in the normal embryo. Indeed, three consistent features of a variety of

preaxonal terrains that have been serially analyzed or reconstructed so far [these include small portions of the presumptive pathways for the corpus callosum and anterior commissure (present study), the optic nerve (Silver and Robb, '79; Silver and Sidman, '80; Krayanek and Goldberg, '81), and peripheral auditory and vestibular nerves (Carney and Silver, '80)1, are that (1) they are often constructed of a certain type or state of glial cell; probably astrocytes (2) the glial cells produce an assorted array of oriented surfaces upon which the axons subsequently grow, and (3) several different types of glial structures can be deployed along the length of the future path of a single fiber system.

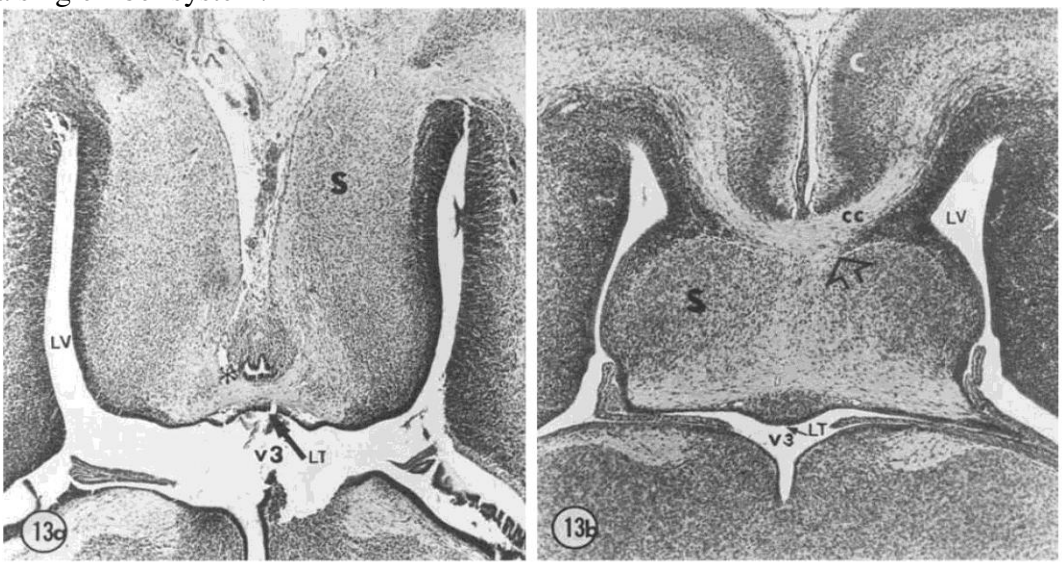


Fig. 13. Horizontal, hematoxylin-stained paraffin sections of the opossum forebrain (day 18) (a) and embryonic mouse forebrain (E 18) (b). The sections are taken through comparable levels. They show the ventricular system (third ventricle, V3; lateral ventricle, LV) and the lamina terminalis (LT). Note the absence of midline septal (S) fusion in the opossum (a) and the presence of septal fusion, the "sling" (open arrow), and the dorsal corpus callosum (CC) in the mouse (b). Note also, in the mouse, the relative increase in the amount of frontal cortex and the relative decrease in the size of the septum. Note, in the opossum, that the ependymal surface of the lateral ventricles is smooth rather than kinked as it is in the mouse. In the opossum, the ependyma of the third ventricle wraps around the anterior commissure to form the preoptic recess (asterisk). × 60.

Thus, in discrete portions of the developing neural margin in birds and mammals primitive radial glial processes become separated by large amounts of extracellular space and, where the processes themselves are aligned in rows, the extracellular geometry is transformed into one of wide-bored "channels" or "tunnels." The substances that fill the spaces have not yet been well characterized. In other regions glial cells migrate from the ependymal zone, become more closely adherent (united one-to-another with myriads of punctum adhaerens type junctions) and, although highly intertwined, can collectivery take on the form of "connecting bridges," "barricades," or "slings." Perhaps the central reason why these specialized glial tissues have eluded discovery for so long is because the cells that generate them are so short lived. In mammals, these glial precursors and the structures they create exist solely during early embryonic stages and, as yet, we have no knowledge of their whereabouts postnatally. The cells either migrate from their positions and become modified to serve other functions (as do the radial glia of the cortex, Schmechel and Rakic, '79) or they may degenerate.

Pioneer fibers only grow through particular regions of their glial environment. Thus, a recurring pattern for the first developing axons (at least in mammals) as they move toward their targets is that they tend to travel along portions of the glial substrata that are relatively rich in cell processes and high in extracellular space. Conversely, early growing axons seem to avoid moving through regions that are more densely aggregated or interwoven, heavily populated with cell nuclei, and poor in extracellular space. Thus, itinerant axons insert themselves between and often perpendicular (but sometimes tangential) to channel-forming glial processes, but they prefer (or are unable to penetrate) the outermost surfaces of more compactly formed structures, such as the "sling."

The later-forming fibers of certain systems also obey rather strict positional rules as they fasciculate with their pioneering predecessors. In the developing callosal system (present study), optic system (Rager and Oeynhausen, '79; Rager, '80; Sapiro et al., '80), and spinal cord (Nordlander and Singer, '82) fasciculating axons appear to have a high affinity for glial endfeet. Thus, newly developing axons usually place themselves between the older (first formed) fibers and the glial limitans. The rostral glial limitans that leads the callosal fibers at the

midline is unusual, in that it is continually changing position as the commissure advances. Since the axons of these fiber systems are generated in sequential order (Silver and Sidman, '80), the subsequent axon-to-axon/glia interactions that occur all along their various routes may constitute a mechanism for maintaining some form of general fiber topography within the developing nerve bundle.

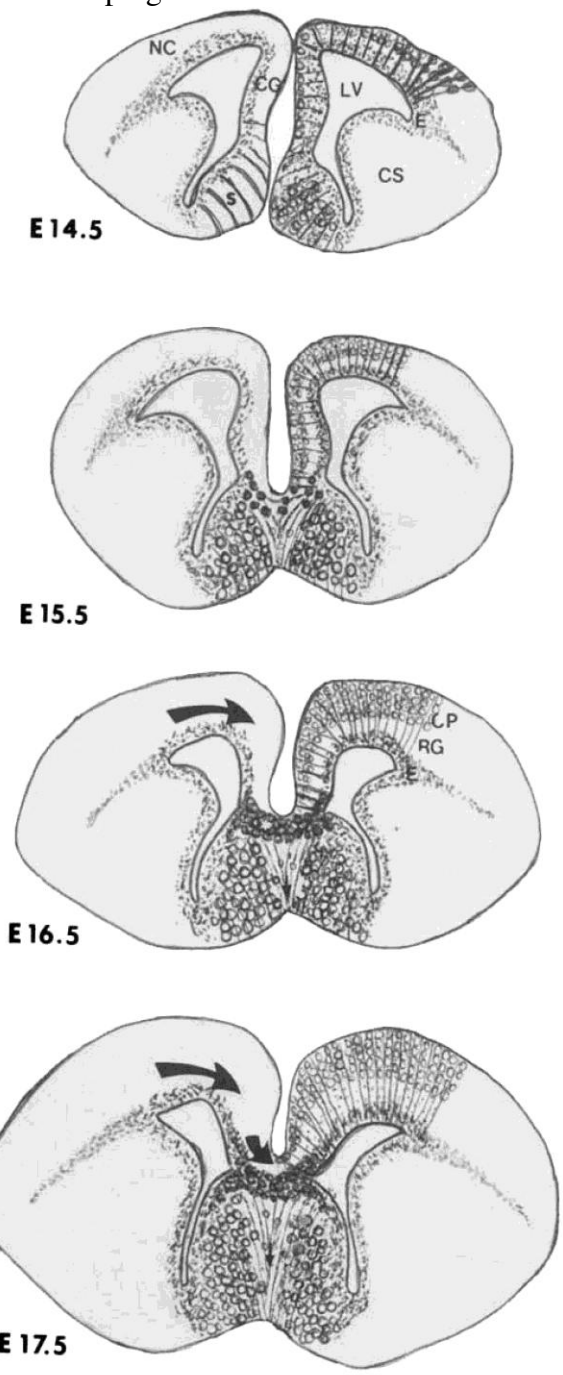


Fig. 14. Summary of ontogenetic events occurring before and during the development of the corpus callosum in C57BL/6J mice (the processes of septal (S) fusion and "sling" (asterisk) formation are depicted). The drawings were made from camera lucida tracings of sections through the normal brain at different stages but all taken 200 μm rostral to the ventricular surface of the lamina terminalis. The lateral to medial route of the callosal fibers, through the oriented interface of cell nuclei and above the "sling," is indicated by the large arrows. The route of the fornix is indicated by the small arrows. The radial glial processes (RG) and septal glia are drawn as fine lines. Their orientations were determined by Golgi analysis (Silver, unpublished) and serial 1-μm sections (see Fig. 3b,c). Septum, S; lateral ventricle, LV; ependyma, E; cortical plate, CP; corpus striatum, CS; cingulate cortex, CG; neocortex, NC.

Although the basic ability of axons to advance along a particular glial pathway is probably mediated locally via some sort of high-affinity, adherent cell surface interaction (Letourneau, '75b; Collins and Garrett, '80) our data, and that of others (Reier and Webster, '74; Reier, '79; Lo and Levine, '80; So, '79; Bohn et al., in press), suggest that such close axon/glia associations can be relatively nonspecific. Thus, when given the opportunity (e.g., after "sling" lesions), *some* of the axons from one system (the callosal) can invade the territory of another (the

fornix or anterior commissure). Interestingly, while in the septum callosal axons behave as if they were of hippocampal origin, mimicking the trajectory of the efferent fibers from this region on route to the hypothalamus (King, '36). Although the cells of origin and the end stations of these presumably misplaced axons are yet unknown, it will be enlightening to our understanding of neuronal specificity in this system (Innocenti et al., '77; Innocenti and Frost, '80) to discover whether or not the acallosal animal has "evolved" a new *synaptically linked,* corticoseptal, cortical association, or corticohypothalamic tract (Silver, in preparation).

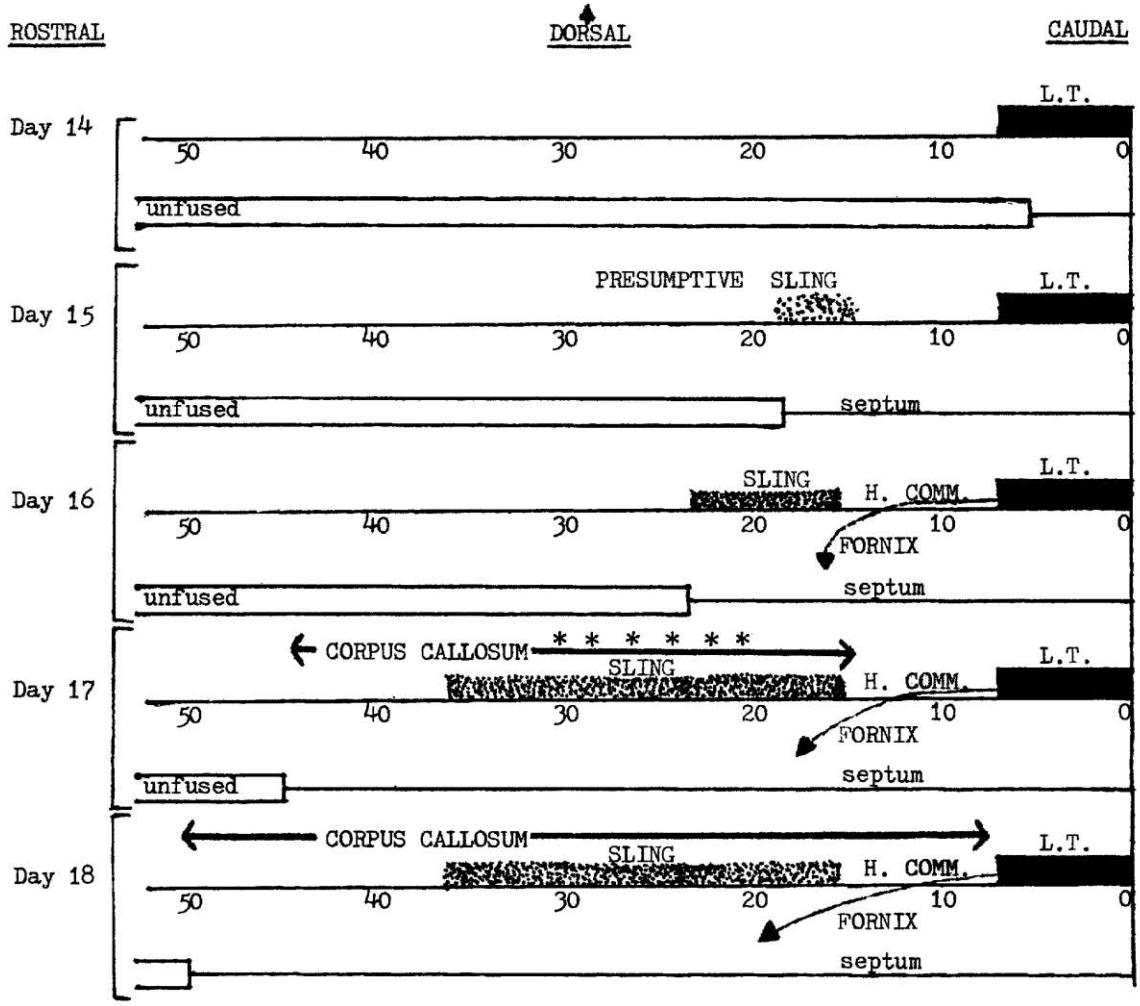


Fig. 15. The locations and development of the corpus callosum, hippocampal commissure, fornix, lamina terminalis (LT), glial "sling," and midline fusion of the septum at successive stages of development in C57BL/6J mice. Each division equals 10 μm, with the zero point representing the rostralmost aspect of the third ventricle (i.e., ventricular surface of LT). Data was collected by counting the number of sections from a given structure to the ventricular surface of LT. Data are averages of three embryos per stage. Asterisks indicate the position where the first "pioneering" callosal fibers arrive at the midline and gather into Probst's bundles (data taken from animal shown in Fig. 11).

After surgical or genetic septal lesions in the embryo, most callosal fibers (whose synaptic terminations, if any, are also unknown) dam up adjacent to the midline and become incorporated within the ectopic longitudinal bundles. The existence of these unusual paired formations is especially perplexing for two reasons. First, there are several tissues that are intact and continuous across the cerebral midline at the time when the callosal whorls are developing. The lamina terminalis, hippocampal commissure (and septum; in lesioned and mutant embryos after it heals, and certainly in embryos cut parasagittally) are all present in acallosal animals. Second, it is difficult to explain these axonal anomalies in terms of an absent chemical attractant emanating from some distant cortical target (Ramon y Cajal, '60). We have no evidence to suggest that the cortex in surgically as well as genetically induced acallosal individuals is unhealthy. Indeed, many other cortical afferent and efferent connections are present in mutated animals (King, '36; Silver, in preparation). Apparently, the primary structure lacking in the developing acallosal brain is the glial "sling" and we would suggest that the acallosal malformation arises because of the local disturbance at this junction.

We propose the following pathogenic scheme. As the pioneering callosal axons approach the altered midline, many (but not all) may fail to recognize any portion of the "sling-free" septum as an appropriate substratum (Sperry, '65; Katz et al., '80). Alternatively, the remaining glia of the severed "sling" might reform into a pair of

bilaterally positioned cul-de-sacs which, tightly constructed with puncta adhaerentia, could block the early passage of most axons (Figs. 7, 8, 11, 12). In either case the bulk of the pioneering fibers are caused to circle at the midline, thereby continually influencing the fasciculating fibers to deflect from their proper course until a thick coil of axons has been formed. The whirling phenomenon is rarely complete, however, and inevitably some of the axons (whose numbers vary in different individuals) can escape the maelstrom by fasciculating upon adjacent, intact fiber pathways that may or may not lead them to the contralateral side of the brain. A very similar example of erratic contact guidance has been demonstrated for the pioneer and later-forming sensory axons in the Hemipteran, *Rhodnius,* following lesions of their epidermal substratum (Wigglesworth, '53).

A critical question remains: If one could harvest embryonic glia from the region of the "sling" well before their properties change and then reintroduce them with proper orientation (a kind of "sling" prosthesis, also see Kromer et al., '81) into the longitudinal cerebral fissure of postnatal acallosal animals, would the Probst's bundle axons sprout, alter their course, and regrow in the proper direction? Indeed, we now have evidence to suggest that such procedures can induce a massive, directed regrowth of Probst's fibers contralaterally (Silver and Ogawa, in press).

Thus, our observations suggest that oriented glial (or presumptive glial) tissues exist in the embryonic neural anlage well in advance of the arrival of axons. Although we do not yet know what organizes the glial cells in the first place, it does appear that these processes may be under morphogenetic control (Silver and Sidman, '80). In addition, we propose that such glial tissues are arranged in the fashion of contiguous pathways and, by their ability to generate a variety of cellular configurations and extra-cellular environments, can influence the direction of movement of axonal growth cones, thereby restricting their pocellular environments, can influence the direction of movement of axonal growth cones, thereby restricting (but *not* specifying) their potential targets. Finally, at the threshold of their destination(s), different types of interactions (fiber-to-dendrite; Rager and Oeynhausen, '79; Fujisawa et. al, '82, or fiber-to-fiber; Constantine-Paton and Law, '78; Macagno, '78; Cook, '79; Gaze et. al, '79) must take place in order to bring the growing tip of the axon to a halt at a particular synaptic location along its pathway.

LITERATURE CITED

Abbie, A.A. (1940) The origin of the corpus callosum and the fate of the structures related to it. J. Comp. Neurol. 70:9-44.

Adler, R., and S. Varon (1981) Neuritic guidance by nonneuronal cells of ganglionic origin. Dev. Biol. 86:69-80.

Bohn, R., P.J. Reier, and E.B. Sourbeer (in press) Axonal interaction with connective tissue and glia substrata during optic nerve regeneration in *Xenopus* larvae and adults. Am. J. Anat.

Bossy, J.G. (1970) Morphological study of a case of complete, isolated, and asymptomatic agenesis of the corpus callosum. Arch. Anat. Histol. Embryo]. 53:289-340.

Carney, P.R., and J. Silver (1980) A mechanism for the guidance of peripheral spiral ganglion cell axons in the developing mouse auditory system. Neurosci. Abs. 6:487.

Caviness Jr., V.S., and P. Rakic (1978) Mechanisms of cortical development: A view from mutations in mice. Ann. Rev. Neurosci. 1:297-326.

Caviness Jr., V.S., M.C. Pinto-Lord, and P. Evrard (1981) The development of laminated pattern in the mammalian neocortex. In T.G. Connelly (ed): Morphogenesis and Pattern Formation. New York: Raven Press, pp. 103-126.

Collins, F., and J.E. Garrett, Jr. (1980) Elongating nerve fibers are guided by a pathway of material released from embryonic nonneuronal cells. Proc. Natl. Acad. Sci. USA 77:6226-6228.

Constantine-Paton, M., and M.I. Law (1978) Eye specific termination bands in tecta of three-eyed frogs. Science 202:639-641.

Cook, J.E. (1979) Interactions between optic fibres controlling the locations of their terminals in the goldfish optic tectum. J. Emb. Exp. Morphol., 52:89-103.

Dennis, M. (1976) Impaired sensory and motor differentiation with corpus callosum agenesis: A lack of collateral inhibition during ontogeny? Neuropsychology 14:455-469.

Egar, M.W., and M. Singer (1972) The role of ependyma in spinal cord regeneration in the urodele *Triturus.* Exp. Neurol. 37:422-430.
Elliot Smith, G. (1896) Morphology of the true "limbic lobe" corpus callosum, septum pellucidum and fornix. J. Anat. Physiol. 30:185-204.
Ettlinger, G., C.B. Blakemore, A.D. Milner, and J. Wilson (1972) Agenesis of the corpus callosum: A behavioral investigation. Brain 95:327-346.
Fujisawa, H., N. Tani, K. Watanabe, and Y.I. Ibata (1982) Branching of regenerating retinal axons and preferential selection of appropriate branches for specific neuronal connection in the newt. Dev. Biol. 90:43-57.
Gaze, R.M., M.J. Keating, A. Ostberg, and S-H. Chung (1979) The relationship between retinal and tectal growth in larual xenopus: Implications for the development of the retino-tectal projection. J. Emb. Exp. Morphol, *53:103-143.*
Gott, P.S., and R.E. Saul (1978) Agenesis of the corpus callosum: Limits of functional compensation. Neurology *28* (12):1272-1279.
Guillery, R.W., H.M. Sobkowicz, and G.L. Scott (1970) Relationships between glial and neuronal elements in the development of long term cultures of the spinal cord of the fetal mouse. J. Comp. Neurol. *140:1-34.*
Hamilton, W.J., J.D. Boyd, and H.W. Mossman (1972) Human Embryology. Baltimore: Williams and Wilkins Co., pp. 484-487.
Harrison, R.G. (1914) The reaction of embryonic cells to solid structures. J. Exp. Zool. 17:521-544.
Harrison, R.G. (1935) The Croonian Lecture: On the origin and development of the nervous system studied by the methods of experimental embryology. Proc. R. Soc. Lond.]Biol.1 118:155-196.
Hatten, M.E., and R.K.H. Liem (1981) Astroglial cells provide a template for the positioning of developing cerebellar neurons in vitro. J. Cell Biol. 90:622-630.
His, W. (1889) Die Formentwicklung des menschlichen Vorderhirns vom Ende des ersten bis zum Beginne des dritten Monates. Abhandl. D. Math. Phy. Kl. Kon Sachs. Akad. Wissench. Bd. 15:675-735.
Hochstetter, F. (1929) Beitrage zur Entwicklungsgeschichte des menschlichen Gehirns. Wien und Leipzig: F. Deutiche.
Horel, J.A., and D.J. Stelzner (1981) Neocortical projections of the rat anterior commissure. Brain Res. *222:1-12.*
Innocenti, G.M., L. Fiore, and R. Caminiti (1977) Exuberant projection into the corpus callosum from the visual cortex of newborn cats. Neurosci. Lett. 4:232-242.
Innocenti, G.M., and D.O. Frost (1980) The postnatal development of visual callosal connections in the absence of visual experience or of the eyes. Exp. Brain Res. 39:365-375.
Ivy, G.O., R.M. Ahers, and H.P. Killackey (1979) Differential distribution of callosal projection neurons in the neonatal and adult rat. Brain Res. 173:532-537.
Ivy, G.O., and H.P. Killackey (1981) Anomalies of the corpus callosum in the mouse (BALB/cJ). Anat. Rec. 199:124.
Johnston, J.B. (1913) The morphology of the septum, hippocampus and pallial commissures in reptiles and mammals. J. Comp. Neurol. 23:371-425.
Kappers, C.U.A. (1908) Eversion and inversion of the dorso-lateral wall in different parts of the brain. J. Comp. Neurol. 18:433.
Kappers, C.U.A., G.C. Huber, and C.C. Crosby (1936) The Comparative Anatomy of the Nervous System of Vertebrates Including Man. Vol. II. New York: Macmillan Co., pp. 1464-1470.
Katz, M.J., R.J. Lasek, and H.J.W. Nauta (1980) Ontogeny of substrate pathways and the origin of the neural circuit pattern. Neuroscience 5:821-833.
King, L.S., and C.E. Keeler (1932) Absence of the corpus callosum, a hereditary brain anomaly of the house mouse. Proc. Natl. Acad. Sci. 18:525-528.
King, L.S. (1936) Hereditary defects of the corpus callosum in the mouse, *Mus musculus.* J. Comp. Neurol. 64:337-363.
Krayanek, S., and S. Goldberg (1981) Oriented extracellular channels and axonal guidance in the embryonic chick retina. Dev. Biol. 84:41-50.
Kromer, L.F., A. Bjorklund, and U. Stenevi (1981) Regeneration of the septohippocampal pathways in adult rats is promoted by utilizing embryonic hippocampal implants as bridges. Brain Res. 210:173-200.

Langelaan, J.W. (1908) On the development of the large commissures of the telencephalon in the human brain. Brain 31:221-241.
Lent, R. (in press) Interhemispheric connections reorganize in hamsters with callosal disgenesis. Nature.
Letoumeau, P.C. (1975a) Possible roles for cell-to-substratum adhesion in neuronal morphogenesis. Dev. Biol. 44:77-91.
Letourneau, P.C. (1975b) Cell-to-substratum adhesion and guidance of axonal elongation. Dev. Biol. 44:92-101.
Levitt, P., M.C. Cooper, and P. Rakic (1981) Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: An ultrastructural immunoperoxidase analysis. J. Neurosci. 1:27-39.
Lo, R.Y.S., and R.L. Levine (1980) Time course and pattern of optic fiber regeneration following tectal lobe removal in the goldfish. J. Comp. Neurol. *191:295-314.*
Loeser, J.D., and E.C. Alvord (1968) Agenesis of the corpus callosum. Brain *91:553-570.*
Luduena, M.A. (1973) Nerve cell differentiation in vitro. Dev. Biol. 33:268-284.
Macagno, E.R. (1978) Mechanism for the formation of synaptic projections in the arthropod visual system. Nature 275:318-320.
Mihalkovics, V. (1877) Entwicklungsgeschichte des Gehirns. Nach Untesuchungen an hoheren Wurbeltieren und den Menechen. Leipzig: W. Engelmann.
Molliver, ME., I. Kostovic, and H. Van der Loos (1973) The development of synapses in cerebral cortex of the human fetus. Brain Res. 50:403-407.
Nauta, W.J.H. and W. Haymaker (1969) In W. Haymaker, E. Anderson, and W.J.H. Nauta (eds): The Hypothalamus. Springfield, Ill.: Charles C. Thomas Publ. pp. 157-160.
Noel, P. (1973) Agenesis of the corpus callosum associated with relapsing hypothermia. Brain 96:359-368.
Nordlander, R.H., and M. Singer (1982) Morphology and position of growth cones in the developing *Xenopus* spinal cord. Dev. Brain Res. 4:181-193.
Nornes, H.O., and G.P. Das (1972) Temporal pattern of neurogenesis in spinal cord: Cytoarchitecture and directed growth of axons. Proc. Natl. Acad. Sci. USA 69:1962-1966.
Overton, J. (1979) Differential response of embryonic cells to culture on tissue matrices. Tissue Cell 11:89-98.
Paterson, J.A., A. Privet, E.A. Ling, and C.P. Leblond (1973) Investigation of glial cells in semithin section. III Transformation of subependymal cells into glial cells, as shown by radioautography after ^{3}H-thymidine injection into the lateral ventricle of the brain of young rats. J. Comp. Neural. 149:83-102.
Peters, A., S. Palay, and H. De F. Webster (1970) The Fine Structure of the Nervous System. New York: Harper and Row.
Probst, M. (1901) Uber den Bau des balkenlosen Grosshirns, sowie uber Mikrogyrie and Heterotopie der grauen Substanz. Arch. F. Psychiatr. *34:709-786.*
Roger, G., S. Lausmann, and F. Gallyas (1979) An improved silver stain for developing nervous tissue. Stain Technol. 54:193-200.
Rager, G., and B. von Oeynhausen (1979) Ingrowth and ramification of retinal fibers in the developing optic tectum of the chick embryo. Exp. Brain Res. 35:217-227.
Rager, G. (1980) Specificity of nerve connections by unspecific mechanisms? Trends Neurosci. *Feb. issue:43-44.*
Rakic, P., and P.I. Yakovlev (1968) Development of the corpus callosum and cavum septi in man. J. Comp. Neurol. 132:45-72.
Rakic, P. (1971) Neuron-glia relationships during granule cell migration in developing cerebellar cortex. A Golgi and electronmicroscopic study in *Macacus rhesus.* J. Comp. Neurol. 141:283-312.
Rakic, P., and R.L. Sidman (1973) Organization of cerebellar cortex secondary to deficit of granule cells in Weaver mutant mice. J. Comp. Neurol. *152:133-162.*
Ramon y Cajal, S. (1960) Studies on Vertebrate Neurogenesis. (trans, L. Guth.) Springfield, Ill.: Charles Thomas Publ.
Reier, P.J., and H. deF. Webster (1974) Regeneration and remyelination of *Xenopus* tadpole optic nerve fibers following transection on crush. J. Neurocytol. 3:592-618.
Reier, P.J. (1979) Penetration of grafted astrocytic scars by regenerating optic nerve axons in *Xenopus* tadpoles. Brain Res. 164:61-68.

Sapiro, J.A., J. Silver, and M. Singer (1980) The patterning of optic axons during early development in *Xenopus laevis.* Invest. Opthalmol. Vis. Sci. 19:247.
Schmechel, D.E., and P. Rakic (1979) A Golgi study of radial glial cells in developing monkey telencephalon: Morphogenesis and transformation into astrocytes. Anat. Embryol. 156:115-152.
Sensenbrenner, M., and P. Mandel (1974) Behavior of neuroblasts in the presence of glial cells, fibroblasts and meningeal cells in culture. Exp. Cell Res. 87:159-167.
Shapira, Y. (1974) Agenesis of the corpus callosum in two sisters. J. Med. Genet. 10:266-269.
Shoukimas, G.M., and J.W. Hinds (1978) The development of the cerebral cortex in the embryonic mouse: An electron microscopic serial section analysis. J. Comp. Neurol. 179:795-830.
Sidman, R.L., J.B. Angevin, and E. Taber-Pierce (1971) Atlas of the Mouse Brain and Spinal Cord. Cambridge: A Commonwealth Fund Book, Harvard Univ. Press.
Sidman, R.L., and P. Rakic (1973) Neuronal migration with special reference to developing human brain: A review. Brain Res. 62:1-35.
Sidman, R.L., and N.K. Wessels (1975) Control of direction of growth during the elongation of neurites. Exp. Neurol. 48:237-251.
Silver, J. (1977) Abnormal development of the suprachiasmatic nuclei of the hypothalamus in a strain of genetically anophthalmic mice. *J.* Comp. Neurol. *176:589-606.*
Silver, J., and R.M. Robb (1979) Studies on the development of the eye cup and optic nerve in normal mice and in mutants with congenital optic nerve aplasia. Dev. Biol. 68:175-190.
Silver, J. (1980) Mechanisms of axonal guidance during formation of CNS commissures. Neurosci. Abs. 6:487.
Silver, J., and R.L. Sidman (1980) A mechanism for the guidance and topographic patterning of retinal ganglion cell axons. J. Comp. Neural. 189:101-111.
Silver, J. (1981) Further studies on the mechanisms of axonal guidance during development of the corpus callosum. Neurosci. Abs. 7:548.
Silver, J., and J. Sapiro (1981) Axonal guidance during development of the optic nerve: The role of pigmented epithelia and other extrinsic factors. J. Comp. Neural. 202:521-538.
Silver, J., and M.Y. Ogawa (in press) Postnatally induced formation of the corpus callosum in acallosal mice using glial coated bridges which span the hemispheres. Neurosci. Abs.
Singer, M., R.H. Nordlander, and M. Egar (1979) Axonal guidance during embryogenesis and regeneration in the spinal cord of the newt. The blueprint hypothesis of neuronal pathway patterning. J. Comp. Neurol. *185:1-22.*
So, K. (1979) Development of abnormal recrossing retinotectal projections after superior colliculus lesions in newborn Syrian hamsters. J. Comp. Neurol. /86:241-258.
Sommer, I., C. Lagenaur, and M. Schachner (1981) Recognition of Bergmann glial and ependymal cells in the mouse nervous system by monoclonal antibody. J. Cell Biol. 90:448-458.
Sotelo, C., and J.P. Changeux (1974) Bergmann fibers and granular cell migration in the cerebellum of homozygous Weaver mutant mouse. Brain Res. 77:484-497.
Sperry, R.W. (1965) Embryogenesis of behavioral nerve nets. In R.L. DeHaan and H. Ursprung (eds): Organogenesis. New York: Holt, Rinehart and Winston. pp. 161-187.
Streeter, G. (1912) Development of the central nervous system. Chap. II. In: Keibel and Mall's Manual of Human Embryology. Philadelphia: J.B. Lippincott and Co.
Sturrock, R.R. (1976) Light microscopic identification of immature glial cells in semithin sections of the developing mouse corpus callosum. *J.* Anat. 122:521-537.
Sturrock, R.R. (1982) A scanning and transmission electron microscope study of the embryonic mouse telencephalon. J. Anat. 134:25-40.
Suitsu, N. (1920) Comparative studies on the growth of the corpus callosum. J. Comp. Neurol. 32:35-60.
Swanson, L.L., and W.M. Cowan (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J. Comp. Neurol. 172:49-84.
Tsai, H.M., *B.B.* Garber, and L.M.H. Larramendi (1981) ^{5}H-thymidine autoradiographic analysis of telencephalic histogenesis in the chick embryo. II. Dynamics of neuronal migration, displacement and aggregation. J. Comp. Neurol. 198:293-306.
Varon, S. (1975) Neurons and glia in neuronal cultures. Exp. Neurol. 48:93-134.

Von Szily, A. (1912) Uber die einleitenden Vorgange bei der ersten Entstehung der nervenfasem im nervus opticus. Graefes Arch. Opthalmol. *81:67-86.*
Wahlsten, D. (1974) Heritable aspects of anomalous myelinated fibre tracts in the forebrain of the laboratory mouse. Brain Res. 68:1-18. Wahlsten, D. (1982) Deficiency of corpus collosum varies with strain and supplier of the mice. Brain Res. 239:329-347.
Ware, R.W., and V. Lopresti (1975) Three dimensional reconstruction from serial sections. Int. Rev. Cytol. 40:325-434.
Warkany, J. (1971) Congenital Malformations. Chicago: Year Book Medical Publ., Inc. pp. 252-254.
Weiss, P. (1934) In *vitro* experiments on the factors determining the course of the outgrowing nerve fiber. J. Exp. Zool. 68:393-448.
Wessels, N.K., P.C. Letourneau, R.P. Nuttall, M. Luduefia-Anderson, and J.M. Geiduschek (1980) Repsonses to cell contacts between growth cones, neurites and ganglionic non-neuronal cells. J. Neurocytol. 9:647-664.
Wigglesworth, V.B. (1953) The origin of sensory neurons in an insect, *Rhodnius prolixus* (Hemiptera). Q. J. Micr. Sci. 94:93-112.
Yanoff, M. (1973) Formaldehyde-glutaraldehyde fixation. Am. J. Ophthalmol. 76:303- 304.