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Cellular and Molecular Tunnels Surrounding the Forebrain Commissures of Human Fetuses

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ABSTRACT

Glial cells and extracellular matrix (ECM) molecules surround developing fiber tracts and are implicated in axonal pathfinding. These and other molecules are produced by these strategically located glial cells and have been shown to influence axonal growth across the midline in rodents. We searched for similar cellular and molecular structures surrounding the telencephalic commissures of fetal human brains. Paraffin-embedded brain sections were immunostained for glial fibrillary acidic protein (GFAP) and vimentin (VN) to identify glial cells; for microtubule-associated protein-2 (MAP-2) and neuronal nuclear protein (NeuN) to document neurons; for neurofilament (NF) to identify axons; and for chondroitin sulfate (CS), tenascin (TN), and fibronectin (FN) to show the ECM. As in rodents, three cellular clusters surrounding the corpus callosum were identified by their expression of GFAP and VN (but not MAP-2 or NeuN) from 13 to at least 18 weeks postovulation (wpo): the glial wedge, the glia of the indusium griseum, and the midline sling. CS and TN (but not FN) were expressed pericellularly in these cell groups. The anterior commissure was surrounded by a $GFAP^+/VN^+$ glial tunnel from 12 wpo, with TN expression seen between the $GFAP^+$ cell bodies. The fimbria showed $GFAP^+/VN^+$ cells at its lateral and medial borders from 12 wpo, with pericellular expression of CS. The fornix showed $GFAP^+$ cells somewhat later (16 wpo). Because these structures are similar to those described for rodents, we concluded that the axon guiding mechanisms postulated for commissural formation in nonhuman mammals may also be operant in the developing human brain. J. Comp. Neurol. 483:375–382, 2005.

Indexing terms: extracellular matrix; corpus callosum; anterior commissure; development

An important issue in central nervous system (CNS) development is the ordered establishment of its connectivity, which consists of the directed growth of billions of axons along stereotyped pathways toward diverse but specific targets. How axonal guidance along such specific routes comes about is of special interest in developmental neurobiology. Such mechanisms are determined by experiments in nonhuman animals and require descriptive confirmation in humans.

As a result of animal experiments, many molecular and cellular cues have been uncovered, which provide signals followed by growing axons. Among them, components of the extracellular matrix (ECM) have been shown to influence axonal growth, either in a permissive or in an instructive way (Lemmon et al., 1992; Letourneau et al., 1994). Proteoglycans (PGs) are major components of the ECM during CNS development (Bicknese et al., 1994; Emerling and Lander, 1996; Margolis and Margolis, 1997), but other molecules have been identified as well (Pires-Neto et al., 1998; Shu and Richards, 2001; Joester and Faissner, 2001).

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It has been proposed that ECM molecules specify the microenvironment that surrounds growing axons and migrating cells by organizing local molecular signals. This idea has been substantiated by the finding that different molecules, which have direct effects on neurite growth and cell adhesion, bind to ECM molecules and are concentrated or reorganized by them (Grumet et al., 1993; Emerling and Lander, 1996; Bennett et al., 1997; Milev et al., 1998). Also, many ECM molecules reportedly have a spatial and temporal expression pattern that is regulated during development; these patterns are associated with the formation and segregation of different fiber tracts (Sheppard et al., 1991; Miller et al., 1995; Bartsch, 1996; Mitrofanis et al., 1997; Pires-Neto et al., 1998). In particular, PGs and tenascins (TNs) are inhibitory for some classes of growing axons (Faissner and Kruse, 1990; Snow et al., 1990b, 1991; Bovolenta and Fernaud-Espinosa, 2000) and permissive for others (Bicknese et al., 1994; Faissner et al., 1994; Götz et al., 1996). Moreover, interactions of PGs with other ECM molecules may change the effects they have on growth cones (Hynds and Snow, 2001; Snow et al., 2002). PGs form molecular barriers that are avoided by axons (Snow et al., 1990a; Steindler, 1993; Katoh-Semba et al., 1995). They may also form tunnels and boundaries that could serve to maintain growing axons packed together, as observed in the anterior commissure (AC; Pires-Neto et al., 1998) and the fornix-hippocampal commissure of developing hamsters (Braga-de-Souza and Lent, 2004). On the other hand, PGs are also present within developing tracts, as is the case with the chondroitin sulfate (CS)-enriched subplate pathway, within which thalamocortical axons must grow in order to innervate their cortical targets (Bicknese et al., 1994).

Axonal boundaries formed by extracellular glycoconjugates are transiently expressed in various developing areas of the CNS and are often associated with glial cells (Steindler, 1993; Garcia-Abreu et al., 1996a,b, 2000; Wu et al., 1998; Pires-Neto et al., 1998; Braga-de-Souza and Lent, 2004), a phenomenon first observed in the roof plate of the spinal cord and of the optic tectum (Snow et al., 1990a). We report here that the expression of ECM molecules associated with glial cells is spatially and temporally related to the emergence of the major forebrain commissures in the developing human brain. The similarity in the expression patterns of these molecules in all mammals studied thus far led us to suggest that these cells and molecules provide the three-dimensional boundaries that contribute to the guidance of growing commissural fibers in the developing human as well.

MATERIALS AND METHODS

Human fetal brains

We have studied a total of 16 human fetuses between 12 and 20 weeks postovulation (wpo), staged by inference based on the mothers' information about their last period (Table 1). The fetuses were obtained after spontaneous abortion. None had any detectable CNS malformation or lesion. Written consent was obtained from parents, and approval for the study was given by the Hôpital Saint'Anne Ethics Committee, in accordance with international regulations (Declaration of Helsinki, 2000).

TABLE 1. Summary of Cases

Case No.	Age (wpo)	Immunohistochemistry
97-38	12	VN, NF
00-242	12	VN, GFAP, MAP-2, NeuN, NF
00-72	12	VN, GFAP, CS, TN
95-34	12	VN, GFAP, CS, TN
93-109	12	VN, GFAP, CS, TN
91-308	13	VN, GFAP, CS, TN
91-255	14	VN, GFAP, CS, TN
99-203	14	VN, MAP-2, NeuN, NF
02-110	15	VN, GFAP, CS, TN
99-53	15	VN, GFAP, MAP-2, NeuN, NF
94-08	16	VN, GFAP, MAP-2, NeuN, NF
00-213	16	VN
02-104	16	VN, GFAP, CS, TN
02-126	16	VN, GFAP, CS, TN
02-345	17	VN, GFAP
02-36	20	VN, GFAP, CS, TN

Immunocytochemistry

The brains were fixed between 24 and 36 hours postmortem in a 10% formaldehyde solution containing 9 g/liter NaCl and 3 g/liter $ZnSO_4$ for variable periods, from 10 to 30 days, depending on the volume of the brain. The entire brains or the hemispheres separately were embedded in paraffin; cut into 7-μm-thick sections in the coronal, horizontal, or parasagittal planes; and mounted on glass slides. Selected sections were immunostained with primary monoclonal antibodies against vimentin (VN; 1:8; Boehringer Mannheim, Mannheim, Germany; or 1:100; Dako, Carpinteria, CA), neuronal nuclear protein (NeuN; 1:500; Chemicon, Temecula, CA), microtubule-associated protein-2 (MAP-2; 1:100; Sigma clone HM2; Sigma, St. Louis, MO), TN (1:25; Novocastra, U.K.), or Neurofilament 70 (NF; 1:400; Dako), CS (1:100; Sigma clone CS56; Sigma) or with a polyclonal antibody against the glial fibrillary acidic protein (GFAP; 1:200–1:400; Dako). All antibodies were diluted in ChemMate antibody diluent (Dako). A Streptavidin-Peroxidase Kit (Coulter, Hialeah, FL) was used to develop the reaction. In brief, slide-mounted sections were incubated for 30 minutes in citrate buffer (pH 6.0) at 98°C in a microwave oven, rinsed in distilled water, incubated in a 3% H_2O_2 solution for 5 minutes, and rinsed again in distilled water. After being washed in phosphate-buffered saline (PBS), they were treated with protein blocking agent (PBA) for 5 minutes (100 μl/slide) at room temperature, without rinsing. Then, they were incubated for 60 minutes at room temperature with the primary antibody, rinsed twice in PBS, and incubated with the biotinylated secondary antibody for 30 minutes at room temperature. To visualize the binding of the secondary antibody, slides were rinsed in PBS (2×5 minutes), incubated in streptavidin-peroxidase complex for 45 minutes, rinsed twice in PBS, and covered for 5 minutes with a freshly prepared diaminobenzidine (DAB) solution (100 μl/slide). Hematoxylin-eosin was used as counterstain.

All sections were examined under a light microscope (Axioplan; Carl Zeiss, Jena, Germany), and some were selected for photographic documentation or drawing with a camera lucida. Photographs were taken digitally using a Zeiss camera coupled to the microscope and optimized for print in Corel Draw 10.0.

RESULTS

ECM molecules and glial and neuronal markers in the developing human telencephalon

In general, the expression of VN in the telencephalon is marked in the proliferative zones surrounding the lateral ventricles and is especially pronounced at the ganglionic eminences (Fig. 1). Labeling is robust between 12 and 14 wpo and declines after wpo 19. GFAP labeling becomes evident from wpo 14 and is strong until at least wpo 20 in regions around the lateral ventricles, especially near the midline (see below), although labeling looks paler than that for VN. By wpo 17, fibers labeled with anti-NF antibody can be seen in the developing subcortical white matter and in the internal capsule, but not in any of the commissures. No staining is visible with markers for neurons, such as NeuN and MAP-2 until at least wpo 17. With regard to ECM molecules, TN is expressed specifically close to the midline and is clearly delineated in the AC and CC as early as 14 wpo. At the same ages, only weak labeling is seen with anti-CSPG immunostaining at the midline. Specific details of labeling at the midline region are described below individually for the three telencephalic commissures.

Commissural tunnel

The human anterior commissure is the first telencephalic commissure that forms across the midline and has been identified in earlier studies around wpo 8 (Rakic and Yakovlev, 1968). Tissue from this early age was not available to us, but the anterior commissure is clearly visible in our earliest brains, at 12–14 wpo (Fig. 1A). From these ages until at least 17 wpo, the anterior commissure is surrounded by a tunnel of GFAP- and VN-positive glial cells (Fig. 1B, arrows), with TN between the cell bodies (not shown). Staining for CSPG yielded negative results.

Fimbria-fornix system pathway

The first hippocampal fibers reportedly cross the midline in humans at approximately 9–10 wpo (Rakic and Yakovlev, 1968). In our material, GFAP- and VN-positive glial cells are seen crossing the fimbria transversely at 12 wpo (Fig. 2A,B). CSPG, but not the other ECM molecules, is lightly expressed pericellularly (not shown). Immunostaining for TN was absent in the fimbria, although it was expressed in the hippocampus (Fig. 2C, arrow) at this age. GFAP-expressing glial cells were seen surrounding the fornix as of 16–17 wpo. In our material, the hippocampal commissures could not be discerned.

Callosal lane

The corpus callosum is the last of forebrain commissures to form across the midline, around 12 wpo in humans (Rakic and Yakovlev, 1968). We were able to discern the glial wedge and the indusium griseum glial wall (Richards, 2002) as early as 14 wpo (Fig. 3A). The presence of a midline sling as described for rodents by Silver and collaborators (1982) was not clearly discerned (Fig. 3A), because the region was often scarcely populated by cells. Cells in the wedge, in the indusium griseum, and at the midline were positive for both GFAP (Fig. 3B) and VN (Fig. 3C), aligning dorsally and ventrally along the growing callosal axons, as has been described for rodents (Shu and Richards, 2001). However, in the human material, no neurons were labeled by either NeuN or MAP-2 at the midline, unlike what has been reported for rats by Shu and collaborators (2003). Both TN (Fig. 3D) and CSPG (not shown) are expressed pericellularly, until at least 20 wpo, between the GFAP-positive glial cells present on either side of the callosal axon bundles.

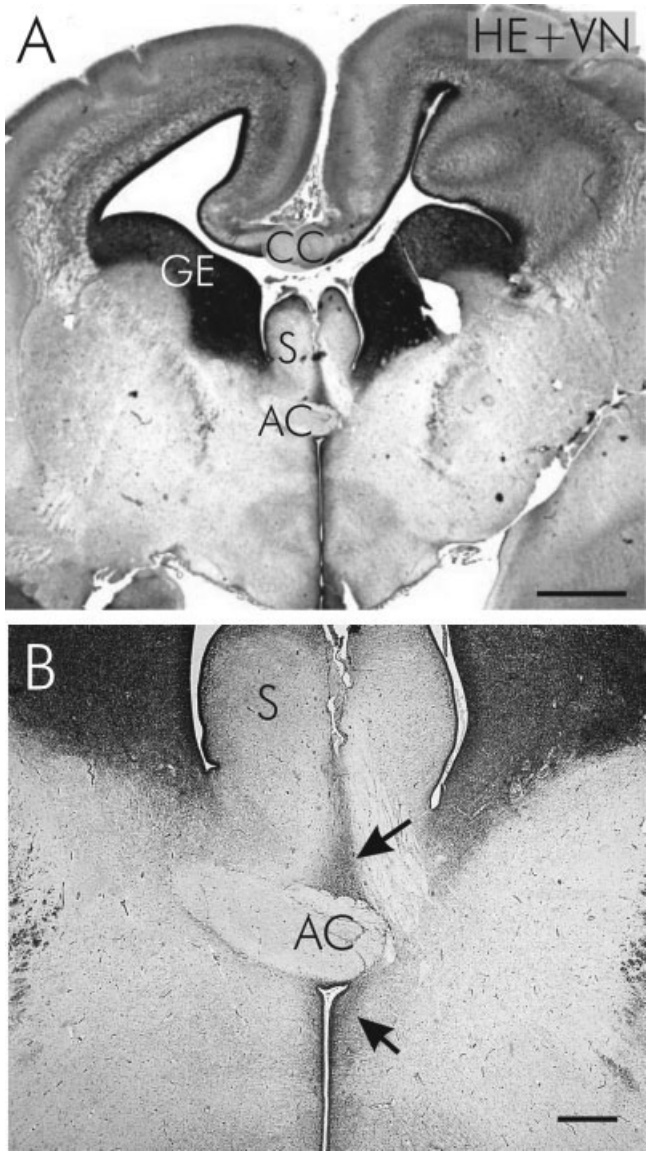
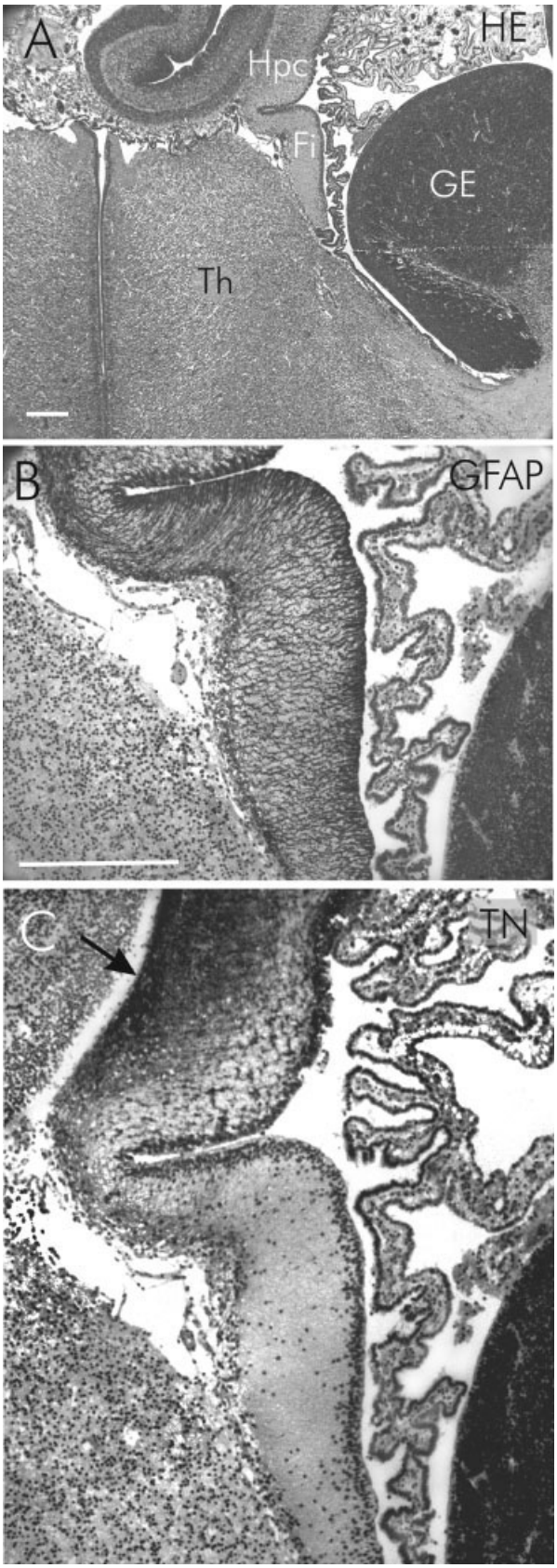


Fig. 1. Coronal sections through the forebrain of a 14 wpo human fetus immunoreacted for vimentin (VN) and stained for hematoxylin-eosin (HE). **B** is a higher magnification of section different from that in **A**. Arrows indicate the glial tunnel surrounding the AC. AC, anterior commissure; CC, corpus callosum; GE, ganglionic eminence; S, septum. Scale bars = 500 μm in A; 1 mm in B.

DISCUSSION

The present observations show that forebrain commissures in humans form in spatial and temporal correspondence with the regulated expression of ECM molecules



along the pathways that delineate these commissures. In the anterior commissure, corpus callosum, and hippocampal fimbria-fornix system, expression of ECM molecules coincides with glial cell clusters, which form tunnels or lanes bordering the fiber tracts.

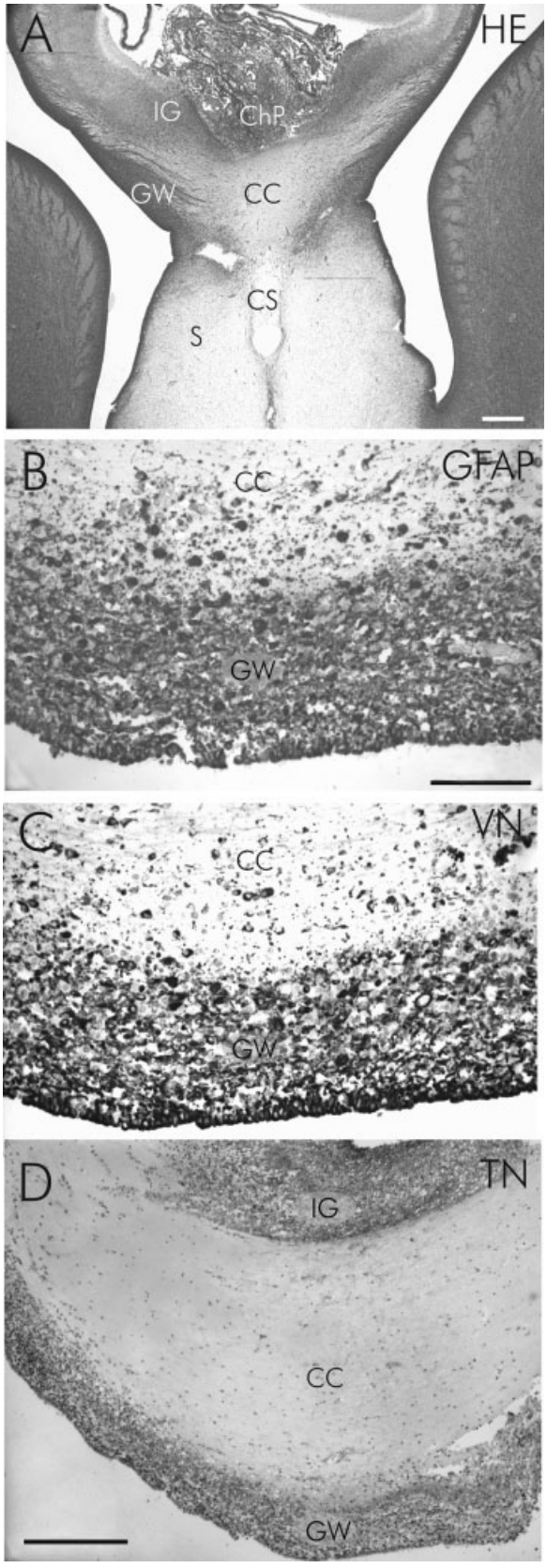
Insofar as these cellular and molecular structures have also been observed in other mammals (Silver et al., 1982, 1993; Pires-Neto et al., 1998; Shu and Richards, 2001; Richards, 2002; Braga-de-Souza and Lent, 2004), and because they participate in the guidance of developing commissural and callosal axons across the midline (Silver et al., 1982; Shu and Richards, 2001), it is likely that they have the same role in human embryonic development as well. The ECM labeling for midline fiber tracts described here and in other studies (Hoffman-Kim et al., 1998; Shu and Richards, 2001; Braga-de-Souza and Lent, 2004) is not evident for fiber tracts that do not cross the midline, such as the internal capsule; therefore, it is likely that this pattern is specifically involved in the guidance of fiber bundles across the midline and that such guidance reflects a general strategy that is developed for commissural axons.

Commissural development and molecular boundaries

The sequence of cellular and molecular events governing the growth of interhemispheric axons across the midplane is "reconstructed" below from data reported from a number of studies. When axons emerge from the somata of commissural cells, their growth cones either are repelled by semaphorins, as in the hippocampal pyramidal layer (Pozas et al., 2001), or are attracted by one semaphorin isoform and then repelled by another, as reported for the rat cerebral cortex (Bagnard et al., 1998); the net result in both cases is a deflection medially and navigation toward the midline. Other attractive molecules, such as netrin-1, are expressed within the fimbria (Barallobre et al., 2000) and reportedly contribute to the orientation of hippocampal commissural axons toward the exact point where they cross the midline. It is conceivable, therefore, that commissural growth cones find at least a permissive, if not an attractive, territory within the fimbria-fornix and the cortical white matter that drives them toward the midline. Other molecules, such as Slit-2, a secreted protein that is recognized by Robo receptors positioned at the tips of cortical growth cones, are reportedly involved in the orientation of callosal fibers toward the midline (Shu and Richards, 2001; Richards, 2002).

Because both netrin and Slit are diffusible molecules, one could question whether and how they are maintained close to their sources, in order to establish a gradient strong enough to be perceived by the growing commissural axons. One possibility is that their diffusion away from

Fig. 2. Adjacent coronal sections through the forebrain of a 12 wpo human fetus, at a level caudal to the corpus callosum, stained for hematoxylin-eosin (HE; **A**) and immunoreacted for glial fibrillary acidic protein (GFAP; **B**) and tenascin (TN; **C**). Note that radial glial cells (in B) cross the fimbria transversely, whereas tenascin (in C) is expressed in the hippocampus (arrow) until the approximate border with the fimbria. Fi, fimbria; GE, ganglionic eminence; Hpc, hippocampus; Th, thalamus. Magnification is the same for B and C. Scale bars = 1 mm in A; 500 μm in B (applies to B,C).



the midline is limited by an adjacent cellular or molecular barrier (e.g., formed by PGs associated with the netrin receptor DCC; see Bennett et al., 1997). Similarly, the question of what makes the callosal and commissural efferents remain within their tracts could be answered by the existence of organizing, growth-inhibitory structures at the borders of these tracts that could not only maintain diffusible molecules within the tracts but also inhibit axonal deviation from the mainstream of growth. Glial cells and their ECM are good candidates for exerting this function, in that they form tunnels, lanes, or simply borders around the fimbria-fornix system (Braga-de-Souza and Lent, 2004), the anterior commissure (Pires-Neto et al., 1998; this work), and the corpus callosum (Shu and Richards, 2001; this work), through which the efferent fibers grow.

Boundaries formed by glial cells and glycoconjugates are transient structures present in different sectors of rodent CNS, such as developing axon tracts, cortical layers, midline nuclei, and other regions important for axonal orientation during growth (Steindler, 1993; Höke and Silver, 1996; Mitrofanis et al., 1997). PGs associated with glial cell clusters were first observed in the roof plate of the spinal cord and superior colliculus of rodents (Snow et al., 1990b), where they act as barriers for dorsal root ganglion axons and retinal fibers during development, preventing them from crossing the dorsal midline (Wu et al., 1998). In the developing midbrain, the tectal midline region expresses and synthesizes higher levels of glycosaminoglycans than the lateral region (Garcia-Abreu et al., 1995, 1996a,b; Hoffman-Kim et al., 1998). CS expression is more concentrated at the specialized raphe glia of the tectal midline and appears after the intertectal fibers have crossed to the opposite side, but prior to the time when retinal axons, which are contained unilaterally, enter the tectum (Hoffman-Kim et al., 1998). In the developing forebrain, CS is present at the subplate and forms a substrate for thalamocortical axons (Bicknese et al., 1994; Miller et al., 1995; Meyer-Puttlitz et al., 1996; Fukuda et al., 1997), segregating them from the cortical efferents that grow in a CS-negative region at the intermediate zone.

CSPGs have also been detected in the developing hippocampus of the rat (Wilson and Snow, 2000), where they are expressed initially in the subplate and marginal zone, then in the neuropil layers of both the hippocampus and the dentate gyrus, but not in the pyramidal cell layers, a distribution complimentary to that described for semaphorins (Pozas et al., 2001). A similar pattern of CS expression has been seen in the newborn hamster hippocampus (Braga-de-Souza and Lent, 2004), except at the precise region where efferent and afferent axons leave and enter the hippocampus, respectively. Evidence for a regulated expression of other ECM molecules such as TNs in

Fig. 3. Coronal sections through the corpus callosum of a 14 wpo human fetus stained for hematoxylin-eosin (HE; **A**) and a 12 wpo fetus immunoreacted for glial fibrillary acidic protein (GFAP; **B**), vimentin (VN; **C**), and tenascin (TN; **D**). B and C show an enlarged view of the glial wedge (GW) under the corpus callosum (CC). D shows a coronal section of another brain at the same age, demonstrating that both the indusium griseum glia (IG) and the GW express tenascin, forming a lane through which the callosal fibers course. ChP, choroids plexus; CS, cavum septum; S, septum. Scale bars = 1 mm in A; 100 μm in B (applies to B,C); 200 μm in D.

the developing nervous system is less abundant (O'Brien et al., 1992; Faissner and Steindler, 1995; Pires-Neto et al., 1998) but confirms the formation of boundaries spatially and temporally associated with the development of fiber tracts.

The influence of ECM molecules on axonal guidance has been shown by many in vitro experiments (for review see Bovolenta and Fernaud-Espinosa, 2000, for CSPGs; Joester and Faissner, 2001, for TNs). The general conclusion from this work is that axons refrain from crossing the borders formed by ECM molecules (Snow et al., 1990a,b, 2001; Landolt et al., 1995; Faissner and Steindler, 1995; Höke and Silver, 1996; Garcia-Abreu et al., 1996a,b), although they may behave differently under certain conditions (Fernaud-Espinosa et al., 1994; Faissner et al., 1994; Götz et al., 1996). In addition, recent evidence indicates that ECM molecules interact with each other (Snow et al., 1996, 2002; Condic et al., 1999; Probstmeier et al., 2000a,b; Oohira et al., 2000; Hynds and Snow, 2001), provoking growth cones to behave differently. ECM molecules also bind small, diffusible molecules, such as growth factors (Friedlander et al., 1994; Milev et al., 1998), cell adhesion molecules (Grumet et al., 1993; Friedlander et al., 1994; Milev et al., 1996), and receptors of ligands that act as attractive signals for axons (Bennet et al., 1997). It is conceivable, therefore, that ECM borders along the commissural tracts are able to bind netrin-1 as well as Slit-1, facilitating their recognition by receptors at the growth cone tips and thus serving to channel the growing efferent axons toward the midline. This suggestion remains to be tested experimentally.

Commissural development in humans

The first identifiable telencephalic commissure in rodents is the anterior commissure, followed by the hippocampal commissure and then the corpus callosum (for review see Braga-de-Souza and Lent, 2004). This orderly timing of commissural development has been observed in several different mammals (Wahlsten, 1981; Ashwell et al.,1996), including humans (Rakic and Yakovlev, 1968), and probably reflects the ventral-to-dorsal and caudal-to-rostral progression of septal fusion at the midline, an important event that may be a prerequisite for commissuration. Septal fusion is a key event that allows the formation of specialized cell structures at strategic points in the telencephalic midplane, before the arrival of the pioneer commissural axons, and appears to be followed by the expression of cellular and molecular signals that direct commissural axons across the midline. In animals with delayed septal fusion, HC and CC formation is defective and retarded (Silver et al., 1982; Wahlsten, 1987; for review see Richards, 2002). This is the case for acallosal mice and acallosal humans, for example, in which a deeper longitudinal fissure separates the hemispheres, and glial structures that might support the navigation of callosal axons across the midline fail to form (Loeser and Alvord, 1968; Silver et al., 1982; Wahlsten, 1987; Hankin et al., 1988; Lassonde et al., 2003). In these instances, callosally projecting axons, being unable to cross the midline, grow ipsilaterally, forming the aberrant Probst bundle (Probst, 1901; Lent, 1981; Wahlsten, 1987; Lassonde et al., 2003) and other anomalous circuits (Tovar-Moll et al., 2004).

In rodents, GFAP-positive glial cells gather at the ventral midplane immediately after septal fusion and form a tunnel with large extracellular spaces along the developing anterior commissure (Silver et al., 1993; Cummings et al., 1997; Pires-Neto et al., 1998, 1999). In the present study, we show this sequence to hold true for humans as well. This cellular tunnel is preceded by the expression of different ECM molecules (Pires-Neto et al., 1998) and may be correlated with the early expression of netrin (Serafini et al., 1996) and Slit proteins (Bagri et al., 2002) in the same locations. Although the glial tunnel surrounding the AC of rodents has been shown to express CS and TN (Pires-Neto et al., 1998), only TN has been detected in humans (this work). For the CC, both TN and CS are expressed in rodents as well as humans.

As midline fusion advances, three different cell structures position themselves at the midline, preceding the arrival of callosal axons: the midline sling (Silver et al., 1982), the glial wedge, and the glia of the indusium griseum (IG; Shu and Richards, 2001). The glial wedge, a cluster of long radial glial processes at the ventricular wall of the corticoseptal boundary, forms a lane with the IG dorsally, where another glial population clusters. Both express GFAP and VN in humans (this work) and in rodents and thus are likely comprised of astrocytes. In rodents, the glia also express Slit-2, a molecule that is repulsive for embryonic cortical axons (Shu and Richards, 2001; Richards, 2002). The midline sling is formed slightly later than the glial wedge and the glia of the IG; it does not express Slit-2. The midline sling was originally described as being composed of glial cells (Silver et al., 1982), but recent work suggests that it may actually be formed by neurons migrating to the opposite hemisphere (Shu et al., 2003). We have searched for the expression of neuronal markers (NeuN and MAP-2) at the midline in our material but were unable to find it. The identity of cells in the midline sling in humans, therefore, remains controversial. In any case, callosal pioneer axons (DeAzevedo et al., 1997; Rash and Richards, 2001) are possibly attracted by netrin released at the midline (Serafini et al., 1996; Barallobre et al., 2000) and are funneled into the glial lane toward the midline by its repulsive, Slit-producing borders. In summary, the cellular and molecular structures surrounding the forebrain commissures in human fetuses are similar to those described for rodents, suggesting that the axon guiding mechanisms postulated for commissural formation in animals may also be operant in the developing human brain.

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LITERATURE CITED

Ashwell KWS, Waite PM, Marotte L. 1996. Ontogeny of the projection tracts and commissural fibers in the forebrain of the tammar wallaby (*Macropus eugenii*): timing in comparison with other mammals. Brain Behav Evol 47:8–22.

Bagnard D, Lohrum M, Uziel D, Puschel AW, Bolz J. 1998. Semaphorins act as attractive and repulsive guidance signals during the development of cortical projections. Development 125:5043–5053.

Bagri A, Marín O, Plump AS, Mak J, Pleasure SJ, Rubenstein JLR, Tessier-Lavigne M. 2002. Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. Neuron 33:233–248.

Barallobre MJ, Del Río JA, Alcántara S, Borrell V, Aguado F, Ruiz M, Carmona MA, Martín M, Fabre M, Yuste R, Tessier-Lavigne M, Soriano E. 2000. Aberrant development of hippocampal circuits and altered neural activity in *netrin 1*-deficient mice. Development 127:4797–4810.

Bartsch U. 1996. The extracellular matrix molecule tenascin-C: expression in vivo and functional characterization in vitro. Prog Neurobiol 49:145–168.

Bennett K.L, Bradshaw J, Youngman T, Rodgers J, Greenfield B, Aruffo A, Linsley PS. 1997. Deleted in colorectal carcinoma (DCC) binds heparin via its fifth fibronectin type III domain. J Biol Chem 272:26940–26946.

Bicknese AR, Sheppard AM, O'Leary DDM, Pearlman AL. 1994. Thalamocortical axons extend along a chondroitin sulfate proteoglycan-enriched pathway coincident with the neocortical subplate and distinct from the efferent path. J Neurosci 14:3500–3510.

Bovolenta P, Fernaud-Espinosa I. 2000. Nervous system proteoglycans as modulators of neurite outgrowth. Prog Neurobiol 61:113–132.

Braga-de-Souza S, Lent R. 2004. Temporal and spatial regulation of chondroitin sulfate, radial glial cells, growing commissural axons, and other hippocampal efferents in developing hamsters. J Comp Neurol 468: 217–232.

Condic ML, Snow DM, Letourneau PC. 1999. Embryonic neurons adapt to the inhibitory proteoglycan aggrecan by increasing integrin expression. J Neurosci 19:10036–10043.

Cummings DM, Malun D, Brunjes PC. 1997. Development of the anterior commissure in the opossum: midline extracellular space and glia coincide with early axon decussation. J Neurobiol 32:403–414.

DeAzevedo LC, Hedin-Pereira C, Lent R. 1997. Callosal neurons in the cingulate cortical plate and subplate of human fetuses. J Comp Neurol 386:60–70.

Emerling D, Lander AD. 1996. Inhibitors and promoters of thalamic neuron adhesion and outgrowth in embryonic neocortex: functional association with chondroitin sulfate. Neuron 17:1089–1100.

Faissner A, Kruse J. 1990. J1/tenascin is a repulsive substrate for central nervous system neurons. Neuron 5:627–637.

Faissner A, Steindler D. 1995. Boundaries and inhibitory molecules in developing neural systems. Glia 13:233–254.

Faissner A, Clement A, Lochter A, Streit A, Mandl C. 1994. Isolation of a chondroitin sulfate proteoglycan with neurite outgrowth promoting properties. J Cell Biol 126:783–799.

Fernaud-Espinosa I, Nieto-Sampedro M, Bovolenta P. 1994. Differential effects of glycosaminoglycans on neurite outgrowth from hippocampual and thalamic neurones. J Cell Sci 107:1437–1448.

Friedlander D, Milev P, Karthikeyan L, Margolis RK, Margolis R.U, Grumet M. 1994. The neuronal chondroitin sulfate proteoglycan neurocan binds to the neural cell adhesion molecules Ng-CAM/L1/NILE and N-CAM and inhibits neuronal adhesion and neurite outgrowth. J Cell Biol 125:669–680.

Fukuda T, Kawano H, Ohyama K, Li H-P, Takeda Y, Oohira A, Kawamura K. 1997. Immunohistochemical localization of neurocan and L1 in the formation of thalamocortical pathway of developing rats. J Comp Neurol 382:141–152.

Garcia-Abreu J, Moura-Neto V, Carvalho SL, Cavalcante LA. 1995. Regionally specific properties of midbrain glia. I. Interactions with midbrain neurons. J Neurosci Res 40:417–477.

Garcia-Abreu J, Silva LCF, Tovar FF, Onofre GR, Cavalcante LA, Moura-Neto V. 1996a. Compartmental distribution of sulfated glycosaminoglycans in lateral and medial midbrain astroglial cultures. Glia 17: 339–344.

Garcia-Abreu J, Cavalcante LA, Silva LCF, Moura-Neto V. 1996b. The extracellular matrix of the midline and non-midline midbrain glia: correlations with neurite growth-supporting abilities. Braz J Med Biol Res 29:1179–1187.

Garcia-Abreu J, Mendes FA, Onofre GR, Freitas MS, Silva LCF, Moura-Neto V, Cavalcante LA. 2000. Contribution of heparan sulfate to the non-permissive role of the midline glia to the growth of midbrain neurites. Glia 29:260–272.

Götz B, Scholze A, Clement A, Joester A, Schutte K, Wigger F, Frank R, Spiess E, Eckblom P, Faissner A. 1996. Tenascin-C contains distinct adhesive, anti-adhesive, and neurite outgrowth promoting sites for neurons. J Cell Biol 132:681–699.

Grumet M, Flaccus A, Margolis RU. 1993. Functional characterization of chondroitin sulfate proteoglycans of brain: interactions with neurons and neural cell adhesion molecules. J Cell Biol 120:815–824.

Hankin M.H, Schneider BF, Silver J. 1988. Death of the subcallosal glial sling is correlated with formation of the cavum septi pellucidi. J Comp Neurol 272:191–202.

Hoffman-Kim D, Lander A.D, Jhaveri S. 1998. Patterns of chondroitin sulfate immunoreactivity in the developing tectum reflect regional differences in glycosaminoglycan biosynthesis. J Neurosci 18:5881–5890.

Höke A, Silver J. 1996. Proteoglycans and other repulsive molecules in glial boundaries during development and regeneration of the central nervous system. Prog Brain Res 108:11.

Hynds DL, Snow DM. 2001. Fibronectin and laminin elicit differential behaviors from SH-SY5Y growth cones contacting inhibitory chondroitin sulfate proteoglycans. J Neurosci Res 66:630–642.

Joester A, Faissner A. 2001. The structure of tenascins in the nervous system. Matrix Biol 20:13–22.

Katoh-Semba R, Matsuda M, Kato K, Oohira A. 1995. Chondroitin sulphate proteoglycans in the rat brain: candidates for axon barriers of sensory neurons and the possible modification by laminin of their actions. Eur J Neurosci 7:613–621.

Landolt RM, Vaughan L, Winterhalter K, Zimmermann DR. 1995. Versican is selectively expressed in embryonic tissues that act as barriers to neural crest cell migration and axon outgrowth. Development 121: 2303–2312.

Lassonde MC, Sauerwein HC, Lepore F. 2003. Agenesis of the corpus callosum. In: Zaidel E, Iacoboni R, editors. The parallel brain. Cambridge, MA: MIT Press. p 357–369.

Lemmon V, Burden SM, Payne HR, Elmslie GJ, Hlavin ML. 1992. Neurite growth on different substrates: permissive versus instructive influences and the role of adhesive strength. J Neurosci 12:818–826.

Lent R. 1981. Neuroanatomical effects of neonatal transection of the corpus callosum in hamsters. J Comp Neurol 223:548–555.

Letourneau PC, Condic ML, Snow DM. 1994. Interactions of developing neurons with the extracellular matrix. J Neurosci 14:915–928.

Loeser J.D, Alvord EC. 1968. Agenesis of the corpus callosum. Brain 91:553–570.

Margolis R.U, Margolis RK. 1997. Chondroitin sulfate proteoglycans as mediators of axon growth and pathfinding. Cell Tissue Res 290:343–348.

Meyer-Puttlitz B, Junker E, Margolis RU, Margolis RK. 1996. Chondroitin sulfate proteoglycans in the developing central nervous system. II. Immunocytochemical localization of neurocan and phosphacan. J Comp Neurol 366:44–54.

Milev P, Maurel P, Häring M, Margolis RK, Margolis RU. 1996. TAG-1/axonin-1 is a high-affinity ligand of neurocan, phosphacan/protein tyrosine phosphatase-ζ/β and N-CAM. J Biol Chem 271:15716–15723.

Milev P, Monnerie H, Popp S, Margolis RK, Margolis U. 1998. The core protein of the chondroitin sufate proteoglycan phosphacan is a high-affinity ligand of fibroblast growth factor-2 and potentiates its mitogenic activity. J Biol Chem 273:21439–21442.

Miller B, Sheppard MA, Bicknese AR, Pearlman AL. 1995. Chondroitin sulfate proteoglycans in the developing cerebral cortex: the distribution of neurocan distinguishes forming afferent and efferent axonal pathways. J Comp Neurol 355:615–628.

Mitrofanis J, Earle KL, Reese BE. 1997. Glial organization and chondroitin sulfate proteoglycan expression in the developing thalamus. J Neurocytol 26:83–100.

Oohira A, Matsui F, Tokita Y, Yamauchi S, Aono S. 2000. Molecular interactions of neural chondroitin sulfate proteoglycans in the brain development. Arch Biochem Biophys 374:24–34.

Pires-Neto MA, Braga-de-Souza S, Lent R. 1998. Molecular tunnels and boundaries for growing axons in the anterior commissure of hamster embryos. J Comp Neurol 399:176–188.

Pires-Neto MA, Braga-de-Souza S, Lent R. 1999. Extracellular matrix molecules play diverse roles in the growth and guidance of central nervous system axons. Braz J Med Biol Res 32:633–638.

Pozas E, Pascual M, Ba-Charvet KTN, Guijarro P, Sotelo C, Chédotal A, Del Río JA, Soriano E. 2001. Age-dependent effects of secreted semaphorins 3A, 3F, and 3E on developing hippocampal axons: in vitro effects and phenotype of semaphoring 3A (–/–) mice. Mol Cell Neurosci 18:26–43.

Probst M. 1901. Ueber den Bau des balkenlosen Grosshirns, sowie uber Mikrogirie und Heterotopie der grauen Substanz. Arch Psychiatr Nervenkr 34:709–786.

Probstmeier R, Stichel CC, Muller HW, Asou H, Pesheva P. 2000a. Chondroitin sulfates expressed on oligodendrocyte-derived tenascin-R are

involved in neural cell recognition. Functional implications during CNS development and regeneration. J Neurosci Res 60:21–36.

Probstmeier R, Braunewell K, Pesheva P. 2000b. Involvement of chodroitin sulfates on brain-derived tenascin-R in carbohydrate-dependent interactions with fibronectin and tenascin-C. Brain Res 863:42–51.

Rakic P, Yakovlev PI. 1968. Development of the corpus callosum and the cavum septi in man. J Comp Neurol 132:45–72.

Rash BG, Richards LJ. 2001. A role for cingulated pioneering axons in the development of the corpus callosum. J Comp Neurol 434:147–157.

Richards LJ. 2002. Axonal pathfinding mechanisms at the cortical midline and in the development of the corpus callosum. Braz J Med Biol Res 35:1431–1439.

Serafini T, Colamarino SA, Leonardo ED, Wang H, Beddington R, Skarnes WC, Tessier-Lavigne M. 1996. Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell 87: 1001–1014.

Sheppard AM, Hamilton SK, Pearlman AL. 1991. Changes in the distribution of extracellular matrix components accompany early morphogenetic events of mammalian cortical development. J Neurosci 11:3928–3942.

Shu T, Richards LJ. 2001. Cortical axon guidance by the glial wedge during the development of the corpus callosum. J Neurosci 21:2749–2758.

Shu T, Li Y, Keller A, Richards LJ. 2003. The glial sling is a migratory population of developing neurons. Development 130:2929–2937.

Silver J, Lorenz SE, Wahlsten D, Coughlin J. 1982. Axonal guidance during development of the cerebral commissures: descriptive and experimental studies, in vivo, on the role of preformed glial pathways. J Comp Neurol 210:10–29.

Silver J, Edwards ME, Levitt P. 1993. Immunocytochemical demonstration of early appearing astroglial structures that form boundaries and pathways along axon tracts in the fetal brain. J Comp Neurol 328:415–436.

Snow DM, Lemmon V, Carrino DA, Caplan AI, Silver J. 1990a. Sulfated proteoglycans in astroglial barriers inhibit neurite outgrowth in vitro. Exp Neurol 109:111–130.

Snow DM, Steindler DA, Silver J. 1990b. Molecular and cellular characterization of the glial roof plate of the spinal cord and optic tectum: a possible role for proteoglycan in the development of axon barrier. Dev Biol 138:359–376.

Snow DM, Watanabe M, Letourneau PC, Silver J. 1991. A chondroitin sulfate proteoglycan may influence the direction of retinal ganglion cell outgrowth. Development 113:1473–1485.

Snow DM, Brown EM, Letourneau PC. 1996. Growth cone behavior in the presence of soluble chondroitin sulfate proteoglycan (CSPG), compared to behavior on CSPG bound to laminin or fibronectin. Int J Dev Neurosci 14:331–349.

Snow DM, Mullins N, Hynds DL. 2001. Nervous system-derived chondroitin sulfate proteoglycans regulate growth cone morphology and inhibit neurite outgrowth: a light, epifluorescence, and electron microscopy study. Microsc Res Techniq 54:273–286.

Snow DM, Smith JD, Gurwell JA. 2002. Binding characteristics of chondroitin sulfate proteoglycans and laminin-1, and correlative neurite outgrowth behaviors in a standard tissue culture choice assay. J Neurobiol 51:285–301.

Steindler DA. 1993. Glial boundaries in the developing nervous system. Annu Rev Neurosci 16:445–470.

Tovar-Moll F, Moll J, Bramati I, Oliveira-Souza R, Andreoiuolo PA, Lent R. 2004. Neuroplasticity in acallosal humans: a diffusion tensor imaging study. Soc Neurosci Abstr 331.16.

Wahlsten D. 1981. Prenatal schedule of appearance of mouse brain commissures. Brain Res Dev Brain Res 1:461–473.

Wahlsten D. 1987. Defects of the fetal forebrain in mice with hereditary agenesis of the corpus callosum. J Comp Neurol 262:227–241.

Wilson MT, Snow DM. 2000. Chondroitin sulfate proteoglycan expression pattern in hippocampal development: potential regulation of axon tract formation. J Comp Neurol 424:532–546.

Wu DY, Schneider GE, Silver J, Poston M, Jhaveri S. 1998. A role for tectal midline glia in the unilateral containment of retinocollicular axons. J Neurosci 18:8344–8355.