Connecting Thalamus and Cortex: The Role of Ephrins

DANIELA UZIEL,[1*] PATRICIA GARCEZ,[1] ROBERTO LENT,[1] CHRISTIANE PEUCKERT,[2] RONNY NIEHAGE,[3] FRANCO WETH,[3] AND JÜRGEN BOLZ[2]

[1]Departamento de Anatomia, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[2]Institut für Allgemeine Zoologie und Tierphysiologie, Friedrich-Schiller-Universität, Jena, Germany
[3]Nachwuchsgruppe Neurogenetik, Universitätsklinikum der Friedrich-Schiller-Universität, Jena, Germany

ABSTRACT

The complex task of wiring up the brain during embryonic development is achieved by a multitude of guidance signals acting in complex combinations to drive growing axons to their proper targets. The somatosensory system provides an extensively studied model system featuring many universal mechanisms of neural development. In rodents, it constitutes an important model to study how precise topographic connections are achieved. Recent evidence suggests that the Eph/ephrin family of guidance molecules is of pivotal importance for the development of the somatosensory system. Members of Eph/ephrin family are thought to be involved in the global presorting of thalamic axons projecting to the cortex, in labeling specific cortical areas for innervation, in providing topographic cues within the target area, and in distinguishing cortical layers for intracortical wiring. The Eph/ephrin system also seems to contribute to the formation of specific corticothalamic feedback projections. So far, the functions of only a few members of the Eph/ephrin family have been examined, but expression analysis indicates complex combinatorial effects of these signaling molecules. Understanding the Eph/ephrin wiring code is expected to yield new insights into the development and plasticity of brain circuits involved in higher functions.

Keywords: somatosensory cortex; ventrobasal complex; pathfinding; development; ephrin

The mature rat brain contains about 200 million neurons (Herculano-Houzel and Lent, 2005) richly connected to form a very precise network that enables its function. Connections between these cells are established during embryonic development and refined at early postnatal ages. During cortical development, even before finishing migration, young neurons extend individual axons that grow in the developing white matter in search of their target (Auladell et al., 1995). Growing axons are thin processes (around 1 μm in diameter) tipped by growth cones, motile structures that express a large variety of receptors recognizing guidance cues distributed in their environment (reviewed by Dickson, 2002; Kiryushko et al., 2004). Growth cone area varies between 60 to 300 μm^2 according to rapidly extending, pausing, and exploratory behaviors (Szebenyi et al., 1998). Such microscopic structures must often navigate through macroscopic distances and make multiple directional choices to be able to establish long projections. The development of such projections can be subdivided into sequential steps, each related to intermediate targets along the fiber tract. Intermediate as

*Correspondence to: Daniela Uziel, MD, PhD, Departamento de Anatomia, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, 21941-590 Rio de Janeiro, Brazil. E-mail: daniuzi@anato.ufrj.br

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well as final targets express different sets of guidance and growth-promoting molecules that direct fibers along their way. Growing axons recognize and innervate their targets with a remarkable precision, establishing highly ordered connections in the nervous system.

The rodent trigeminal system provides an excellent model for studying how these precise connections are formed at different levels of this pathway and how they ultimately generate sensory perception leading to behavior. Mystacial whiskers are arranged in a highly stereotypical pattern forming five rows in the rodent snout. Whisker hair follicles are surrounded by peripheral endings of trigeminal sensory neurons, which detect movement of the vibrissae. The axons of these neurons terminate in the trigeminal nuclei of the brainstem, forming clusters called barrelettes, a pattern that replicates the array of whiskers on the snout. This point-to-point precision is maintained through all levels of processing, so that projections to the ventrobasal complex (VB) of the thalamus are also segregated in clusters called barreloids. The VB neurons then project to the primary somatosensory cortex (S1), where their axon terminals form patches in layer 4 that are surrounded by cortical neurons, the so-called barrels (Woolsey and Van der Loos, 1970; Welker and Van der Loos, 1986). Neurons from S1 reciprocally innervate thalamic nuclei and also connect the secondary somatosensory cortex, motor cortex, contralateral somatosensory cortex, striatum, and pons (Akers and Killackey, 1978; Carvell and Simons, 1987; Welker et al., 1988; Deschênes et al., 1998). Although less well studied, reciprocal connections from S1 to VB are also topographically organized and are likely to be of comparable importance for function, constituting a feedback loop of sensory information.

In most species, sensory thalamic afferents innervate cortical areas during the embryonic period. In the last decade, several groups have studied the development of thalamocortical pathways in order to elucidate the rules for topographic matching between these two structures. It is now clear that growing thalamic fibers are presorted very early during development, already along their way to the telencephalon (Molnar et al., 1998b), and are guided by strategically located molecular cues (for recent reviews, see Garel and Rubenstein, 2004; Vanderhaeghen and Polleux, 2004). In this review, we will correlate the time schedule for the outgrowth of thalamocortical and corticothalamic axons with the expression of ephrins and discuss their potential roles for the establishment of connections between thalamus and cortex and in the formation of whisker maps.

TIME SCHEDULE FOR DEVELOPMENT OF THALAMOCORTICAL AND CORTICOTHALAMIC PROJECTIONS IN THE MOUSE

To better relate the expression of guidance molecules and the development of thalamocortical and corticothalamic projections, we compiled an abridged time schedule of neurogenesis and axogenesis of cortical layers 4, 5, and 6 (the site for subcortical recipient and projecting cells), and of the thalamic ventrobasal complex in the mouse (Fig. 1).

Thalamic cells that will form the ventrobasal nucleus are born between embryonic day 10 (E10) and E13 (Angevine, 1970). The developmental sequence of axonal extension has been examined using DiI labeling techniques (Senft and Woolsey, 1991; Agmon et al., 1993; Auladell et al., 2000; Deng and Elberger, 2003). Thalamocortical fibers first penetrate the internal capsule as early as E12, and 1 day later the pioneer thalamocortical axons reach the neocortical intermediate zone. At E14, afferents invade the subplate (Auladell et al., 2000). As first described in monkeys and cats (Rakic, 1977; Ghosh and Shatz, 1992), before thalamocortical fibers enter their appropriate cortical targets, they accumulate and wait in the subplate zone. The exact duration of the waiting period in rodents is still somewhat controversial (Catalano et al., 1991, 1996; Molnar et al., 1998a,b; Deng and Elberger, 2003), because it is much shorter than in larger mammals. Stellate cells in layer 4 are the main target of thalamocortical afferents. As those cells are only born between E13 and E16 (Caviness, 1982; Polleux et al., 1997), thalamocortical axons start to invade layer 4 only after the waiting period within the following days, when most layer 4 cells have migrated out from the ventricular zone to reach their final position in the cortical plate.

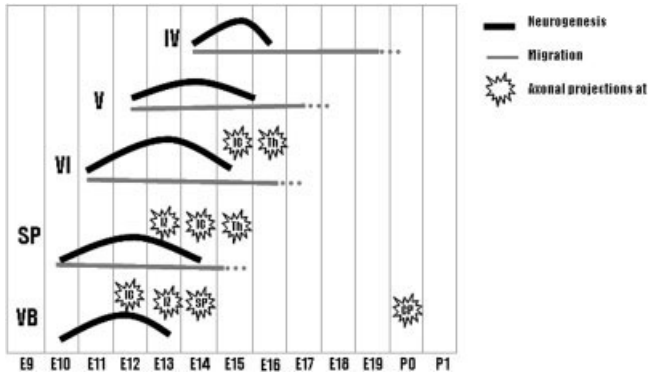

Fig. 1. Time schedule for neurogenesis and axogenesis of deep cortical layers, subplate, and the ventrobasal complex of the thalamus. The dark curves represent the time course of neurogenesis. The gray lines show the migration period. The stars point to the moment when layer VI, subplate (SP), and ventrobasal (VB) axons first reach the intermediate zone (IZ), internal capsule (IC), thalamus (Th), subplate (SP), and cortical plate (CP).

Using anterograde DiI tracing, Senft and Woolsey (1991) identified the earliest fibers in layer 4 at postnatal day 0 (P0), in accordance with Agmon et al. (1993), who placed a DiI crystal in the cortical plate at P0 and were able to detect labeled cells in VB. Rebsam et al. (2002) took advantage of a transient expression of the serotonin transporter (5-HTT) in VB axons to study thalamocortical projections by immunohistochemistry. According to these authors, a network of 5-HTT–positive fibers is already visible in layer 6 at P0. At P2, thalamocortical fibers form two continuous tangential bands in layers 4 and 6, respectively. Later, from P2 to P5, a refinement of thalamocortical arbors takes place in the somatosensory cortex, forming patched barrel arbors in layers 4 and 6 (Rebsam et al., 2002).

Birth dates of cortical layers in different cortical areas in mice were described by Caviness (1982) and recently detailed by Polleux et al. (1997), who performed [^{3}H]-thymidine injections from E10.5 to E18.5. Neurons that

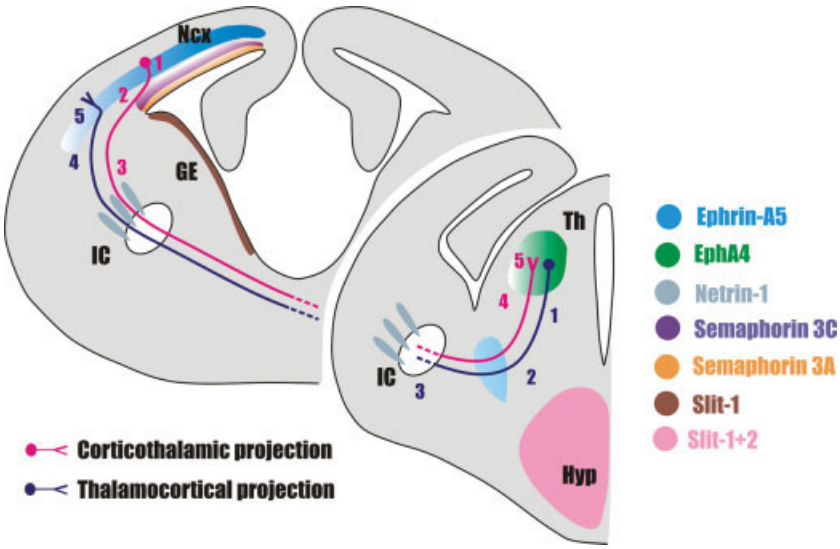

Fig. 2. The five steps in the trajectory of growing thalamocortical and corticothalamic fibers as described in the text. The expression of guidance molecules is related to each of these steps: Slit (Bagri et al., 2002) to the emergence of thalamic axons out of the diencephalon and in the ventral telencephalon, an ephrin-A5 gradient in the ventral telencephalon for axonal presorting, Netrin-1 as an attractive factor for both populations in the internal capsule (Braisted et al., 2000), semaphorins 3A and 3C for cortical fibers to penetrate the intermediate zone (Bagnard et al., 1998), and EphA4 in the thalamus and ephrin-A5 in the cortex for the establishment of topographic connections (Gao et al., 1998; Mackarehtschian et al., 1999; Vanderhaeghen et al., 2000). Th, thalamus; Hyp, hypothalamus; IC, internal capsule; GE, ganglionic eminence; Ncx, neocortex.

form layer 6 are born between E10.5 and E15 (Polleux et al., 1997), and they arrive at their final position around E17 (Caviness, 1982). Layer 5 cells are born between E12 and E15.5 (Polleux et al., 1997) and migrate to the cortical plate until at least E18. It is believed that the first cell population to extend corticothalamic axons are the subplate neurons, followed shortly afterward by axons from the developing cortical plate (McConnell et al., 1989; De Carlos and O'Leary, 1992; Auladell et al., 2000). At E12, cortical axonal processes extend laterally and course in the intermediate zone parallel to the pial surface. By E13.5, the first CT axons cross the pallial-subpallial boundary and navigate laterally toward the lateral ganglionic eminence. At E14, they reach the internal capsule and arrive within the thalamus on the subsequent day (Metin and Godement, 1996; Auladell et al., 2000).

A SMALL STEP FOR AN AXON, A GIANT LEAP FOR BRAIN CIRCUITRY

Growing axons that form long projections in the nervous system are attracted or repelled by guidance cues expressed by intermediate and final targets. The existence of intermediate targets has been reported in the development of several fiber bundles, including the thalamocortical and corticothalamic projections (Garel and Rubenstein, 2004). The long pathway between cortex and thalamus can be fragmented into single steps in which different guidance molecules act (Fig. 2). For instance, dorsal thalamic cells that will constitute thalamic relay nuclei for sensory processing information extend axons that must (1) grow ventrally toward the thalamic border, (2) turn laterally to exit the thalamus, (3) form and penetrate the internal capsule to reach the cortical intermediate zone, and (4) then choose their correct cortical area to (5) innervate it. Intermediate targets can also be defined for corticofugal axons as well, independently of their subcortical destinations. These axons must first (1) grow ventrally toward the intermediate zone, then (2) turn laterally, and then (3) exit the dorsal telencephalon through the internal capsule. Corticofugal axons innervating the thalamus, once in there, must (4) choose the correct thalamic nucleus (or nuclei) to (5) innervate it. For each of these steps, diffusible and substrate-bound factors expressed by intermediate targets act as directional cues for specific populations of growing axons.

Some of the guidance molecules produced by these intermediate targets have been characterized and identified (Fig. 2). In the case of corticofugal projections, axons first are attracted to the intermediate zone by semaphorin 3C expressed in the subventricular zone, but they never penetrate the proliferative zones because they are repelled by semaphorin 3A expressed by ventricular zone cells (Bagnard et al., 1998). The molecules that guide the following step in this pathway—to turn laterally in the intermediate zone—are still unknown, but the signals that direct cortical fibers to reach the thalamus are probably the same that orient thalamic axons through the internal capsule into the telencephalon. Cell-cell interactions between thalamic and cortical axons determine fasciculation or even retraction of growing fibers, being essential in the development of this pathway (Molnar and Blakemore, 1995; Bagnard et al., 2001).

A similar interplay between attractive and repulsive guidance molecules in the first steps after axonal emergence was revealed for thalamocortical fibers. Thalamocortical axons make a lateral turn at the ventral border of the thalamus as a result of the balance between attractive cues in the internal capsule (Netrin-1) and repulsive cues

in the hypothalamus (Braisted et al., 1999; Bagri et al., 2002) and ganglionic eminences (Metin and Godement, 1996; Braisted et al., 1999, 2000; Bagri et al., 2002). This hypothesis is strongly supported by studies of the thalamocortical pathway in mutant mice strains (*ebf1*, *dlx1*, and *dlx2* knockouts) with abnormal ganglionic eminences. A population of thalamic axons is misrouted and penetrates the amygdala ($ebf1^{-/-}$), or stalls in the developing basal ganglia ($dlx1^{-/-}$ and $dlx2^{-/-}$), and shifts its rostrocaudal distribution in cortical regions (Garel et al., 2002). Recent data from Niehage et al. (2005) point to the expression of some ephrins and Eph receptors in the hypothalamus, which may also contribute as repulsive cues to avoid thalamic fibers to extend ventrally. Moreover, the expression of ephrins in the cortex and Eph receptors by thalamic cells seems to play an important role for thalamic axons to choose their correct cortical target area (Gao et al., 1998; Mackarehtschian et al., 1999; Vanderhaeghen et al., 2000; Uziel et al., 2002); this will be discussed later in this review.

WHO IS WHO IN THE EPH/EPHRIN FAMILY

The Eph receptors constitute the largest known family of receptor tyrosine kinases. Currently, 16 Eph receptors and 8 ephrin ligands have been identified in vertebrates. Group A ligands (ephrin-A1 to A5) are GPI-linked proteins that bind promiscuously to group A receptors (EphA1 to A8), whereas group B ligands (ephrin-B1 to B3) are transmembrane proteins that bind promiscuously to group B receptors (EphB1 to B4, EphB6). Cross-binding between A-receptors and B-ligands and vice versa occurs to a limited extent (Lemke, 1997; Klein, 2004). A striking feature of the ephrin system is that signaling can also occur via the ligands. This so-called reverse signaling has been initially described for the group B ligands, but there is now recent evidence that group A ligands can also mediate a cellular response after contact with Eph receptors (reviewed in Davy and Sorriano, 2005). Eph receptors and their ligands have been implicated as mediators of a variety of developmental events and their role in axonal guidance has received special attention (for recent reviews, see Klein, 2004; Vanderhaeghen and Polleux, 2004). Members of the Eph/ephrin act by modulating cell affinity to substrates or to neighboring cells by regulating cytoskeleton proteins (Noren and Pasquale, 2004).

In the developing central nervous system, ephrin ligands and their receptors are extensively expressed and contribute to the formation of connections between brain regions (see, for example, Tuzi and Gullick, 1994; Drescher et al., 1995; Gao et al., 1998; Mackarehtschian et al., 1999; Vanderhaeghen et al., 2000; Uziel et al., 2002; Dufour et al., 2003). In order to identify candidate molecules of the Eph/ephrin family, which might contribute to the development of the thalamocortical system, we performed a comprehensive in situ hybridization screening survey for transcripts of all known members of the family at critical developmental stages of the somatosensory and frontal cortical pathways (Niehage et al., 2005). All A and B ephrin ligands, seven out of eight EphA receptors, and three out of five EphB receptors were found to be expressed in these systems between E14 and P6. The expression patterns are highly differential in space and time. Specific combinations of Eph/ephrin molecules characterize each telencephalic layer as well as individual thalamic nuclei at different developmental stages. This complexity of the expression patterns supports a view of the Eph/ephrin system being of fundamental importance for thalamocortical brain wiring.

EPHRINS DIRECT THALAMIC AXONS TO FIND THEIR CORTICAL TARGET

In the past years, the role of ephrins in the development of retinotectal projections has been extensively explored. Pioneering studies from Drescher et al. (1995) identified a graded expression of ephrins (originally called repulsive axonal guidance signal or RAGS) in the optic tectum, and further work from this and other groups brought insight into their mechanism of action and intracellular cascades (see, for example, Nakamoto et al., 1996; Monschau et al., 1997; Yates et al., 2001; Hansen et al., 2004; Knoll and Drescher, 2004; Rashid et al., 2005). It is now generally accepted that in the retina and optic tectum, ephrins and Eph receptors can act as repulsive guidance cues distributed in opposing gradients.

The global topography of thalamocotical projections might be established by a similar mechanism as in the retinotectal system. Receptors EphA3, A4, and A5 are present in E13–15 dorsal thalamus forming a gradient of high rostromedial to low caudolateral expression. At the same time, there is a complementary transient gradient of ephrin-A5 expression in the ventral telencephalon (Dufour et al., 2003). This might lead to a presorting of thalamic axons after they leave the thalamus and grow through the ventral telencephalon toward the cortex. Thus, thalamic fibers from anterior nuclei, which express high EphA receptor concentrations, are prevented to reach the posterior cortex, because of high concentration of ephrin A in the posterior ventral telencephalon. This global early anterior-posterior sorting may be a prerequisite for the precise innervation of specific cortical areas.

Cortical areas are characteristic functional units of the cerebral cortex, which were defined by cytoarchitectural and functional features. Each cortical area receives projections from specific thalamic nuclei. For instance, the mediodorsal and laterodorsal thalamic nuclei project to limbic cortical areas (Domesick, 1972; Van Groen and Wyss, 1992), whereas the lateral thalamic nuclei, such as the lateral geniculate, the ventrobasal complex, and the ventrolateral nucleus, project respectively to primary visual, somatosensory, and motor areas (for review, see Lopez-Bendito and Molnar, 2003). In the last decade, many theories have been proposed to explain to what extent areal identity exists innately or is influenced by the arrival of thalamic projections and neuronal activity. Considerable evidence suggests that at least the initial cortical parcellation is regulated by molecular determinants intrinsic to the cortex and therefore independent of extrinsic information. Thus, much effort was concentrated in defining molecular labels that would characterize these emerging cortical areas. Most of these markers are differentially expressed between cortical areas before thalamic afferents have invaded the cortical plate [LAMP (Barbe and Levitt, 1992; Pimenta et al., 1996), H2Z1 (Cohen-Tannoudji et al., 1994), Tbr-1 (Bulfone, et al., 1995), cadherins (Nakagawa et al., 1999)] or even in the complete absence of thalamocortical projections (Miyashita-Lin et al., 1999; Nakagawa et al., 1999). Postnatal thalamic innervation and local environmental cues can, however, al-

ter the expression of some of these proteins (Gitton et al., 1999). Early expression of area-specific molecules suggests that they may also participate in guidance of axons to grow into the appropriate cortical area, but it is not yet clear if they also contribute to intra-areal topography. The establishment of mature connections is probably the result of an interplay between early expressed guidance molecules and activity of thalamic afferents and cortical circuits (Crowley and Katz, 2000; Iwasato et al., 2000; Erzurumlu and Kind, 2001).

Ephrins have been implicated to contribute to the interareal specificity of thalamocortical connections. For instance, during the embryonic period, ephrin-A5 is expressed in the subplate and cortical plate of several neocortical areas, but not in adjacent limbic cortical regions, where LAMP is expressed (Gao et al., 1998; Mackarehtschian et al., 1999; Vanderhaeghen et al., 2000). In vitro, ephrin-A5 inhibits the outgrowth and acts as a repulsive cue for axons that normally innervate limbic cortical areas, but has no effect on thalamic fibers projecting to neocortical areas (Gao et al., 1998; Mann et al., 2002). Placing crystals of DiI into S1 of ephrin-A5 knockout and wild-type animals, we described a miswired projection from dorsomedial thalamic nuclei to S1 at P8. These results suggest that in the absence of ephrin-A5, thalamic fibers that normally innervate limbic regions reroute and invade neighboring neocortical areas (Uziel et al., 2002). Dufour et al. (2003) looked for miswiring of thalamic axons in older (P14 to P18) ephrin-A5 knockout animals, but had negative results. This may suggest that these abnormal connections are transient, being eliminated and corrected before adulthood. However, in the same study, these authors reported ectopic labeling patterns in the ventrolateral nucleus after injections of DiI in S1 of ephrin-A5/EphA4 double knockouts. Based on these indirect evidences, we suggest a synergistic role of ephrin-A5 and LAMP to direct limbic thalamic axons toward the cingulate cortex and to avoid the S1 region (Mann et al., 1998; Bolz et al., 2004).

GRADIENTS OF EPHRINS IN S1 AND FORMATION OF BODY MAP

Ephrins and Eph receptors are not only involved in interareal topography, but also in intra-areal topography in which a map representing the entire body surface is formed. Using in situ hybridization assays, Vanderhaeghen et al. (2000) described a graded distribution (medial > lateral) of ephrin-A5 in the cortical plate between E18 to P3 and EphA4 in the thalamus (ventromedial > dorsolateral) at similar developmental stages. They hypothesized that somatotopic map orientation in S1 could be achieved by a repellent ligand-receptor interaction in thalamocortical innervation, because the orientation of the EphA4 gradient is such that levels are highest in the part corresponding to the snout, represented laterally in S1, where ephrin-A5 concentration is low. In addition, Vanderhaeghen et al. (2000) demonstrated in vitro that axons from the ventromedial third of VB grow preferentially on stripes lacking ephrin-A5. Again, as first shown for the retinotectal system, cells expressing high levels of receptors connect with regions that show low levels of ligands. To test for any disturbance of thalamocortical path-finding in animals lacking ephrin-A5, these authors employed anterograde labeling, but were unable to find any gross abnormality in topographic arrangement or area specificity. Vanderhaeghen and collaborators (2000) found, however, significant changes in barrel dimensions. Barrels in the medial portion of S1 were contracted, whereas barrels in the lateral regions of S1 were expanded. To further investigate the functional impact of this deformed barrelfield, Prakash et al. (2000) used optical imaging of young adult mice to study the organization of S1 in ephrin-A5 knockout animals. The representation of medial barrels appeared more compressed and more overlapping in ephrin-A5 knockout mice in comparison to controls, confirming a functional distortion of the map. Prakash et al. (2000) also performed retrograde injections in S1 after optical recordings to examine if functional distortions could be due to finer changes in barreloid-to-barrel connection patterns. They reported a normal staining in VPM and a preserved spatial topography of barreloids, with no indications of gross abnormalities in the point-to-point specificity of thalamic connections associated to the absence of ephrin-A5. Corticothalamic projections were, however, not investigated.

EPHRINS MEDIATE LAYER-SPECIFIC WIRING

Different classes of molecules, such as neurotrophic factors, cell adhesion molecules, semaphorins, and ephrins, are expressed in layer-specific patterns during development. These factors are believed to regulate the targeting and branching of specific populations of axons (Bolz et al., 2004). Particularly striking is the layer-specific expression of the Eph/ephrin family during early postnatal development. For instance, ephrin-A5 is expressed robustly in layer 4 and 6 when thalamic axons arborize in the cortex. In vitro, ephrin-A5 acts as a branch-promoting factor for thalamic fibers projecting to neocortical areas: thalamic axons grown on membrane carpets of layer 4 cells or ephrin-A5–producing cells have more branches than on layer 5 or control substrates (Mann et al., 2002). In vivo experiments have shown that thalamic fibers from ephrin-A5 knockout animals stop normally in layer 4, but their pattern of arborization is altered, showing less complex arbors (Muehlfriedel et al., 2000). This suggests that ephrin-A5 in layer 4 is not a stop signal for thalamic axons, but rather acts as a branch-promoting factor. So, this molecule regulates arborization of thalamic terminals in the correct cortical target layer, as first suggested by Castellani et al. (1998) for the establishment of intracortical connections.

So far very little is known about the molecular signals that define individual cortical layers. Previous work from Castellani et al., 1998; Castellani and Bolz, 1999 revealed that the pattern of intracortical projections is due to a layer-specific distribution of extracellular molecule: neurotrophin-3 and ephrin-A5 promote axonal branching of layer 6 axons, which target neurotrophin-3 and ephrin-A5 expressing layers in vivo (layer 4), and that it inhibits branching of layers 2/3 axons, which avoid layer 4. Many other members of the Eph/ephrin family show a layer-specific distribution during postnatal stages when thalamocortical arbors and intrinsic cortical connections are elaborated (Niehage et al., 2005). The possible functional importance of these molecules for the interlaminar cortical wiring is not known.

CONNECTING CORTEX TO THALAMUS: ARE EPHRINS ALSO INVOLVED IN DEVELOPMENT OF RECIPROCAL PROJECTIONS?

It is generally accepted that all neocortical areas receive inputs from the thalamus and reciprocate them. Although there is a point-to-point projection from VB to S1, projections from the barrel cortex back to the thalamus are not restricted to the corresponding barreloids in VB. Instead, corticothalamic projections originating from a single barrel contact a group of barreloids belonging to one arc. Axons from the barrel cortex also innervate the reticular and posterior thalamic nuclei as well as the midbrain superior colliculus, in which projections from a series of barrels converge to a common termination site (Welker et al., 1988). The corticothalamic feedback loop is established early in development by the same time layer 4 cells are being innervated by thalamic axons (Auladell et al., 2000).

Corticothalamic projections arise exclusively from pyramidal cells in layers 5 and 6. According to Clasca et al. (1995), layer 5 axons are the first to project to the thalamus, and they are followed by the development of layer 6 projections (Clasca et al., 1995; see also Sur and Leamey, 2001).

Deschênes et al. (1998) postulated that not all the connections between cortex and thalamic nuclei are reciprocal. These authors divide descending projections to the thalamus in two different categories. One, layer 6 cells project to the sensory thalamic nuclei and distribute branches to the reticular nucleus. Two, layer 5 cells primarily send axons to the brainstem or to the spinal cord. However, these axons also give branches to higher-order intralaminar and association nuclei, but not to the reticular or to the sensory relay nuclei. Thalamic projections from layer 6 cells are the most numerous. It is also possible to identify subpopulations of cells in layer 5 that project to different targets. For example, callosal cells in the infragranular laminae are small pyramidal neurons located in lower layer 5 and upper layer 6, whereas subcortical projecting cells are large pyramidal neurons situated predominantly in the upper regions of layer 5 (Hersch and White, 1982; Hubener and Bolz, 1988). These large layer 5 cells are those having collateral projections into higher-order thalamic nuclei (reviewed in Jones, 2002; Sherman and Guillery, 2002). This suggests that differential expression of Eph/ephrin family members in layers 5 and 6, and even within layer 5 subpopulations, could match complementary expression profiles in different thalamic nuclei to guide growing corticothalamic axons to their appropriate destination.

Results of our in situ hybridization screening survey indeed support the notion that Eph/ephrins are involved in the formation of the corticothalamic projection. At P6, the upper half of layer 5 and layers 2/3 express EphA5 (Fig. 3). EphA5 expression can already be detected in the cortical plate at E16 of the somatosensory cortical area. At E18, when layer 6 can first be discerned in mice, it becomes evident that this layer is actually not labeled with EphA5 riboprobes. At these embryonic stages, the VB complex, but not the surrounding higher-order nuclei, expresses ephrin-A5. It is therefore conceivable that layer 6 axons, being devoid of EphA5, can invade the ephrin-A5 expressing VB complex. Somatosensory layer 5 axons, however, by virtue of their EphA5 expression, avoid VB and are diverted to higher-order associative nuclei, which are ephrin-A5–negative.

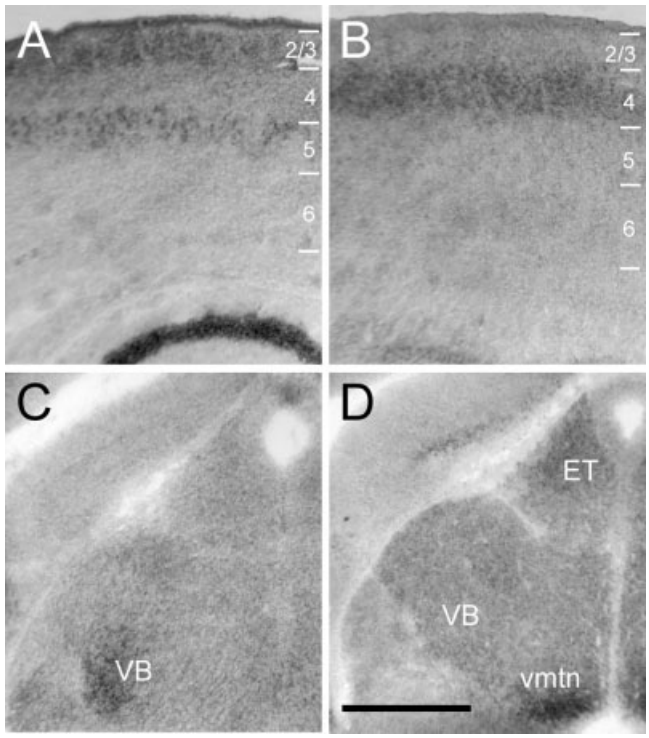

Fig. 3. Examples of the highly differential expression of Eph/ephrin genes during thalamocortical development. Expression patterns are visualized by in situ hybridization with digoxigenin-labeled antisense RNA probes on coronal cryostat sections of P6 cortex (**A** and **B**) and E16 thalamus (**C** and **D**) of the mouse. A: At P6, EphA5 is selectively expressed in layers 2/3 and the upper region of layer 5 of the somatosensory cortex (S1). B: At the same time, EphA8 is a differential marker of cortical layer 4. C: At E16, ephrin-A5 is expressed in the ventrobasal thalamic nucleus (VB). D: At the same time, VB does not express ephrin-B3, which instead is prominent in the epithalamus (ET) and ventromedial associative nuclei (vmtn). Scale bar = 500 μm.

CONCLUSIONS

This review discusses the temporal and spatial distribution of Eph receptors and ligands, and how they may influence the development of thalamocortical connections of the somatosensory system. Gradient distribution of Eph receptors in the thalamus and ephrins in the ventral telencephalon contributes to an early sorting of fibers, even before entering the cerebral cortex. Differential expression of subtypes of ephrins in the dorsal telencephalon provides cues for area-specific targeting of thalamocortical projections. Moreover, gradients of ephrins within specific cortical areas are instrumental for intra-areal topographic mapping. We provide new data showing that ephrin expression confined to individual cortical layers contribute not only to the laminar specificity of thalamocortical projections and intrinsic cortical circuits but also to corticofugal connections. A more detailed understanding of the Eph/ephrin wiring code is expected to lead to better insights into the development of the architecture of the brain circuitry involved in higher functions.

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LITERATURE CITED

Agmon A, Yang LT, Odowd DK, Jones EG. 1993. Organized growth of thalamocortical axons from the deep tier of terminations into layer-IV of developing mouse barrel cortex. J Neurosci 13:5365–5382.

Akers RM, Killackey HP. 1978. Organization of corticocortical connections in parietal cortex of rat. J Comp Neurol 181:513–537.

Angevine JB Jr. 1970. Time of neuron origin in the diencephalon of the mouse: an autoradiographic study. J Comp Neurol 139:129–187.

Auladell C, Perez-Sust P, Super H, Soriano E. 2000. The early development of thalamocortical and corticothalamic projections in the mouse. Anat Embryol 201:169–179.

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.

Bagnard D, Chounlamountri N, Puschel AW, Bolz J. 2001. Axonal surface molecules act in combination with semaphorin 3A during the establishment of corticothalamic projections. Cereb Cortex 11: 278–285.

Bagri A, Marin 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.

Barbe MF, Levitt P. 1992. Attraction of specific thalamic input by cerebral grafts depends on the molecular identity of the implant. Proc Natl Acad Sci USA 89:3706–3710.

Bolz J, Uziel D, Muhlfriedel S, Gullmar A, Peuckert C, Zarbalis K, Wurst W, Torii M, Levitt P. 2004. Multiple roles of ephrins during the formation of thalamocortical projections: maps and more. J Neurobiol 59:82–94.

Braisted JE, Tuttle R, O'Leary DDM. 1999. Thalamocortical axons are influenced by chemorepellent and chemoattractant activities localized to decision points along their path. Dev Biol 208:430–440.

Braisted JE, Catalano SM, Stimac R, Kennedy TE, Tessier-Lavigne M, Shatz CJ, O'Leary DDM. 2000. Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. J Neurosci 20:5792–5801.

Bulfone A, Smiga SM, Shimamura K, Peterson A, Puelles L, Rubenstein JLR. 1995. T-brain-1: a homolog of brachyury whose expression defines molecularly distinct domains within the cerebral-cortex. Neuron 15:63–78.

Carvell GE, Simons DJ. 1987. Thalamic and corticocortical connections of the 2nd somatic sensory area of the mouse. J Comp Neurol 265:409–427.

Castellani V, Yue Y, Gao PP, Zhou RP, Bolz J. 1998. Dual action of a ligand for Eph receptor tyrosine kinases on specific populations of axons during the development of cortical circuits. J Neurosci 18: 4663–4672.

Castellani V, Bolz J. 1999. Opposing roles for neurotrophin-3 in targeting and collateral formation of distinct sets of developing cortical neurons. Development 126:3335–3345.

Catalano SM, Robertson RT, Killackey HP. 1991. Early ingrowth of thalamocortical afferents to the neocortex of the prenatal rat. Proc Natl Acad Sci USA 88:2999–3003.

Catalano SM, Robertson RT, Killackey HP. 1996. Individual axon morphology and thalamocortical topography in developing rat somatosensory cortex. J Comp Neurol 367:36–53.

Caviness VS. 1982. Neocortical histogenesis in normal and reeler mice: a developmental-study based upon [H-3] thymidine autoradiography. Dev Brain Res 4:293–302.

Clasca F, Angelucci A, Sur M. 1995. Layer-specific programs of development in neocortical projection neurons. Proc Natl Acad Sci USA 92:11145–11149.

Cohen-Tannoudji M, Babinet C, Wassef M. 1994. Early determination of a mouse somatosensory cortex marker. Nature 368:460–463.

Crowley JC, Katz LC. 2000. Early development of ocular dominance columns. Science 290:1321–1324.

Davy A, Soriano P. 2005. Ephrin signaling in vivo: look both ways. Dev Dyn 232:1–10.

De Carlos JA, O'Leary DDM. 1992. Growth and targeting of subplate axons and establishment of major cortical pathways. J Neurosci 12:1194–1211.

Deng JB, Elberger AJ. 2003. Corticothalamic and thalamocortical pathfinding in the mouse: dependence on intermediate targets and guidance axis. Anat Embryol 207:177–192.

Deschênes M, Veinante P, Zhang ZW. 1998. The organization of corticothalamic projections: reciprocity versus parity. Brain Res Rev 28:286–308.

Dickson BJ. 2002. Molecular mechanisms of axon guidance. Science 298:1959–1964.

Domesick VB. 1972. Thalamic relationships of the medial cortex in the rat. Brain Behav Evol 6:457–483.

Drescher U, Kremoser C, Handwerker C, Loschinger J, Noda M, Bonhoeffer F. 1995. In-vitro guidance of retinal ganglion-cell axons by rags, a 25 kda tectal protein related to ligands for eph receptor tyrosine kinases. Cell 82:359–370.

Dufour A, Seibt J, Passante L, Depaepe V, Ciossek T, Frisen J, Kullander K, Flanagan JG, Polleux F, Vanderhaeghen P. 2003. Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. Neuron 39:453–465.

Erzurumlu RS, Kind PC. 2001. Neural activity: sculptor of "barrels" in the neocortex. Trends Neurosci 24:589–595.

Gao PP, Yue Y, Zhang JH, Cerretti DP, Levitt P, Zhou RP. 1998. Regulation of thalamic neurite outgrowth by the Eph ligand ephrin-A5: implications in the development of thalamocortical projections. Proc Natl Acad Sci USA 95:5329–5334.

Garel S, Yun K, Grosschedl R, Rubenstein JLR. 2002. The early topography of thalamocortical projections is shifted in Ebf1 and Dlx1/2 mutant mice. Development 129:5621–5634.

Garel S, Rubenstein JLR. 2004. Intermediate targets in formation of topographic projections: inputs from the thalamocortical system. Trends Neurosci 27:533–539.

Ghosh A, Shatz CJ. 1992. Pathfinding and target selection by developing geniculocortical axons. J Neurosci 12:39–55.

Gitton Y, Cohen-Tannoudji M, Wassef M. 1999. Role of thalamic axons in the expression of H-2Z1, a mouse somatosensory cortex specific marker. Cereb Cortex 9:611–620.

Hansen MJ, Dallal GE, Flanagan JG. 2004. Retinal axon response to ephrin-as shows a graded, concentration-dependent transition from growth promotion to inhibition. Neuron 42:717–730.

Herculano-Houzel S, Lent R. 2005. Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. J Neurosci 25:2518–2521.

Hersch SM, White EL. 1982. A quantitative study of the thalamocortical and other synapses in layer IV of pyramidal cells projecting from mouse SmI cortex to the caudate-putamen nucleus. J Comp Neurol 211:217–225.

Hubener M, Bolz J. 1988. Morphology of identified projection neurons in layer 5 of rat visual cortex. Neurosci Lett 94:76–81.

Iwasato T, Datwani A, Wolf AM, Nishiyama H, Taguchi Y, Tonegawa S, Knopfel T, Erzurumlu RS, Itohara S. 2000. Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. Nature 406:726–731.

Jones EG. 2002. Thalamic circuitry and thalamocortical synchrony. Philos Trans R Soc Lond B Biol Sci 357:1659–1673.

Kiryushko D, Berezin V, Bock E. 2004. Regulators of neurite outgrowth: role of cell adhesion molecules. Ann NY Acad Sci 1014: 140–154.

Klein R. 2004. Eph/ephrin signaling in morphogenesis, neural development and plasticity. Curr Opin Cell Biol 16:580–589.

Knoll B, Drescher U. 2004. Src family kinases are involved in EphA receptor-mediated retinal axon guidance. J Neurosci 24:6248–6257.

Lemke G. 1997. A coherent nomenclature for Eph receptors and their ligands. Mol Cell Neurosci 9:331.

Lopez-Bendito G, Molnar Z. 2003. Thalamocortical development: how are we going to get there? Nat Rev Neurosci 4:276–289.

Mackarehtschian K, Lau CK, Caras I, McConnell SK. 1999. Regional differences in the developing cerebral cortex revealed by ephrin-A5 expression. Cereb Cortex 9:601–610.

Mann F, Zhukareva V, Pimenta A, Levitt P, Bolz J. 1998. Membrane-associated molecules guide limbic and nonlimbic thalamocortical projections. J Neurosci 18:9409–9419.

Mann F, Peuckert C, Dehner F, Zhou RP, Bolz J. 2002. Ephrins regulate the formation of terminal axonal arbors during the development of thalamocortical projections. Development 129:3945–3955.

McConnell SK, Ghosh A, Shatz CJ. 1989. Subplate neurons pioneer the 1st axon pathway from the cerebral-cortex. Science 245:978–982.

Metin C, Godement P. 1996. The ganglionic eminence may be an intermediate target for corticofugal and thalamocortical axons. J Neurosci 16:3219–3235.

Miyashita-Lin EM, Hevner R, Wassarman KM, Martinez S, Rubenstein JLR. 1999. Early neocortical regionalization in the absence of thalamic innervation. Science 285:906–909.

Molnar Z, Blakemore C. 1995. How do thalamic axons find their way to the cortex. Trends Neurosci 18:389–397.

Molnar Z, Adams R, Goffinet AM, Blakemore C. 1998a. The role of the first postmitotic cortical cells in the development of thalamocortical innervation in the reeler mouse. J Neurosci 18:5746–5765.

Molnar Z, Adams R, Blakemore C. 1998b. Mechanisms underlying the early establishment of thalamocortical connections in the rat. J Neurosci 18:5723–5745.

Monschau B, Kremoser C, Ohta K, Tanaka H, Kaneko T, Yamada T, Handwerker C, Hornberger MR, Loschinger J, Pasquale EB, Siever DA, Verderame MF, Muller BK, Bonhoeffer F, Drescher U. 1997. Shared and distinct functions of RAGS and ELF-1 in guiding retinal axons. EMBO J 16:1258–1267.

Muehlfriedel S, Uziel D, Zarbalis K, Aschoff AP, Wurst W, Bolz J. 2005. Development of thalamocortical arbors in the barrel cortex of ephrin-A5 knockout mice. Program No. 217.21. Abstract/Itinerary Planner. New Orleans, LA: Society for Neuroscience.

Nakagawa Y, Johnson JE, O'Leary DDM. 1999. Graded and areal expression patterns of regulatory genes and cadherins in embryonic neocortex independent of thalamocortical input. J Neurosci 19:10877–10885.

Nakamoto M, Cheng HJ, Friedman GC, McLaughlin T, Hansen MJ, Yoon CH, Oleary DDM, Flanagan JG. 1996. Topographically specific effects of ELF-1 on retinal axon guidance in vitro and retinal axon mapping in vivo. Cell 86:755–766.

Niehage R, Peuckert C, Bolz J, Weth F. 2005. Developmental expression of ephrin/Eph wiring molecules in mouse homologs of schizophrenia-related brain regions. Program No. 485.5. Abstract/Itinerary Planner. Washington, DC: Society for Neuroscience.

Noren NK, Pasquale EB. 2004. Eph receptor-ephrin bidirectional signals that target Ras and Rho proteins. Cell Signalling 16:655–666.

Pimenta AF, Reinoso BS, Levitt P. 1996. Expression of the mRNAs encoding the limbic system-associated membrane protein (LAMP): 2, fetal rat brain. J Comp Neurol 375:289–302.

Polleux F, Dehay C, Kennedy H. 1997. The timetable of laminar neurogenesis contributes to the specification of cortical areas in mouse isocortex. J Comp Neurol 385:95–116.

Prakash N, Vanderhaeghen P, Cohen-Cory S, Frisen J, Flanagan JG, Frostig RD. 2000. Malformation of the functional organization of somatosensory cortex in adult ephrin-A5 knock-out mice revealed by in vivo functional imaging. J Neurosci 20:5841–5847.

Rakic P. 1977. Prenatal development of visual-system in rhesus-monkey. Philos Trans R Soc Lond B Biol Sci 278:245.

Rashid T, Upton AL, Blentic A, Ciossek T, Knoll B, Thompson ID, Drescher U. 2005. Opposing gradients of ephrin-as and epha7 in the superior colliculus are essential for topographic mapping in the mammalian visual system. Neuron 47:57–69.

Rebsam A, Seif I, Gaspar P. 2002. Refinement of thalamocortical arbors and emergence of barrel domains in the primary somatosensory cortex: a study of normal and monoamine oxidase a knock-out mice. J Neurosci 22:8541–8552.

Senft SL, Woolsey TA. 1991. Growth of thalamic afferents into mouse barrel cortex. Cereb Cortex 1:308–335.

Sherman SM, Guillery RW. 2002. The role of the thalamus in the flow of information to the cortex. Philos Trans R Soc Lond B Biol Sci 357:1695–1708.

Sur M, Leamey CA. 2001. Development and plasticity of cortical areas and networks. Nat Rev Neurosci 2:251–262.

Szebenyi G, Callaway JL, Dent EW, Kalil K. 1998. Interstitial branches develop from active regions of the axon demarcated by the primary growth cone during pausing behaviors. J Neurosci 18:7930–7940.

Tuzi NL, Gullick WJ. 1994. Eph, the largest known family of putative growth-factor receptors. Br J Cancer 69:417–421.

Uziel D, Muhlfriedel S, Zarbalis K, Wurst W, Levitt P, Bolz J. 2002. Miswiring of limbic thalamocortical projections in the absence of ephrin-A5. J Neurosci 22:9352–9357.

Vanderhaeghen P, Lu Q, Prakash N, Frisen J, Walsh CA, Frostig RD, Flanagan JG. 2000. A mapping label required for normal scale of body representation in the cortex. Nat Neurosci 3:358–365.

Vanderhaeghen P, Polleux F. 2004. Developmental mechanisms patterning thalamocortical projections: intrinsic, extrinsic and in between. Trends Neurosci 27:384–391.

Van Groen T, Wyss JM. 1992. Projections from the laterodorsal nucleus of the thalamus to the limbic and visual cortices in the rat. J Comp Neurol 324:427–448.

Welker E, Van der Loos H. 1986. Quantitative correlation between barrel-field size and the sensory innervation of the whiskerpad: a comparative study in six strains of mice bred for different patterns of mystacial vibrissae. J Neurosci 6:3355–3373.

Welker E, Hoogland PV, Vanderloos H. 1988. Organization of feedback and feedforward projections of the barrel cortex: a pha-l study in the mouse. Exp Brain Res 73:411–435.

Woolsey TA, Van der Loos H. 1970. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17:205–242.

Yates PA, Roskies AL, McLaughlin T, O'Leary DDM. 2001. Topographic-specific axon branching controlled by ephrin-As is the critical event in retinotectal map development. J Neurosci 21:8548–8563.