Independent parcellation of the embryonic visual cortex and thalamus revealed by combinatorial *Eph/ephrin* gene expression

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The visual cortex in primates is parcellated into cytoarchitectonically, physiologically, and connectionally distinct areas: the striate cortex (V1) and the extrastriate cortex, consisting of V2 and numerous higher association areas [1]. The innervation of distinct visual cortical areas by the thalamus is especially segregated in primates, such that the lateral geniculate (LG) nucleus specifically innervates striate cortex, whereas pulvinar projections are confined to extrastriate cortex [2–8]. The molecular bases for the parcellation of the visual cortex and thalamus, as well as the establishment of reciprocal connections between distinct compartments within these two structures, are largely unknown. Here, we show that prospective visual cortical areas and corresponding thalamic nuclei in the embryonic rhesus monkey (*Macaca mulatta*) can be defined by combinatorial expression of genes encoding Eph receptor tyrosine kinases and their ligands, the ephrins, prior to obvious cytoarchitectonic differentiation within the cortical plate and before the establishment of reciprocal connections between the cortical plate and thalamus. These results indicate that molecular patterns of presumptive visual compartments in both the cortex and thalamus can form independently of one another and suggest a role for EphA family members in both compartment formation and axon guidance within the visual thalamocortical system.

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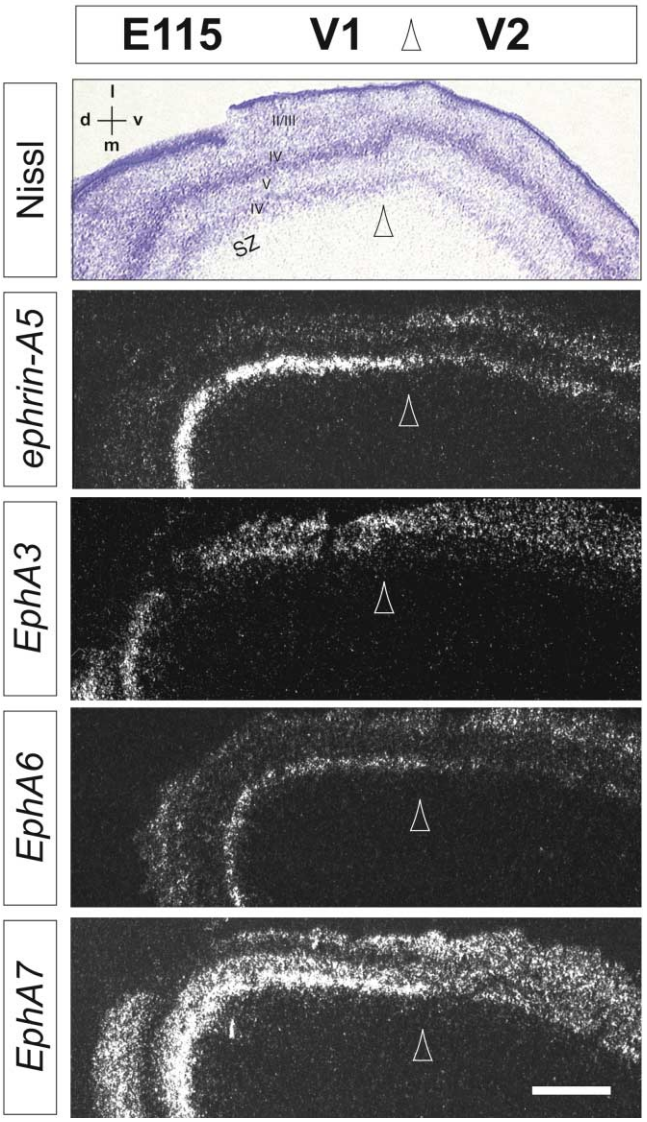
Results and discussion

In primates, more than half of the neocortex and its associated thalamic nuclei are involved in the processing of visual information [1]. The large forebrain and prolonged gestation (165 days) of the Macaque monkey allows high spatiotemporal resolution of cellular and molecular events involved in the development of the visual thalamocortical system. As yet, however, little is known about the molecular mechanisms involved in the parcellation of the primate visual cortex and corresponding thalamic nuclei. Our previous studies [9,10] demonstrated that *EphA* and *ephrin-A* (*EphA* family) genes exhibit patterning across the developing Macaque neocortex. In the present study, we examined whether *EphA* family members could differentiate between specific visual cortical areas and thalamic nuclei and investigated the relationship between this expression and the formation of their reciprocal connections [3–8].

We started by examining the expression pattern of *EphA* family genes at embryonic day 115 (E115), an age when the neocortex is synaptically connected with the thalamus and displays areal cytoarchitectonic differences, especially the sharp border between striate and extrastriate cortex (the V1/V2 border). An analysis of different *EphA* family genes revealed distinct patterns of expression that matched the cytoarchitectonic V1/V2 border (Figure 1). For example, *ephrin-A5* expression was prominent in the dorsal compartment, notably the striate cortex, but less apparent in the more ventral V2 region (Figure 1). Well-defined laminar differences also existed: while *ephrin-A5* was distributed primarily in layers V and VI of striate cortex, it was expressed by both supra- and infragranular layer in extrastriate cortex (Figure 1). Potential receptors for ephrin-A5 were also differentially expressed between V1 and V2 (Figure 1).

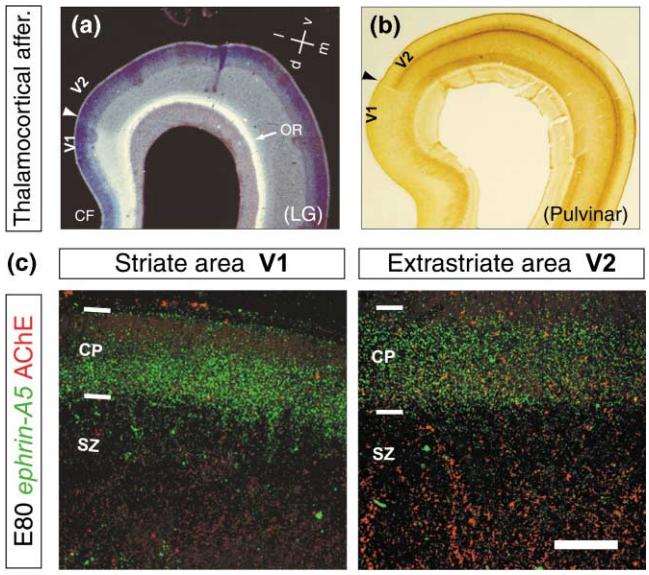
We next analyzed whether such molecular differences between visual areas existed earlier, such as E80, when the prospective neocortex consists of a dense, homogenous sheet of immature neurons (cortical plate, CP) without overt areal cytoarchitectonic differences [2,7,8,11]. Moreover, neurons of the CP are still being generated, and thalamic fibers are just beginning to invade the CP after waiting in the subplate zone [3,7,8]. While the site of the future V1/V2 border isn't morphologically apparent at E80 or any other earlier age, its location can be predicted by the selective labeling of thalamocortical fibers that sit below the CP in the intermediate and subplate zones, using acetylcholinesterase (AChE) histochemistry (Figure 2a,b). AChE historeactivity transiently marks afferent fibers originating within the pulvinar, a thalamic nucleus that selectively innervates extrastriate areas but not the striate cortex (which is eventually innervated by AChE-negative LG afferents) [7] (Figure 2a,b). Consistent with previous

Figure 1



EphA family gene expression marks distinct domains within the E115 primate visual neocortex. A representative border between area V1 and V2 in the Nissl-stained E115 CP (top) and in situ hybridizations [3] corresponding to *ephrin-A5*, *EphA3*, *EphA6*, and *EphA7* on similar sections (below). The arrowheads mark the V1/V2 border. Other *Eph* receptor expression patterns were more prominent in V2 (data not shown). d, dorsal; l, lateral; m, medial; SZ, subplate zone; v, ventral. The scale bar represents 900 μm.

Figure 2

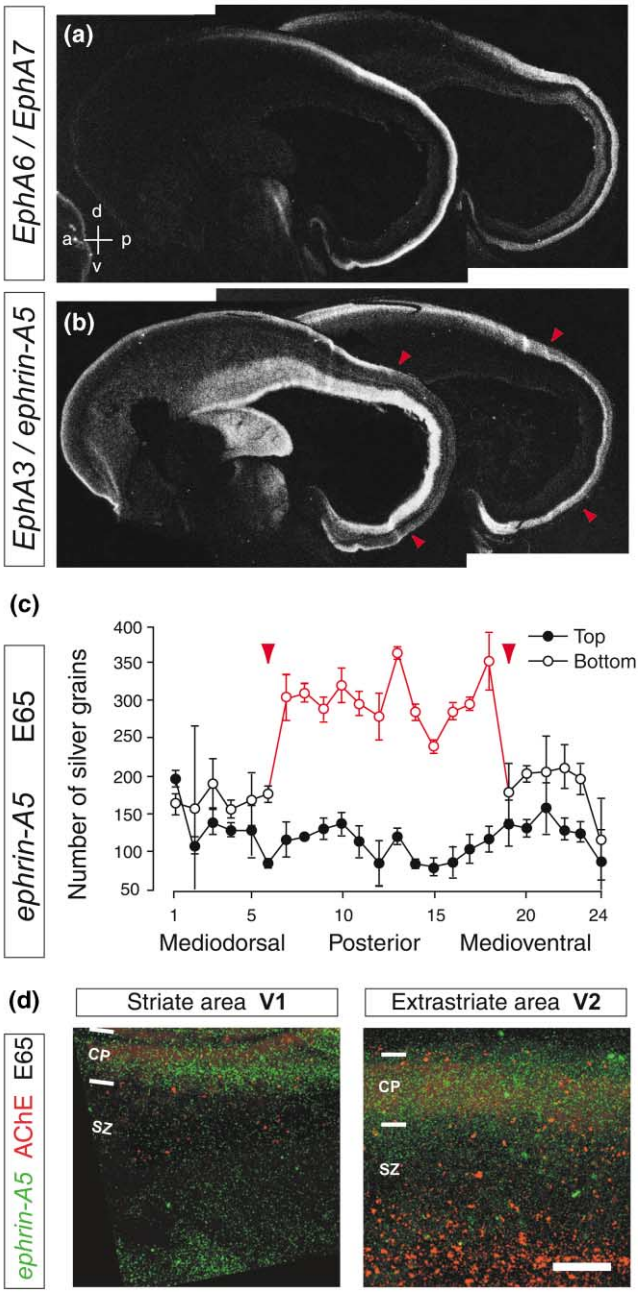


Differences in *ephrin-A5* expression within the cortical plate (CP) correspond to prospective striate and extrastriate cortex at E80. **(a)** A coronal section of an embryonic monkey cerebral wall in which one eye was injected with a mixture of [^{3}H]proline and [^{3}H]fucose [6], illustrating inputs from the lateral geniculate (LG) nucleus that terminate in prospective area V1. **(b)** A similar coronal section stained for AChE historeactivity [9], highlighting pulvinar fibers that reside within the intermediate and subplate zones of prospective area V2, is shown. Note that in monkey, (a) and (b) are mutually exclusive. **(c)** AChE (red) and *ephrin-A5* (green) were imaged from adjacent sections of E80 cerebral wall and merged using Adobe Photoshop. *Ephrin-A5* expression is throughout the CP (the limits of the CP are indicated by white bars in each panel) in V2 but is restricted to the CP's deepest strata in V1. CF, calcarine fissure; d, dorsal; l, lateral; m, medial; OR, optic radiation; SZ, subplate zone; v, ventral. The scale bar represents 1 mm (a,b) and 300 μm (c).

work, AChE historeactivity was detected in axons residing within the intermediate and subplate zones of the future extrastriate cortex but was absent from the neighboring cerebral wall representing prospective striate cortex (Figure 2c). *Ephrin-A5* expression in the AChE-rich cerebral wall was diffuse throughout the CP (Figure 2c). In contrast, in the AChE-poor occipital cerebral wall, *ephrin-A5* expression was concentrated within the deepest strata of the CP (Figure 2c). Thus, *ephrin-A5*'s expression represented a molecular difference between future striate and extrastriate CPs at E80. Since a small proportion of thalamic fibers had already innervated the CP at this age, however, such differences in expression could have been due to either cues originating within the thalamus or to molecular biases housed within the visual CP.

To evaluate the influence of reciprocal connections between the cortex and thalamus on differential gene expression in these structures, we examined *Eph* family expression at E65, before all of the neurons that eventually receive input from the thalamus had been generated (prospective layer IV), and, thus, patterned thalamocortical connectivity had been established [2–8,11]. Patterning of expression within the CP was obvious at E65 [9,10]: *EphA6* and *EphA7* were expressed in overlapping but distinct gradients that selectively labeled the posterior half of the CP, a region that corresponded spatially to future visual cortex (Figure 3a). Furthermore, the expression of another receptor, *EphA3*, and a ligand, *ephrin-A5*, demar-

Figure 3

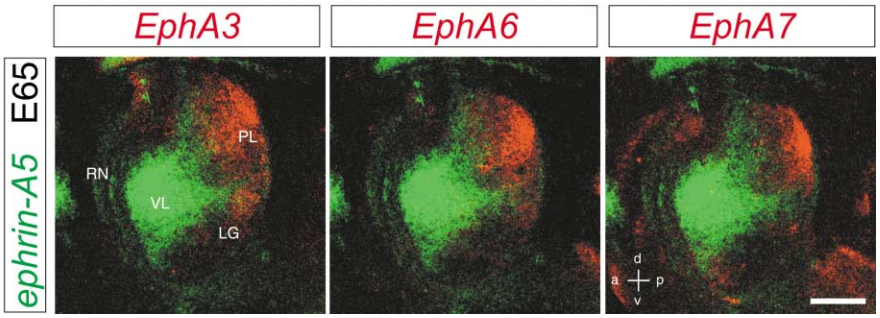


The expression patterns of *EphA* family members differentiate between prospective striate and extrastriate cortex at E65. In situ hybridization was performed with probes corresponding to **(a)** *EphA6* (left) and *EphA7* (right) and **(b)** *EphA3* (left) and *ephrin-A5* (right) using adjacent sagittal sections of E65 monkey brain. *EphA6* expression represents a posterior and steep gradient that is embedded within *EphA7*'s more extensive and shallow gradient (a). *EphA3* and *ephrin-A5* delineate a compartment within the posterior CP, in which expression changes abruptly (b, arrowheads). **(c)** *Ephrin-A5* expression within the superficial CP (top) remains constant along the anteroposterior axis, while its deep-strata expression (bottom) is induced 2-fold at the borders marked with arrowheads in (b), with the same border indicated in (c). Silver grains were quantitated by imaging the CP at different anteroposterior positions and counting the number of silver grains within a 250 μm × 250 μm area. Numbers reflect the subtraction of background levels of silver grains. **(d)** AChE (red) and *ephrin-A5* (green) were imaged from adjacent sections of E65 cerebral wall and merged using Adobe Photoshop. *Ephrin-A5* expression is throughout the CP (the limits of the CP are indicated by white bars in each panel) in V2 but is restricted to the CP's deepest strata in V1. a, anterior; d, dorsal; p, posterior; SZ, subplate zone; v, ventral. The scale bar represents 400 μm (a,b) and 300 μm (d).

cated distinct compartments within the *EphA6*- and *EphA7*-positive prospective visual CP: E*phA3* expression comprised a plus-minus pattern, with levels high anteriorly and absent posteriorly, whereas *ephrin-A5* expression respected the same border, but its expression varied according to prospective laminae (Figure 3b). At this stage, levels of *ephrin-A5* expression were constant within the most superficial CP along the anteroposterior axis but increased selectively in the deepest strata of the posterior CP (Figure 3b,c). Again, we used AChE historeactivity to visualize the location of pulvinar fibers, which have entered the intermediate zone but have not yet invaded the CP at E65 [7]. As we observed previously, *ephrin-A5* was uniformly expressed in the CP overlying pulvinar fibers but was restricted to the deepest strata of the CP in AChE-poor cerebral wall (prospective layers V and VI). This data, in combination with extrapolation from localization at older ages, led us to conclude that differences in expression of *ephrin-A5* and *EphA3* at E65 corresponded to distinctions between presumptive striate and extrastriate cortex. Thus, *Eph* family gene expression distinguished between the two regions at a time when there were no other known areal landmarks within the visual CP. In addition, these molecular differences existed in the absence of reciprocal synaptic connections between the CP and thalamus, indicating that they emerge independently. Interestingly, thalamic fibers do reside within the subplate zone at these early ages. Thus, while mature patterns of connections certainly do not exist, it is intriguing to consider whether subplate interactions influence or are influenced by Eph family members.

Next, we analyzed whether corresponding patterns of *Eph* family expression existed in the thalamus at E65. Despite the lack of reciprocal connections between the CP and thalamus at this age [4], we observed patterning of *ephrin-A5* and select *Eph* receptors within the developing thalamus. *Ephrin-A5* was predominantly expressed within the ventrolateral nucleus that would eventually innervate somatosensory CP, whereas *EphA3*, *EphA6*, and *EphA7* were most abundant in the pulvinar and to a lesser extent in the geniculate body that would innervate visual CP (Figure 4). Moreover, distinct gradients of *EphA6* and *EphA7* expression were present in the pulvinar. This patterned expression within the thalamus, in conjunction with the complex neocortical expression we have documented, supports a model in which combinatorial *Eph* family expression could

Figure 4



EphA gene family expression marks distinct thalamic nuclei. In situ hybridizations using probes that correspond to *EphA3* (left, red), *EphA6* (middle, red), and *EphA7* (right, red) show complementary compartments with *ephrin-A5* (green, all panels) within the E65 thalamus. *EphA3*, *EphA6*, and *EphA7* are present within the pulvinar and geniculate nuclei, while the ventral complex expresses *ephrin-A5*. Similar to their expression in the neocortex, *EphA6* and *Eph-A7* are expressed in overlapping but distinct gradients in the pulvinar: *EphA6* is present throughout and in a shallow gradient (middle panel), while *EphA7* is posteriorly restricted and displays a steeper gradient (right panel). a, anterior; d, dorsal; GE, ganglionic eminence; LG, lateral geniculate body; p, posterior; PL, pulvinar; RN, reticular nucleus; v, ventral; VL, ventrolateral complex. The scale bar represents 200 μm.

independently establish cellular groupings within the thalamus and cortex and then influence reciprocal innervation between these structures.

In this study, we have shown that distinct compartments within the visual CP and thalamus can be defined in embryonic primates by the distribution of *Eph* family gene expression. The resulting combinations are remarkable in that they are generated by a single family of signaling molecules, albeit one that can have complex roles in proliferation, migration, pattern formation, and axon guidance in other neural systems [12–15]. Only recently, however, have the roles of Eph family members been examined in the developing cerebral cortex [9,10,16–19]. Our results show that *Eph* family member expression can distinguish prospective visual cortical areas and thalamic nuclei before the emergence of cytoarchitectonic differentiation of the CP and the establishment of reciprocal connections, similar to expression of other gene families in the rodent [20,21]. Given the potent roles of Eph family members in other systems [12–15], it is possible that the combinatorial patterns that we have uncovered in the embryonic primate visual system could provide the necessary information for both the establishment of visual cortical maps as well as proper thalamocortical connections.

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References

1. Van Essen DC, Anderson CH, Felleman DJ: **Information processing in the primate visual system: an integrated systems perspective.** *Science* 1992, **255:**419-423.
2. Rakic P: **The specification of cortical areas**. *Science* 1988, **241:**170-176.
3. Rakic P: **Prenatal genesis of connections subserving ocular dominance in the rhesus monkey.** *Nature* 1976, **261:**467-471.
4. Shatz CJ, Rakic P: **The genesis of efferent connections from the visual cortex of the fetal rhesus monkey.** *J Comp Neurol* 1981, **196:**287-307.
5. Snider CJ, Dehay C, Berland M, Kennedy H, Chalupa LM: **Prenatal development of retinogeniculate axons in the macaque monkey during segregation of binocular inputs.** *J Neurosci* 1999, **19:**220-228.
6. Meissirel C, Wikler, KC., Chalupa, LM., Rakic, P: **Early divergence of magnocellular and parvocellular functional subsystems in the embryonic primate visual system.** *Proc Natl Acad Sci* 1997, **94:**5900-5905.
7. Kostovic I, Rakic P: **Development of prestriate visual projections in the monkey and human fetal cerebrum revealed by transient cholinesterase staining.** *J Neurosci* 1984, **4:**25-42.
8. Dehay C, Giroud P, Berland M, Smart I, Kennedy H: **Modulation of the cell cycle contributes to the parcellation of the primate visual cortex.** *Nature* 1993, **366:**464-466.
9. Donoghue MJ, Rakic P: **Molecular gradients and compartments in the embryonic primate cerebral cortex.** *Cereb Cortex* 1999, **9:**586-600.
10. Donoghue MJ, Rakic P: **Molecular evidence for the early specification of presumptive functional domains in the embryonic primate cerebral cortex.** *J Neurosci* 1999, **19:**5967-5979.
11. Rakic P: **Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition.** *Science* 1974, **183:**425-427.
12. O'Leary DD, Wilkinson DG: **Eph receptors and ephrins in neural development.** *Curr Opin Neurobiol* 1999, **9:**65-73.
13. Xu Q, Mellitzer G, Robinson V, Wilkinson DG: **In vivo cell sorting in complementary segmental domains mediated by Eph receptors and ephrins.** *Nature* 1999, **399:**267-271.
14. Karam SD, Burrows RC, Logan C, Koblar S, Pasquale EB, Bothwell M: **Eph receptors and ephrins in the developing chick cerebellum: relationship to sagittal patterning and granule cell migration.** *J Neurosci* 2000, **20:**6488-6500.
15. Conover JC, Doetsch F, Garcia-Verdugo J-M, Gale NW, Yancopoulos GD, Alvarez-Buylla A: **Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone.** *Nature Neurosci* 2000, **3:**1091-1097.
16. Castellani V, Yue Y, Gao PP, Zhou R, Bolz J: **Dual action of a ligand for Eph receptor tyrosine kinases on specific populations of axons during the development of cortical circuits.** *J Neurosci* 1998, **18:**4663-4672.
17. Gao PP, Yue Y, Zhang JH, Cerretti DP, Levitt P, Zhou R: **Ephrin-dependent growth and pruning of hippocampal axons.** *Proc Natl Acad Sci USA* 1998, **95:**5329-5334.
18. Vanderhaeghen P, Lu Q, Prakash N, Frisen J, Walsh CA, Frostig RD,

et al.: **A mapping label required for normal scale of body representation in the cortex.** *Nat Neurosci* 2000, **3:**358-365.
19. Mackarehtschian K, Lau CK, Caras I, McConnell SK: **Regional differences in the developing cerebral cortex revealed by ephrin-A5 expression.** *Cereb Cortex* 1999, **9:**601-610.
20. Miyashita-Lin EM, Hevner R, Wasserman KM, Martinez S, Rubenstein JL: **Early neocortical regionalization in the absence of thalamic innervation.** *Science* 1999, **285:**906-909.
21. Nakagawa Y, Johnson JE, O'Leary DDM: **Graded and areal expression patterns of regulatory genes and cadherins in embryonic neocortex independent of thalamocortical input.** *J Neurosci* 1999, **19:**10877-10885.