Cooperation of intrinsic and extrinsic signals in the elaboration of regional identity in the posterior cerebral cortex

Fatiha Nothias*, Gord Fishell and Ariel Ruiz i Altaba

Understanding the compartmentalization of the neocortex (isocortex) of the mammalian brain into functional areas is a challenging problem [1–3]. Unlike pattern formation in the spinal cord and hindbrain, it does not involve the specification of distinct cells types: distinct areas differ in their patterns of connectivity and cytoarchitecture. It has been suggested that signals intrinsic to the neocortical neuroepithelium specify regional fate [3]. Alternatively, spatial patterning might be imposed by extrinsic cues such as thalamocortical projections [4–6]. Recent results highlight the ability of early precursor cells of the telencephalic neuroepithelium to 'remember' their spatial position from times before thalamic innervation [7–12]. An influence from the thalamus, however, cannot be ruled out as there is a precise invasion of the correct cortical areas by the corresponding projections [13,14]. Furthermore, cortical neuronal progenitors have been proposed to adopt new connection patterns after transplantation [6,7], as well as when the thalamic input is rerouted [15,16]. Here, we describe the transient expression of the homeobox gene *Otx2* in the posterior, prospective visual, neocortex and use it to analyze the establishment of posterior cortical fate. The results suggest that whereas intrinsic cortical information is sufficient to specify regional fate, extrinsic signals from the thalamus are involved in the expansion or maintenance of the population of cells expressing *Otx2* but not in regionalization.

Address: The Skirball Institute, Developmental Genetics Program and Department of Cell Biology, NYU Medical Center, 540 First Avenue, New York, New York 10016, USA.

Present address: *Institut Alfred Fessard, CNRS, Gif sur Yvette, France.

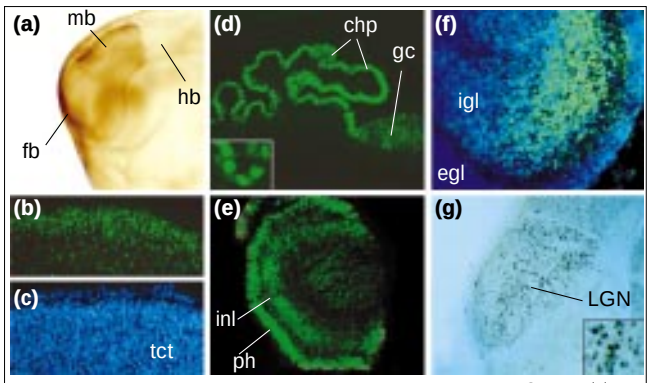
Correspondence: Ariel Ruiz i Altaba
E-mail: ria@saturn.med.nyu.edu

Received: 20 November 1997
Revised: 14 January 1998
Accepted: 19 February 1998

Published: 30 March 1998

Current Biology 1998, 8:459–462
http://biomednet.com/elecref/0960982200800459

Figure 1

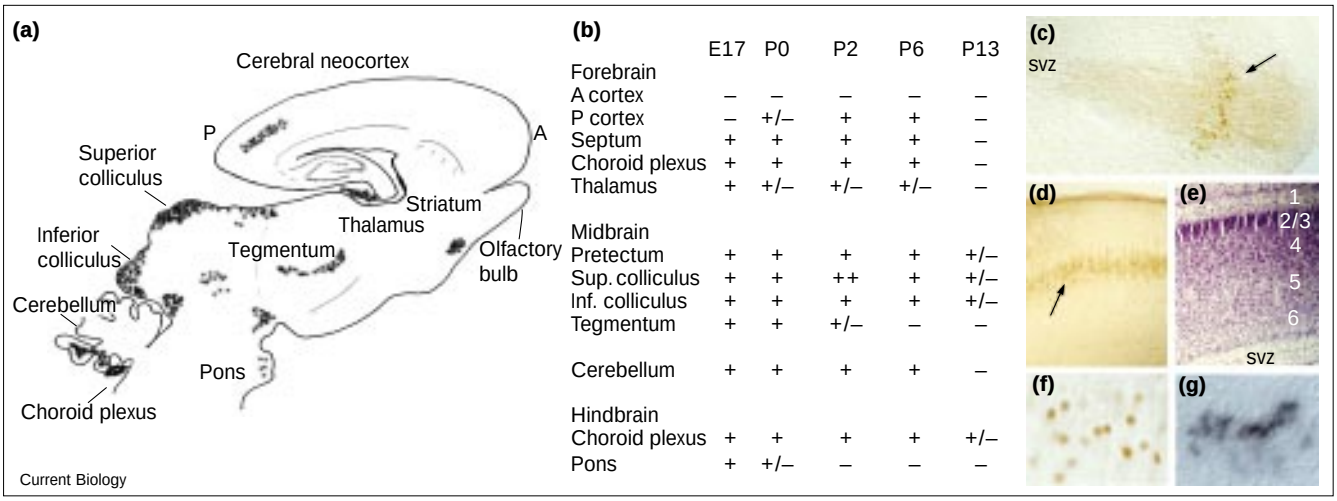


Expression of Otx2 protein in the developing brain and visual centers. (**a**) Expression of Otx2 (epitope MMSYLKQPPYAVNGLSLTASGMDL-LHQSV, in single letter amino-acid code) in a stage ~34 frog (*Xenopus laevis*) tadpole seen in side view after peroxidase immunocytochemistry. Labeling is in the forebrain (fb) and midbrain (mb) but not in the hindbrain (hb). (**b**) Expression of Otx2 in superficial cells in the tectum (tct; or superior colliculus) of an embryonic day 17 (E17) Sprague–Dawley rat embryo in a sagittal section after fluorescence immunocytochemistry. (**c**) The same field as in (b) showing the position of DAPI-labeled nuclei. (**d**) Expression of Otx2 in choroid plexus (chp) and adjacent granule cells (gc) of the cerebellum as seen in a sagittal section of an E17 rat embryo. (**e**) Expression of Otx2 in the developing eye of a 24 h zebrafish embryo seen in cross-section. Expression is detected in the photoreceptor layer (ph) and inner layer (inl). (**f**) Expression of Otx2 in the posterior cerebellum of a postnatal day 6 (P6) rat as seen in a sagittal section. The image shows double labeling of Otx2 in green and cell nuclei in blue (DAPI stain). Expression is high in the internal granular layer (igl) and very weak or absent in the external germinal layer (egl). (**g**) Expression of Otx2 in the developing lateral geniculate nucleus (LGN) of a P6 rat seen in cross-section. Note the higher expression in the ventral region. The insets in (d,g) show Otx2 in nuclei.

Results and discussion

Labeling of vertebrate embryos with an anti-Otx2 polyclonal antibody showed Otx2 in the nuclei of the expected cells, given the pattern of *Otx2* mRNA expression [17–25] (Figure 1a and data not shown), with the exception of the chick Hensen's node [21], suggesting translational regulation [26]. Within the embryonic murine brain, sites of Otx2 expression included the tectum, retina, choroid plexus and thalamus [18,24,25,27] (Figure 1b–e,g). In the postnatal brain, Otx2-expressing cells included those in the colliculi and posterior cerebellum (Figures 1f,2a,2b). By 13 days after birth, little Otx2 expression remained (Figure 2b). Thus, whereas at early embryonic stages Otx2 is detected within the entire midbrain and forebrain regions, by late embryonic and early postnatal stages, there is predominant expression in areas of the brain involved in processing visual information: the retina, lateral geniculate nucleus (LGN) and superior colliculus.

Figure 2



Expression of Otx2 protein in the visual cortex of postnatal animals. (a) Diagram of the position of Otx2-expressing cells (shaded areas) in a lateral sagittal section of a P6 rat brain; the anterior (A) and posterior (P) poles are indicated. The lateral geniculate nucleus is not included. (b) Summary of the expression (+) of Otx2 in different regions of the rat brain during late embryonic to early postnatal stages. (c) Localization of Otx2 (arrow) in the posterior neocortex of P4 rats. Faint cytoplasmic staining was detected in regions where nuclear labeling was found. (d) Localization of the Otx2-expressing cells (arrow) in the neocortex to the lower layer 4 or upper layer 5 by (e) staining an adjacent section with cresyl violet; numbers refer to cortical layers. (f,g) High magnification micrographs of the expression of (f) Otx2 in the occipital neocortex in P6 rats and (g) *Otx2* mRNA in P5 CD1 mice. *Otx2* expression in mouse visual cortex was similar to that in rats (P6 rats are an equivalent age to P5 mice). (c,e) The subventricular zone (svz) is indicated.

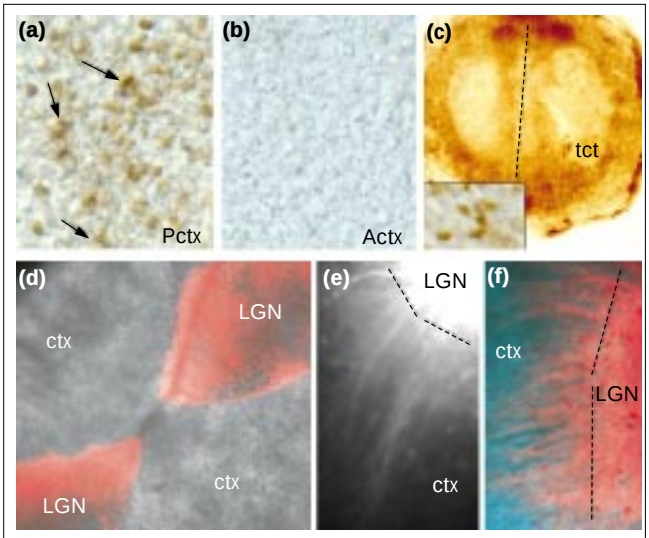
A novel site of Otx2 expression was identified in the posterior cerebral cortex of postnatal rats and mice. This contrasts with the widespread expression of *Otx1* throughout the neocortex [27]. Otx2 was found in a restricted population of neurons located in layer 4 and/or the upper portion of layer 5 (Figure 2a,c–f). We confirmed that *Otx2* was expressed in the cortex by *in situ* hybridization (Figure 2g) and by nested reverse transcriptase (RT)–PCR analysis using primers from throughout the coding region (data not shown). The cortical area harboring the Otx2-positive neurons was identified as visual cortex by retrograde labeling from the cortex to the LGN (data not shown).

To investigate posterior cortical fate specification, we assayed single cortical explants as well as co-cultures of cortex and thalamus for Otx2 expression. Pieces of telencephalic neuroepithelium — from rats at embryonic day 13–14 (E13–E14), which is before thalamic innervation — were excised, avoiding the olfactory area, septum and the ganglionic eminences. Anterior and posterior cortical pieces were similarly excised from older rat embryos (E15.5–E17; after thalamic innervation). Single telencephalic explants were cultured and assayed for Otx2 expression at a time when *Otx2* mRNA is normally expressed in the posterior neocortex: postnatal day 2–6 (P2–P6). Specific nuclear labeling was observed in 10% of the E13.5 posterior cortical pieces examined but not in any of the anterior ones ($n = 15$; Figure 3a,b). Sensitive RT–PCR analysis confirmed the selective expression of *Otx2* in 20% of posterior cortical explants taken before or after innervation (Figure 4a). As controls, tectal and choroid plexal explants expressed *Otx2* (Figures 3c,4). Explants of posterior thalamus, including the LGN, were also positive for *Otx2* although at E13–E14 only 60% of explants expressed *Otx2* (Figure 4). Thus, signals intrinsic to the neuroepithelium can induce *Otx2* expression and the innervation that occurred before the E15.5–E17 explants were prepared is insufficient to increase *Otx2* expression.

To test the requirement of maintained innervation for *Otx2* expression, we co-cultured posterior cortex with anterior or posterior thalamus at E13–E14 and E15.5–E17, grew the recombinates to P2–P6 (Figure 3d) and assayed cortical *Otx2* expression by RT–PCR (Figure 4b). At early stages, thalamic axons do not innervate cortical explants [28,29]. Consistent with this, co-culture of thalamus with E15.5–E17, but not E13–E14, posterior cortex increased the incidence of cortical *Otx2* expression (Figure 4a). Axonal tracts crossed into the cortex from both anterior and posterior co-cultured E15–E17.5 thalamic explants (80%, $n = 5$; Figure 3e,f).

The enhanced *Otx2* expression in E15.5–E17 cortical explants induced by co-cultured thalamus was independent of the origin of the explant. Both anterior and posterior thalamus induced an increase in cortical *Otx2* expression to 75% and 67% of cases, respectively (Figure 4). Because levels of Otx2 protein did not appear

Figure 3



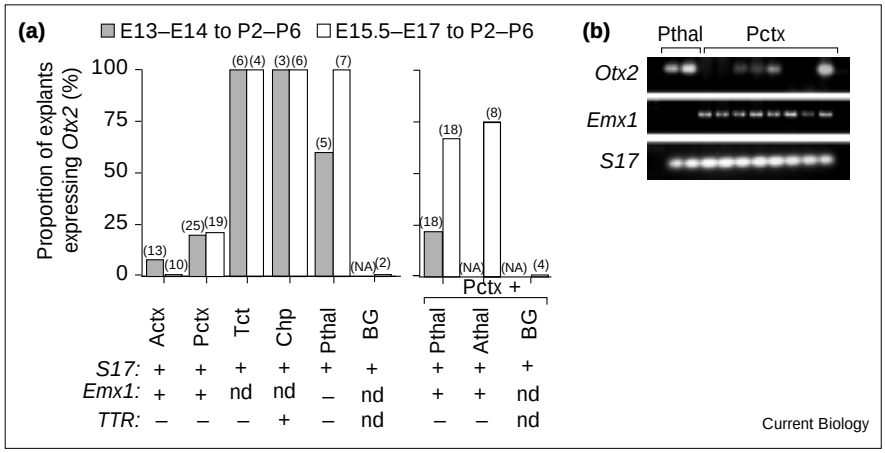
In vitro culture of cortex and thalamus: Otx2 protein expression and innervation. (**a**,**b**) Expression of Otx2 (arrows) in cell nuclei of (a) posterior (Pctx) but not (b) anterior (Actx) cortical explants grown from E13.5 to P6. (**c**) Expression of Otx2 in a tectal explant (tct) grown from E15.5 to P6. The dashed line depicts the dorsal axis of symmetry. The inset shows nuclear Otx2. (**d**) Labeling of posterior thalamic (LGN) pieces with PKH – a dye that labels the surface of the explants – before co-culture with cortical explants (ctx) shows the borders between thalamus (red) and cortex. (**e**,**f**) Axonal ingrowth, labeled using DiI crystals, from LGN co-cultured with ctx. Similar ingrowth was detected in co-cultures of anterior thalamus and cortex.

to vary in cells from normal brain versus those in explants, the increase in RNA levels probably reflects an increase in the number of *Otx2*-expressing cells. As only the posterior thalamic explant contains LGN neurons that normally innervate the visual cortex, this result indicates that whereas innervation is required for robust cortical *Otx2* expression, the identity of the innervating thalamic axons is not important. Nevertheless, the observed increase in cortical *Otx2* expression appears to be specific for the thalamus, as E17 basal ganglia explants (consisting mostly of striatum) did not mimic the effects of the thalamus (Figure 4a). This is important as only the thalamus normally innervates the neocortex. Thalamocortical afferents might, therefore, exert a global trophic influence on their target.

It has been suggested that distinct cortical areas have specific attractive properties for the corresponding thalamic axons [30,31]. This specificity results in the specific innervation of cortical targets by the appropriate thalamic axons [13,14]. However, *in vitro* (this work, [28]) and *in vivo* [15,16] thalamic afferents can be enticed to innervate incorrect targets without the respecification of cortical areal phenotypes *in vitro* (this work) or *in vivo* [32], although such respecification can happen [6]. Pattern formation in the neocortex can thus be thought of as a threefold problem: how specific areas acquire distinct identities before innervation, how thalamic axons recognize specific cortical targets, and how cortical and thalamic neurons of a given system develop in parallel to form a coherent unit.

Previous experiments have suggested that somatosensory neocortical fates are determined intrinsically [11]. Our experiments support this idea and show that visual neocortex identity is also determined intrinsically but that the development of the posterior cortex is influenced by extrinsic mechanisms. Only thalamus taken at E15.5 or later could induce Otx2 expression in neocortex, coincident with the time when thalamic innervation occurs

Figure 4



Quantitation of the number of explants expressing *Otx2* mRNA. (**a**) Histograms showing the proportion of E13–E14 or E15.5–E17 explants grown *in vitro* to P2–P6 that express *Otx2*. Expression was assessed either in explants taken from anterior and posterior cortex (Actx and Pctx), tectum (Tct), choroid plexus (Chp), posterior thalamus (Pthal) or basal ganglia (BG) and grown alone, or from Pctx explants after co-culture with Pthal, anterior thalamus (Athal) or BG. Numbers in brackets are the numbers of explants examined. As controls for RNA recovery, cortical identity and presence of Chp cells, we assayed for the expression of the ubiquitous ribosomal *S17*, cortex-specific *Emx1* [18] and *TTR* genes, respectively (the results are indicated below the histogram; nd, not determined). A small fraction of the samples of Actx from E13.5 animals expressed background levels of *Otx2*. Co-cultured tissues were removed and RNA from each type of tissue extracted separately. NA; not applicable. (**b**) Example of an RT–PCR assay showing the expression of *Otx2* in Pthal explants and 4 out of 8 Pctx explants derived from thalamocortical co-cultures; *Emx1* and *S17* controls are shown.

[28,29], suggesting that connections between the thalamus and cortex might expand or maintain the Otx2-positive population by providing permissive inducing and/or trophic factors. As the identity of the thalamic explant co-cultured with posterior neocortex was not important for enhanced *Otx2* expression, the identity of the neocortical area is not dependent on information provided by thalamocortical connections. The thalamus could be involved in orchestrating the coherent development of thalamus and neocortex, however. The patterning of the neocortical neuroepithelium could therefore be fundamentally similar to that of the neural plate, in which intrinsic signals from within the neuroepithelium and extrinsic signals from the adjacent mesoderm cooperate in pattern formation [33]. Finally, the patterns of expression of Otx2 in the retina, LGN and visual neocortex (this work, [25,27]) raise the possibility that Otx2 is functionally involved in the patterning of the visual system.

Supplementary material

Additional methodological data are published with this paper on the internet.

Acknowledgements

We thank L. Philipson for unconditional support. Anti-Otx2 antibodies were raised by A.R.A. when he was a post-doc with T. Jessell and his support and encouragement are deeply appreciated. We are grateful to N. Dahmane for comments on the manuscript, A. Simeone for the mouse *Otx2* sequence, R. Costa for a *transthyretin* cDNA, J. Kohtz for the *S17* and *Emx1* primers and S. Mazan and P. Brulet for the unsuccessful use of *Otx2 : LacZ* mice. This work was supported by grants from the Association pour la Recherche Médicale (F.N.), the National Institutes of Health NS32993 (G.F.), the March of Dimes (A.R.A.), the Skirball Institute (G.F., A.R.A.) and the Pew Scholars Program in the Biomedical Sciences (A.R.A.).

References

1. O'Leary DDM: Do cortical areas emerge from a protocortex? *Trends Neurosci* 1989, 12:400-406.
2. Shatz CJ: Dividing up the neocortex. *Science* 1992, 258:237-238.
3. Rakic P: Specification of cerebral cortical areas. *Science* 1988, 241:170-176.
4. Welker E, van der Loos H: Is a real extent in sensory cerebral cortex determined by peripheral innervation density? *Exp Brain Res* 1986, 63:650-654.
5. O'Leary DDM, Schlaggar BL, Tuttle R: Specification of neocortical areas and thalamocortical connections. *Annu Rev Neurosci* 1994, 17:419-439.
6. Schlaggar BL, O'Leary DDM: Potential of visual cortex to develop an array of functional units unique to somatosensory cortex. *Science* 1991, 252:1556-1560.
7. Barbe MF, Levitt P: The early commitment of fetal neurons to the limbic cortex. *J Neurosci* 1991, 11:519-533.
8. Arimatsu Y, Miyamoto M, Nihonmatsu I, Hirata K, Uratani Y, Hatanaka Y, Takiguchi-Hayashi K: Early regional specification for a molecular neuronal phenotype in the rat neocortex. *Proc Natl Acad Sci USA* 1992, 89:8879-8883.
9. Ferri RT, Levitt P: Cerebral cortical progenitors are fated to produce region-specific neuronal populations. *Cerebral Cortex* 1993, 3:187-198.
10. Arimatsu Y, Nihonmatsu I, Hirata K, Takiguchi-Hayashi K: Cogeneration of neurons with a unique molecular phenotype in layers V and x VI of widespread lateral neocortical areas in the rat. *J Neurosci* 1994, 14:2020-2031.
11. Cohen-Tannoudji M, Babinet C, Wassef M: Early determination of a mouse somatosensory cortex marker. *Nature* 1994, 368:460-463.
12. Ferri RT, Eagelson KL, Levitt P: Environmental signals influence expression of a cortical areal phenotype ***in vitro*** independent of effects on progenitor cell proliferation. *Dev Biol* 1996, 175:184-190.
13. Shatz CJ, Ghosh A, McConnell SK, Allendoerfer KL, Friauf E, Antonini A: Pioneer neurons and target selection in cerebral cortical development. *Cold Spring Harbor Symp Quant Biol* 1990, LV:469-480.
14. Bölz J, Novak N, Götz M, Bonhoeffer T: Formation of target-specific neuronal projections in organotypic slice cultures from rat visual cortex. *Nature* 1990, 346:359-362.
15. Woolsey TA, Wann JR: Areal changes in mouse cortical barrels follow vibrissal damage at different postnatal ages. *J Comp Neurol* 1976, 170:53-66.
16. Metin C, Frost DO: Visual responses of neurons in somatosensory cortex of hamsters with experimentally induced retinal projections to somatosensory thalamus. *Proc Natl Acad Sci USA* 1989, 86:357-361.
17. Simeone A, Acampora D, Gulisano M, Stornaiuolo A, Boncinelli E: Nested expression domains of four homeobox genes in developing rostral brain. *Nature* 1992, 358:687-690.
18. Boncinelli E, Gulisano M, Broccoli V: Emx and Otx homeobox genes in the developing mouse brain. *J Neurobiol* 1993, 24:1356-1366.
19. Li Y, Allende ML, Finkelstein R, Weinberg ES: Expression of two zebrafish orthodenticle-related genes in the embryonic brain. *Mech Dev* 1994, 48:229-244.
20. Mercier P, Simeone A, Cotelli F, Boncinelli E: Expression pattern of two otx genes suggests a role in specifying anterior body structures in zebrafish. *Int J Dev Biol* 1995, 39:559-573.
21. Bally-Cuif L, Gulisano M, Broccoli V, Boncinelli E: c-otx2 is expressed in two different phases of gastrulation and is sensitive to retinoic acid treatment in chick embryo. *Mech Dev* 1995, 49:49-63.
22. Pannese M, Polo C, Andreazzoli M, Vignali R, Kablar B, Barsacchi G, Boncinelli E: The ***Xenopus*** homologue of Otx2 is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* 1995, 121:707-720.
23. Blitz IL, Cho KWY: Anterior neurectoderm is progressively induced during gastrulation: the role of the ***Xenopus*** homeobox gene orthodenticle. *Development* 1995, 121:993-1004.
24. Mallamaci A, Di Blas E, Briata P, Boncinelli E, Corte G: OTX2 homeoprotein in the developing central nervous system and migratory cells of the olfactory area. *Mech Dev* 1996, 58:165-178.
25. Bovolenta P, Mallamaci A, Briata P, Corte G, Boncinelli E: Implication of Otx2 in pigment epithelium determination and neural retina differentiation. *J Neurosci* 1997, 17:4243-4252.
26. Acampora D, Mazan S, Lallemand Y, Avantaggiato V, Maury M, Simeone A, Brulet P: Forebrain and midbrain regions are deleted in Otx2-/- mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* 1995, 121:3279-3290.
27. Frantz GD, Weimann JM, Levin ME, McConnell SK: Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. *J Neurosci* 1994, 14:5725-5740.
28. Molnar Z, Blakemore C: Lack of regional specificity for connections formed between thalamus and cortex in co-culture. *Nature* 1991, 351:475-477.
29. Götz M, Novak N, Bastmeyer M, Bölz J: Membrane-bound molecules in rat cerebral cortex regulate thalamic innervation. *Development* 1992, 116:507-519.
30. Barbe MF, Levitt P: Attraction of specific thalamic input by cerebral grafts depends on the molecular identity of the implant. *Proc Natl Acad Sci USA* 1992, 89:3706-3710.
31. Ghosh A, Shatz CJ: A role for subplate neurons in the patterning of connections from thalamus to neocortex. *Development* 1993, 117:1031-1047.
32. Ebrahimi-Gaillard A, Roger M: Development of spinal cord projections from neocortical transplants heterotopically placed in the neocortex of newborn hosts is highly dependent on the embryonic locus of origin of the graft. *J Comp Neurol* 1996, 365:129-140.
33. Ruiz i Altaba A: Pattern formation in the vertebrate neural plate. *Trends Neurosci* 1994, 17:233-243.

Supplementary material

Cooperation of intrinsic and extrinsic signals in the elaboration of regional identity in the posterior cerebral cortex

Fatiha Nothias, Gord Fishell and Ariel Ruiz i Altaba

Current Biology 30 March 1998, 8:459–462

Materials and methods

Animals

Sprague–Dawley rats and CD1 mice were purchased from Charles River or Taconic Farms or bred in the Skirball mouse facility. Pregnant rats and mice were euthanized by an overdose of nembutal. Embryo dissection was performed in L15-air media on ice. The morning after conception was counted as E0.5. Frog (*Xenopus laevis*) embryos were obtained from our facility and grown to the desired Nieuwkoop and Faber stage. Chick embryos were obtained by incubating fertilized eggs (Spafas, Inc) to the desired Hamburger and Hamilton stage in a humidified incubator. Zebrafish embryos were obtained from a pet aquarium. Embryos were processed by standard techniques [S1].

In vitro *explant culture and tissue labeling*

Embryonic brains were dissected free from the meninges and other membranes and dissected according to clear morphological landmarks [S2]. Brain explants were cultured *in vitro* on floating synthetic membranes (0.4 μm; Falcon #3090) over serum-free F12/DMEM (1:1) media with N2 and B27 (GIBCO) supplements, mito C (Collaborative Research), glutamine and penicillin/streptomycin for up to the equivalent of P4–P6. These long incubations (8–14 days) did not require change of media.

In E13.5 explants, choroid plexus progenitors were sometimes taken with the cortical pieces and these differentiated well in culture. However, we were able to eliminate choroid plexal cells by dissection from our cortical explants before RNA extraction as confirmed by the absence of *transthyretin* (*TTR*), a specific marker of choroid cells [S3], from our cortical samples.

Surface labeling of thalamic explants prior to culture was performed by briefly rinsing the explants in L15 media containing DNase (0.1 mg/ml) and incubating for 2–5 min in PKH dye as suggested by the manufacturer (Sigma). The boundary of labeled and unlabeled cells in cortex–thalamus co-cultures corresponded to the morphological boundary between the cortical and thalamic explants.

Labeling of thalamic axons growing into the cortex in co-cultures was performed by localized labeling with DiI crystals in the thalamic explant one day before harvest.

Immunocytochemistry

An anti-Otx2 polyclonal antibody was raised in rabbits by injecting a KLH-coupled peptide. The peptide (MMSYLKQPPYAVNGLSLTASG-MDLLHQSVC) was derived from the amino-terminal end of frog Otx2 carrying an added carboxy-terminal cysteine for coupling to activated KLH (Pierce). Rabbit sera was used at 1/8000.

Whole-mount and immunocytochemistry was performed on MEMFA-fixed embryos by standard techniques using peroxidase-coupled secondary antibodies and the DAB/H_2O_2 reaction.

Immunocytochemistry on frozen sections of embryonic samples was performed on MEMFA-fixed tissues equilibrated in 30% sucrose in phosphate buffer and frozen with Tissue-Tek (Fisher). Secondary antibodies coupled to fluorescein (Boehringer-Mannheim) were used along with a pinch of PPDA to retard bleaching and DAPI as nuclear counterstain.

Immunocytochemistry of post-natal brains was performed by perfusing the animals with fresh 4% paraformaldehyde pH 8.0 followed by dissection of the brain and overnight postfixation in 4% paraformaldehyde. The fixed brains were embedded in Tissue-Tek, frozen and sections (50 μm) cut in a cryostat. These sections were washed in PBS containing 0.1% Triton X-100 and processed for whole-mount immunocytochemistry as described above and mounted on a glass slide under a coverslip with Aquamount (Miles).

Images were recorded on film using an Axiophot Zeiss microscope or digitally with a Princeton Instruments cooled CCD (KAF 1400 chip) camera.

RT–PCR, clones and in situ *hybridization*

In situ hybridization with a mouse *Otx2* cDNA [S4] was performed on fresh-frozen sections [S5]. A chick O*tx*2 cDNA was isolated from a stage 14 cDNA library using a frog O*tx2* PCR fragment as probe at low stringency. This chick cDNA clone was used to make antisense digoxigenin-labeled RNA probes to test for the presence of *Otx2* RNA as control for antibody labeling.

For RNA extraction, the explants were lifted from the membranes after culture and transferred in a minimal volume of media into eppendorf tubes and either quickly frozen or processed for RNA extraction by the guanidinium/acid phenol method. First strand cDNA was synthesized using random hexamers and Superscript reverse transcriptase (BRL). Primers for RT–PCR were as follows: Otx2-U, 5′-TAAAGCAACCGC-CTTACGC-3′; Otx2-D, 5′-GTTGATTTTCAGTGCCACC-3′; S17-U, 5′-AAGCTCCGCAACAAGATAGC-3′; S17-D, 5′-TGAAGGTTG-GACAGACTGCC-3′; Emx1-U, 5′-CGAGAAGAACCACTACGTGG-3′; Emx1-D, 5′-AGGTGACATCGATGTCCTCC-3′; TTR-U, 5′-TGGTTTTCACAGCCAACG-3′; TTR-D, 5′-GAGTCTCTCAAT-TCTGG-3′. All reactions were carried out at 55°C for 40 cycles and run in ethidium-bromide-containing 2% agarose gels.

References

S1. Stern C, Holland P: *Essential Developmental Biology – A Practical Approach*. Oxford: Oxford University Press; 1993.

S2. Fishell G, Mason CA, Hatten ME: Dispersion of neural progenitors within the ventricular zone of the cerebral cortex. *Nature* 1993, 362:636-638.

S3. Costa RH, van Dyke TA, Yan C, Kuo F, Darnell JE Jr: Similarities in transthyretin gene expression and differences in transcription factors: liver and yolk sac compared to choroid plexus. *Proc Natl Acad Sci USA* 1990, 87:6589-6593.

S4. Simeone A, Acampora D, Gulisano M, Stornaiuolo A, Boncinelli E: Nested expression domains of four homeobox genes in developing rostral brain. *Nature* 1992, 358:687-690.

S5. Scharen-Wiemers N, Gerlin-Moser A: A single protocol to detect transcripts of various types and expression levels in neural tissue and cultured cells: ***in situ*** hybridization using digoxigenin-labeled cRNA probes. *Histochemistry* 1993, 100:431-440.