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Pax6-dependent regulation of adhesive patterning, *R-cadherin* expression and boundary formation in developing forebrain

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SUMMARY

Mutations in the gene for the transcription factor, *Pax6*, induce marked developmental abnormalities in the CNS and the eye, but the cellular mechanisms that underlie the phenotype are unknown. We have examined the adhesive properties of cells from the developing forebrain in *Small eye*, the *Pax6* mutant mouse. We have found that the segregation normally observed in aggregates of cortical and striatal cells in an in vitro assay is lost in *Small eye*. This correlates with an alteration of in vivo expression of the homophilic adhesion molecule, *R-cadherin*. Moreover, the boundary between cortical and striatal regions of the telencephalon is dramatically altered in *Small eye*: radial glial fascicles do not form at the border, and the normal expression of *R-cadherin* and *tenascin-C* at the border is lost. These data suggest a link between the transcription factor, *Pax6*, *R-cadherin* expression, cellular adhesion and boundary formation between developing forebrain regions.

Key words: *Small eye* mutant mice, patterning, cortex, striatum, radial glia, RC2, tenascin-C

INTRODUCTION

The molecular mechanisms that generate regional and cellular diversity in the developing central nervous system are largely unknown. Transcription factors, signalling and adhesion molecules are all likely to be involved in these mechanisms, and the manner in which the pattern of expression of many of these genes anticipates prospective brain regions is consistent with such a role (for review: Krumlauf, 1994; McMahon 1992; Redies and Takeichi, 1996). How the different elements of these mechanisms might interact, however, is less well understood. The present study aims at elucidating a functional link between transcription factors and adhesion molecules co-expressed in a region-specific manner in the developing brain.

The analysis of genes, such as from the *Hox, Emx, Otx, Dlx, Pax, POU* (reviewed in Lumsden and Krumlauf, 1996; Mansouri et al., 1994; Rosenfeld, 1991; Rubenstein and Puelles, 1994;), zinc-finger (reviewed in Beato, 1989) or *Wnt* and *FGF* gene families (reviewed in Nusse and Varmus, 1992; Baird, 1994), has confirmed their important role in the specification of developing brain regions. For example, the elimination of *Hoxa-1* by homologous recombination results in a deletion of rhombomeres 5 and 4, while the disruption of the zinc-finger gene *Krox-20* leads to a loss of the territory of rhombomeres 3 and 5 (reviewed in Lumsden and Krumlauf, 1996). Furthermore, the targeted inactivation of *Hoxb-1* changes the identity of rhombomere 4 (Studer et al., 1996). After targeted mutation of the *Emx2* gene, the dentate gyrus is entirely missing and the hippocampus and medial limbic cortex are also abnormal (Pellegrini et al., 1996; Yoshida et al., 1997), whereas the elimination of *Otx1* affects severely the cortical morphogenesis and the cerebellar foliation and leads to a spontaneous epileptic behaviour (Acampora et al., 1996).

Mutation of the *Pax6* gene generates a variety of CNS abnormalities in brain and eye development in various vertebrates including humans (reviewed in Tremblay and Gruss, 1994). *Pax6* is a member of the evolutionary conserved *Pax* gene family of transcriptional regulators and its expression is mostly confined to the developing CNS and eye (Walther and Gruss, 1991). The naturally occurring *Small eye* mutation (*Sey*) is a point mutation within the *Pax6 g*ene. It results in a protein that is truncated between the two DNA-binding domains (the paired domain and the paired type homeodomain) and is assumed to be non functional (Hill et al., 1991). Homozygous mice die soon after birth having failed to develop eyes or a nasal cavity (Hogan et al., 1986). They also have a malformed cerebral cortex (Schmahl et al., 1993). The cellular mechanisms underlying these malformations have not yet been thoroughly investigated, although failures in neural cell migration have been observed in the rat *Small eye* mutation (Matsuo et al., 1993). Recently we demonstrated severe patterning defects in the *Small eye* mutant forebrain in regions where *Pax6 i*s specifically expressed (Stoykova et al., 1996). We found that the morphogenesis of the hypothalamo-telencephalic transition zone was distorted, and that the specification of nuclei and structures in the ventral thalamus was abolished. In the wild-type telencephalon, *Pax6* and *Dlx1* are expressed in a complementary pattern in the cortex and the basal ganglia, and thereby delineate the boundary between the lateral ganglionic eminence (LGE) and the cortex (CTX). In the *Small eye*

mutant, the expression of *Dlx1* is distorted at this and other expression borders (Stoykova et al., 1996). These observations prompted the suggestion that loss of *Pax6* function leads to a distortion of forebrain boundaries.

In the present study, we have examined the behaviour of cells from the two forebrain regions that appear compromised (cortex and striatum). This follows our recent study, which showed that cells from these forebrain regions differ in their adhesive properties (Götz et al., 1996). In a short-term adhesion assay, cells from a given region prefer to adhere to other cells from the same region and to segregate from cells of the adjacent domain. This property is dependent on Ca^{2+}-dependent adhesion molecules, and two such homophilic molecules, R-cadherin (Matsunami and Takeichi, 1995) and Lewis X (Götz et al., 1996), are located exclusively in the cortex and thus may mediate the differential adhesion. Similar region-specific segregation has been observed for other telencephalic (Whitesides and LaMantia, 1995) and rhombencephalic regions (Wizenmann and Lumsden, 1997). These in vitro results have shown that cells of prospective brain regions differ in their adhesive properties and suggested that region-specific adhesion might be a fundamental aspect of pattern formation in the developing brain in vivo. This hypothesis predicts a link between the patterning instructed by transcription factors, the region-specific segregation in vitro and the delineation of adjacent brain regions in vivo. We tested this hypothesis by analysing the region-specific segregation and boundary formation between distinct regions in the *Small eye* mutant in comparison to the wild type.

MATERIALS AND METHODS

Animals

The allele of the *Small eye* mouse used in this work is *Sey*, a spontaneous mutation originally described by Roberts (1967) and Hogan et al. (1986) on a C57BL/6J × DBA/2J background. Heterozygous *Sey* parents were crossed to obtain homozygous, heterozygous and control wild-type embryos. The day of vaginal plug was considered as embryonic day (E) 0.5. In some experiments, we included heterozygous animals from the small eye allele Sey^{Dey} (Jackson Laboratories, Theiler et al., 1978), which has a large chromosomal deletion including the *Pax6* region.

Short-term aggregation assay

Experiments were performed as previously described in Götz et al. (1996). The lateral and medial ganglionic eminence (for simplification in the text referred to as 'striatum') and neocortical anlage ('cortex') were isolated in Dulbecco's modified medium (DMEM) containing 10 mM Hepes, and dissociated in Ca/Mg-free HBSS containing 1 mM EDTA (dissociation buffer). After several washes in chemically defined medium (Bottenstein and Sato, 1979), cells were labelled with the fluorescent cell tracker dyes (Molecular Probes, green: C2925, blue: A2110; red: C2927; final concentration 10 μM) as described previously (Götz et al., 1996). After removal of the staining solution and several washes, cells of each fraction were mixed in equal numbers (200 μl of 1×10^6 cells/ml) in 24-well plates and incubated at 37°C in 5% CO_2 on a rotary shaker (70-80 revs/minute) incubator for 60 minutes. Aggregates were then fixed in 3.7% formalin and mounted as described previously (Götz et al., 1996).

Analysis was performed after fixation overnight and aggregates were classified according to the following criteria as described in Götz et al. (1996):

– as 'pure' when only 1-3 cells in an aggregate exhibit a different fluorescence.

– as 'clustered' when cells of different fluorescence are arranged in separate clusters within the same aggregate (Fig. 1E-G). Four and more cells of the same fluorescence next to each other were regarded as a 'cluster'.

– as 'mixed' when none of the above criteria was appropriate and cells with different fluorescence were intermingled (see Fig. 1A-D).

The 'pure' and 'clustered' aggregates were considered as 'segregated', and their frequency as a percentage of all aggregates was designated the segregation ratio. Experiments with high proportion of dead cells, weak aggregation, incomplete or equivocal fluorescent labelling were excluded from the analysis. Data were collected from a total of 9 different experiments and the aggregates were analysed double-blind.

The mutant and WT aggregates were also analysed in size and number and were found to be comparable in these parameters. In order to quantify the size of aggregates, we defined small (more than 10 and less than 30 cells/aggregate) and large (more than 30) aggregates. WTctx/WTstr formed 17% (n=96) and *Sey*ctx/*Sey*str formed 20% (n=193) large aggregates. When the total number of aggregates per well was assessed, we found a mean of 253 (n=3289, 13 wells) for the WT aggregates and a mean of 232 (n=3020, 13 wells) aggregates per well for mutant aggregates.

BrdU labeling and immunohistochemistry

In order to label cells on their day of birth, time-mated heterozygous *Sey* mice were injected intraperitoneally with 14 mg bromodeoxyuridine (BrdU, Sigma) per 100 g body weight (Miller and Nowakowski, 1988). Animals were killed after 1 hour by cervical dislocation, the brain was removed in ice-cold phosphate-buffered saline (PBS), pH 7.4, embedded in Tissue Tek and immediately frozen on dry ice. Frontal or sagittal cryostat sections were cut at 10 μm thickness. For BrdU detection, sections were fixed for 10 minutes in 4% paraformaldehyde in PBS (pH 7.5) and then processed as described previously (Götz and Bolz, 1992; BrdU antiserum, IgG1, 1:10, Bioscience, Switzerland). The monoclonal antibody RC2 (IgM; kindly provided by P. LePrince) was used to detect radial glial cells at a 1:60 dilution after fixation in 2% paraformaldehyde in PBS, pH 7.5 for 30 minutes. The monoclonal antibody directed against β-tubulin III (IgG2b; Sigma) was used at 1:100 after fixation in methanol at –20°C for 5 minutes. Secondary rhodamine- or fluorescein-coupled antibodies were used at 1:50 (for further description see Götz and Bolz, 1992). For detection of the Lewis X antigen, we used the monoclonal antibody RB11.2 (IgM; Götz et al., 1996) and the avidin-biotin enhancement of the Vectastain Elite kit (Vector) and a standard DAB reaction (Vektor). After washing, slides were mounted in the glycerol-based mounting medium Mowiol (Aldrich). Serial sections of WT and *Sey/Sey* brains were stained simultaneously to compare the staining intensity. At least two different WT and mutant brains were analysed at each stage (E13.5-E18.5), except for Lewis X, which was examined at E14.5.

In situ hybridisation

Sectioning, in situ hybridisation, washing and emulsion autoradiography were performed as previously described (Stoykova and Gruss, 1994). ^{35}S-labelled sense and anti-sense RNA probes were synthesised in the presence of two radioactive nucleotides according to the suppliers instructions (Promega) from linearised plasmid templates as described for *Pax6* in Walther and Gruss (1991), *Emx1/2* in Simeone et al. (1992), *Otx1/2* in Simeone et al. (1993), *Prox 1* in Oliver et al. (1993), *R-cadherin* in Matsunami and Takeichi (1995), *N-cadherin* in Redies and Takeichi (1993) and *tenascin-C* in Götz et al. (1997). Two independent in situ analyses were performed for each stage on serial sagittal and transverse sections from WT and *Sey/Sey* littermate embryos with all the probes mentioned above (see also Stoykova et al., 1996). Signals were compared at corresponding levels in WT and

Sey/Sey brains processed in the same in situ hybridisation experiment. The terminology for the prosomeric subdivision is according to Puelles and Rubenstein (1993) and the rat brain atlases of Paxinos et al. (1991) and Altman and Bayer (1995) were used for the regional identification.

RESULTS

Segregation of cortical and striatal cells *Small eye* mutant mice

First, we examined whether the segregation of cortical and striatal cells is altered in the *Small eye* mutant using the previously described short-term aggregation assay (Götz et al., 1996). Cortical and striatal cells (see Methods) from wild-type (WT) and *Sey/Sey* littermates were isolated, labelled with different fluorescent dyes and allowed to aggregate for an hour on a rotary shaker. Aggregates were classified as either 'pure', 'clustered' or 'mixed' as before (Götz et al., 1996) and the segregation ratio was calculated as described in the Methods.

When cortical and striatal cells from E13.5 WT mice were mixed, 71% of all aggregates (n=249) were segregated, i.e. clustered or pure (Figs 1A-D, 2B). These results are in good agreement with our previous data from rat and chick telencephalon (Götz et al., 1996). In contrast, however, when cortical and striatal cells from *Sey/Sey* littermates were mixed, only 37% of all aggregates (n=861) were segregated (Fig. 2B) and most aggregates were mixed (see example in Fig. 1E-G). This is a significant reduction (χ^2 test, P=0.01) compared to the segregation ratio of WT cortical and striatal cells. A comparably low segregation ratio was observed when cortical and striatal cells from heterozygous *Small eye* (*Sey/WT*) or *Dickies's Small eye* (Sey^{Dey}/WT) were mixed (*Sey*/WT: 43% segregated aggregates, n=150; heterozygous animals with severe eye defects were selected. Sey^{Dey}/WT: 47%, n=164). The low percentage of segregated aggregates formed by cortical and striatal cells from *Sey/Sey* mutants is not significantly different from the negative control when two samples of WT striatal cells were differently labelled and mixed with each other (37%, n=484; Fig. 2B). These results suggest that the cortical/striatal segregation is attenuated in *Sey/Sey* mutant mice.

Next, we analysed the developmental profile of the adhesive behaviour to ask whether segregation might appear at later stages in the *Sey/Sey* mutants. Corticostriatal aggregates from WT and *Sey/Sey* littermates ranging from E12.5-E15.5 were assessed. Throughout this developmental window, the majority of aggregates was mixed with a segregation ratio of 38% (n=1823). In contrast, WT cells displayed the developmental profile previously described (Götz et al., 1996) – between E12.5 and E14.5, most WT cortico-striatal aggregates were segregated (70%, n=418) but, by E15.5, most aggregates were mixed and only 35% were segregated (n=103; Fig. 2A). As depicted in Fig. 2A, the segregation ratio of E15.5 *Sey/Sey* cortical and striatal cells remained as low as at earlier stages (37%, n=444). Taken together, throughout the developmental stages analysed (E12.5-15.5) cells from *Sey/Sey* cortex and striatum predominantly mix in contrast to the developmentally regulated region-specific adhesion in the WT animals.

The failure of cortico-striatal segregation observed in the *Sey/Sey* mutant implies that cells have altered adhesive prop-

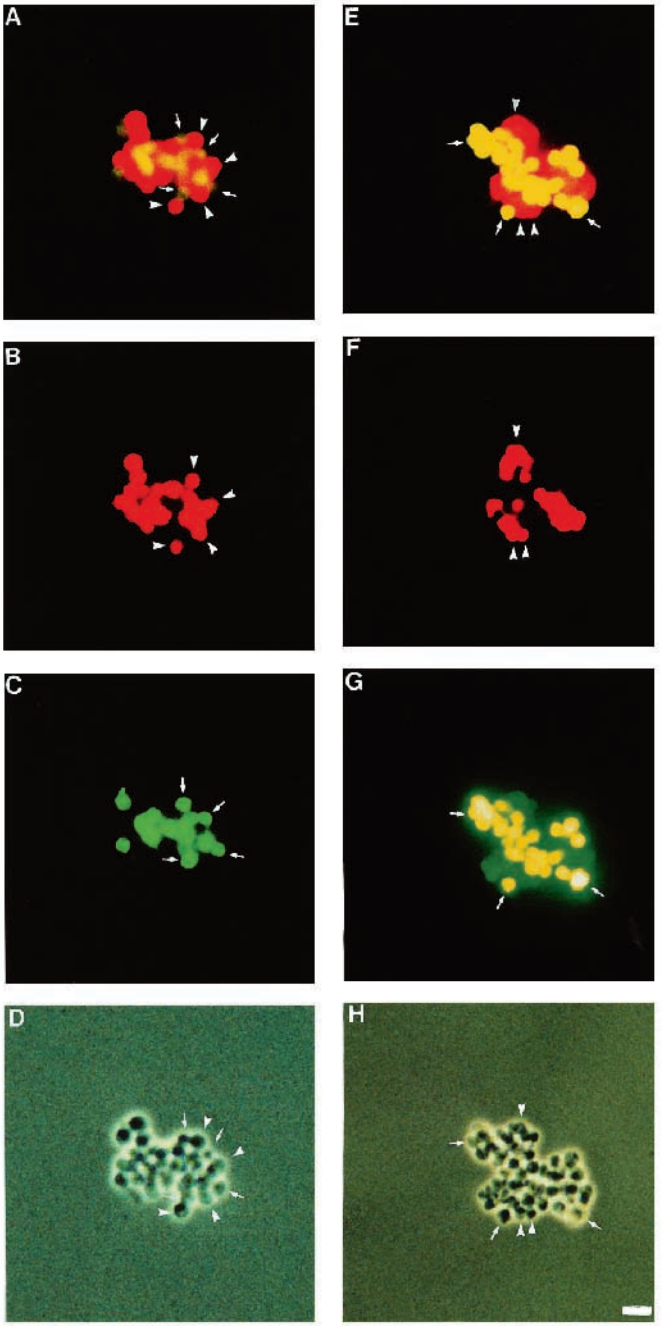


Fig. 1. Fluorescence micrographs of aggregates of wild-type (WT) and *Sey/Sey* cortical and striatal cells labeled with different fluorescent dyes isolated at embryonic day 13.5. (D,H) The phase contrast of the respective aggregregates, photographed in green (C,G), red (B,F) and green+red fluorescence (A,E). Green fluorescent cells appear yellow in the double filter (A,E). To illustrate the distinct labeling of cells arrows and arrowheads in A,D and E,H indicate examples of cells that are labeled either by red (arrowheads in B,F) or green (arrows in C,G) fluorescent dyes. (A-D) Example of an aggregate formed by green fluorescent *Sey/Sey* cortical and red fluorescent *Sey/Sey* striatal cells. This aggregate was classified as mixed. (E-H) Example of an aggregate formed by red fluorescent WT cortical and green fluorescent WT striatal cells. This aggregate was classified as segregated. Scale bar: 25 μm.

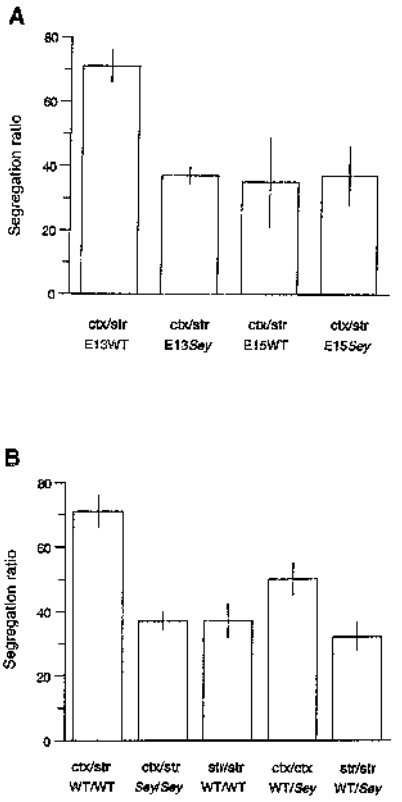


Fig. 2. Quantitative analysis of aggregates formed by wild-type (WT) and *Sey/Sey* cortical and striatal cells. Aggregates were assessed as segregated or mixed, and the proportion of segregated aggregates is expressed as a percentage of the aggregates examined (segregation ratio). Error bars depict the standard error of the mean (s.e.m.). (A) The histogram depicts the composition of aggregates formed by cortical and striatal cells from WT and *Sey*/*Sey* littermates at E13.5 and E15.5, respectively. The data are derived from four different experimental batches with a total number of 357 aggregates assessed for E13.5 WT ctx/str; 855 for E13.5 *Sey/Sey* ctx/str; 103 for E15.5 WT/WT ctx/str and 444 for E15.5 *Sey*/*Sey* ctx/str. Note that cortical and striatal cells from *Sey*/*Sey* predominantly mix, whereas cortical and striatal cells from WT littermates predominantly segregate at E13.5 and mix at E15.5. (B) The histogram depicts the composition of aggregates formed by WT and *Sey/Sey* cortical and striatal cells isolated at E13.5. The first two bars are identical to the first two bars in A for direct comparison. The third bar str/str represent the negative control (striatal cells labeled with different dyes and mixed, *n*=484). The fourth and the fifth bars represent the segregation ratio of cells from homotypic regions of the WT and *Sey* with a total number of 514 aggregates for ctx/ctx WT/*Sey* and 412 the for str/str WT/*Sey*. Note that *Sey*/*Sey* striatal cells mix with WT striatal cells similar to the negative control (WT str/str), whereas *Sey*/*Sey* cortical cells exhibit a significantly higher segregation ratio from WT cortical cells (χ^2 test, *P*=0.01).

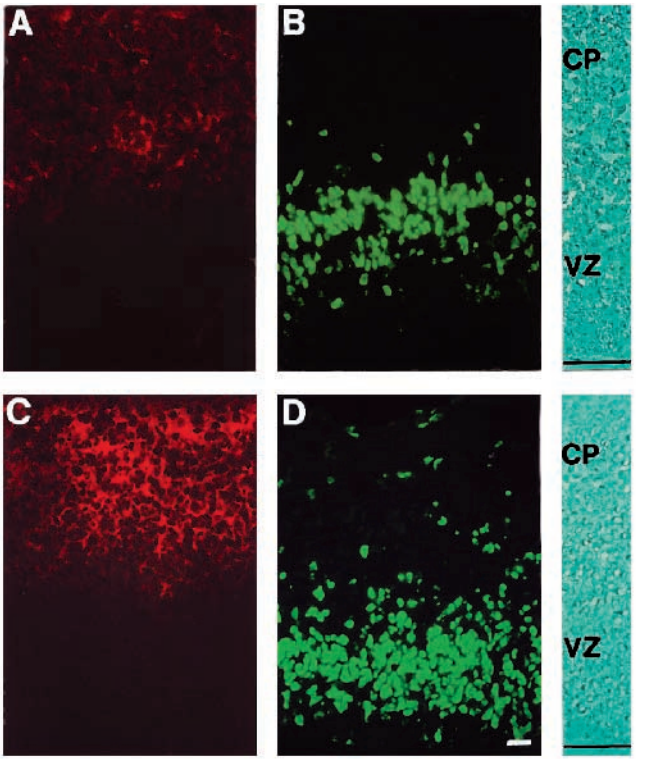


Fig. 3. Fluorescent micrographs of transverse sections of WT and *Sey*/*Sey* cortex at embryonic day E13.5. Corresponding sections were stained for differentiated neurons (anti-tubulin, A,C) and BrdU (B,D; BrdU-pulse 1 hour). Cortical layers were assessed using phase contrast and are indicated on the right (CP, cortical plate; VZ, ventricular zone). The black line indicates the border to the ventricle. Note that tubulin-positive, differentiated neurons and BrdU-pulse-labeled cells are located at similar positions in WT and *Sey*/*Sey* mutant cortex. Scale bar: 50 μm.

erties. Since *Pax6* is expressed by cortical but not striatal cells (Walther and Gruss, 1991; Stoykova and Gruss, 1994), we postulated that the adhesive properties of just the cortical cells might have been altered. To test this, we mixed WT and *Sey/Sey* cells from the same region. When E13.5 *Sey/Sey* and WT striatal cells were mixed, they failed to segregate (segregation ratio 32%, *n*=412; Fig. 2B). Similar results were observed with E15.5 cells (segregation ratio 30%, *n*=56). In contrast, when *Sey/Sey* and WT cortical cells were mixed, a significant proportion of the aggregates segregated. This was true of both E13.5 and E15.5 cells (segregation ratio E13.5 = 50%, *n*=514, Fig. 2B: E15.5 = 54%, *n*=433; χ^2 test, *P*=0.01). Interestingly, the segregation ratio of WT and *Sey/Sey* cortical cells is intermediate between the negative control (37%) and the high segregation ratio of WT cortical and striatal cells (72%; Fig. 2B). These results indicate that the adhesive properties of the *Sey/Sey* cortical cells are changed so that they now segregate from WT cortical cells, but that the striatal cells of the *Sey/Sey* brain still mix with WT striatal cells.

Differentiation of WT and *Sey/Sey* cortex are comparable during the stages analysed in the adhesion assay

Next we asked whether the adhesive differences of *Sey/Sey* cortical cells might be caused by alterations in the differentiation of WT and mutant cortical cells. We used BrdU-pulse labelling to detect dividing precursor cells and an antibody directed against β-tubulin III to detect differentiating neurons. During normal cortical development, precursor cells are located in the ventricular zone (VZ) and generate postmitotic neurons that migrate towards the pial surface and form the cortical plate (CP). Accordingly, in sections through the cortex from both WT and *Sey/Sey* littermates at E13.5, β-tubulin-stained cells were observed in the cortical plate, whereas BrdU-

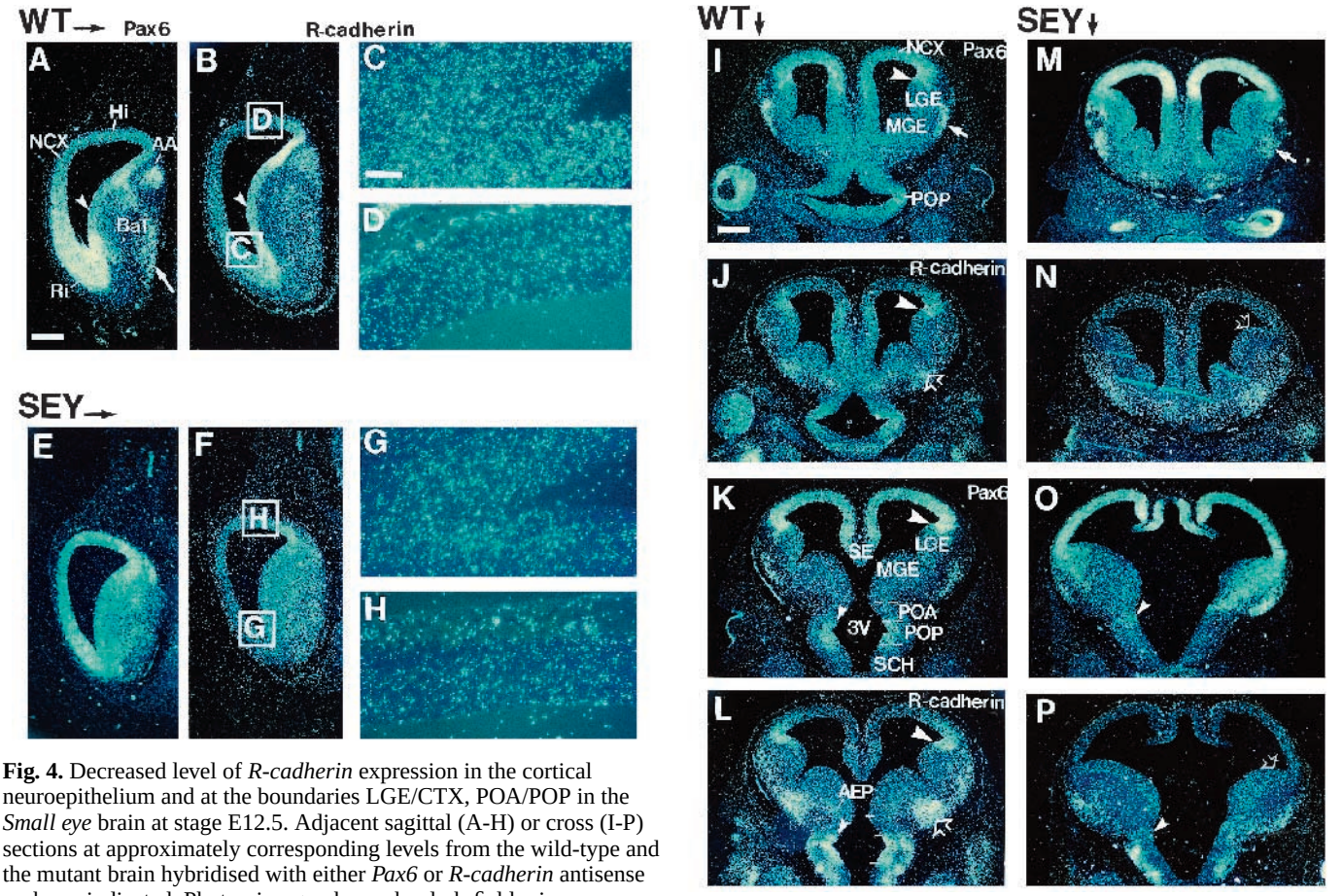


Fig. 4. Decreased level of *R-cadherin* expression in the cortical neuroepithelium and at the boundaries LGE/CTX, POA/POP in the *Small eye* brain at stage E12.5. Adjacent sagittal (A-H) or cross (I-P) sections at approximately corresponding levels from the wild-type and the mutant brain hybridised with either *Pax6* or *R-cadherin* antisense probe as indicated. Photomicrographs are by dark-field microscopy from WT (A-D; I-L) and *Sey/Sey* brain (E-H; M-P); C,G and D,H are higher magnifications of the neuroepithelium of the anterior rhinencephalon (Ri) and of the hippocampal region (Hi) in the cortical fields as indicated in B, F in the wild-type and in the mutant brain, respectively. (A,B) At lateralmost sagittal sections, the expression of *Pax6* and *R-cadherin* overlap in the ventricular zone of (from rostral to caudal): the anterior rhinencephalon (Ri), corticostriatal border (LGE/CTX, arrowhead) and amygdala (AA). The arrow in A points to a differentiating field in the basal rhinencephalon, strongly labelled by the *Pax6* probe. The big arrowheads in I-L indicate the expression border LGE/CTX, which is delineated by the *R-cadherin* and the *Pax6* expression domain on the cortical side. Note that this expression border does not coincide with the morphological cortico-striatal sulcus but extends over the lateromost field of the VZ of the LGE. Note that, in the *Sey/Sey* sections, *R-cadherin* expression is reduced to background levels at the border LGE/CTX (open arrows in N,P). Small arrowheads in K and L depict a second expression boundary, outlined by the coexpression of *R-cadherin* and *Pax6* in POP but not in POA. Note that in *Sey/Sey* (small arrowheads in O,P) the expression of *R-cadherin* is abolished in the POP region. The bigger open arrows in J, L point to the presumptive differentiating striatum, the arrows in I,M to the presumable region of the piriform cortex. AA, amygdala; AEP, anterior endopeduncular area; BaT, basal telencephalon; Hi, hippocampus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCX, neocortex POA, anterior preoptic area; POP, posterior preoptic area; SCH, suprachiasmatic area; 3V, third ventricle. Scale bars: in A (B,E,F) 300 μm; in C (D,G,H) 150 μm; in I (I-P) is 150 μm.

pulse-labelled precursor cells were located in the ventricular zone (Fig. 3A-D). We could detect only small differences in the ventricular zone of the *Sey/Sey* cortex as compared to the wild type: in the mutant VZ, more BrdU-labelled cells seem to be located closer to the ventricular surface. This observation merits further investigation, which is beyond the scope of this study. In the context of this study, the significant point is that, at the time when the adhesive behaviour of the mutant cortical cells is significantly altered, there are no obvious differences in the differentiation state of WT and *Sey/Sey* cortices.

Colocalized and specific expression domains of *R-cadherin* and *Pax6* in developing mouse forebrain

The intriguing similarity between the expression of *R-cadherin* and *Pax6* at early developmental stages (Gänzler and Redies, 1995; Matsunami and Takeichi, 1995) and the altered adhesive properties of the cortical cells in the *Small eye* brain prompted us to examine the effect of the *Pax6* mutation on the expression of the homophilic adhesion molecule *R-cadherin*. We first analysed the expression of the two genes on serial sagittal and cross sections in WT brain by in situ hybridisation analysis.

At E12.5 (preplate stage), *R-cadherin* and *Pax6* were both strongly expressed in the proliferative neuroepithelium of neocortex, hippocampus, subiculum, amygdala and anterior rhinencephalon and in a portion of the lateralmost striatal neuroepithelium (Fig. 4A,B). Both genes are expressed in the ventricular zone of the lateral cortex, but expression of each ceases abruptly at the boundary between the cortex and the lateral ganglionic eminence (LGE: Fig. 4 I/J, K/L). Thus the expression

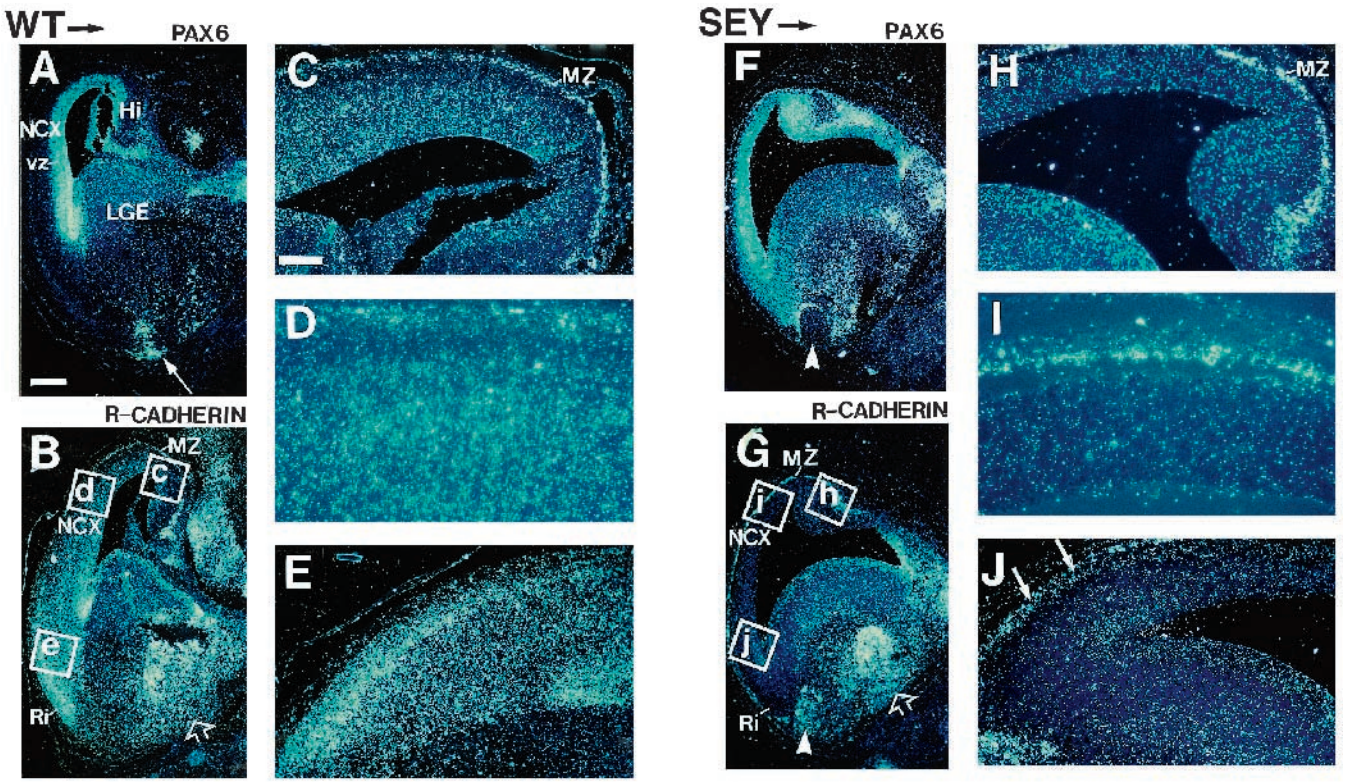


Fig. 5. Decreased level of *R-cadherin* expression in developing cortex, except for the marginal zone, in the *Small eye* brain at stage E14.5. Adjacent sagittal sections from wild-type (A-E) and *Sey/Sey* brain (F-J) at approximately corresponding lateral level were hybridised with specific probes for *Pax6* (A,F) and *R-cadherin* (B-E; G-J). C-E and H-J are higher magnification pictures of the *R-cadherin* hybridisation signal detected in posterior, middle and rostral cortical areas in the wild-type and in the mutant brain, as indicated in the B and G, respectively. In H-J, note the reduced level of *R-cadherin* expression in the *Sey/Sey* cortex, except in the marginal zone (MZ); the arrows in J point to *R-cadherin*-positive cells in the presumptive superficial layers of the lateral cortical plate in the *Small eye* mutant. The arrow in A indicates a *Pax6*-positive differentiating field in the presumable region of the olfactory bulb in the anterior rhinencephalon. The open arrow in B and G point to a *R-cadherin*-positive region in the basal telencephalon in the wild-type and mutant brain, respectively, which includes the presumptive region of pallidum and a differentiating field in the posterior rhinencephalon. The arrowheads in F and G point to the vesicle-like structure in the mutant telencephalic wall filled with *R-cadherin* (G)-positive cells. Scale bars: in A (B,F,G) 300 μm; in C (D,E,H,I,J) 150 μm.

of the two genes helps delineate precisely this boundary between embryonic cortex and striatum. They help delineate another boundary in the preoptic area. Both genes are expressed in the VZ of the posterior preoptic area (POP), while *R-cadherin* alone is also expressed in the mantle zone of this region (Fig. 4K,L). The transcripts of both genes are absent from the adjacent region of the anterior preoptic area (POA), thus outlining a second expression boundary (POA/POP).

In addition, there are regions where one of these genes is expressed and the other not. The *R-cadherin* expression in the mantle zone of the POP has already been noted. Within the basal telencephalon, this gene is also expressed within the presumptive globus pallidus (Fig. 4J,L), while *Pax6* is strongly expressed in the presumptive anterior olfactory nucleus and the piriform cortex. The identity of these different nuclei was confirmed at E18.5 when their morphology is clearer and the gene expression pattern is still clear (Figs 7A,B,F,G, 8).

At early cortical plate stage (E14.5), the pattern of expression seen earlier is maintained (Fig. 5). Consistent with the reported data (Matsunami and Takeichi, 1995), *R-cadherin* is faintly expressed in medial cortical regions (data not shown). The *Pax6* expression in the cortical ventricular and subventricular (SVZ) zones remains. In addition, *R-cadherin* expression is now detected throughout the cortical thickness including the cortical plate (Fig. 5C-E).

We also examined the expression of the *Pax6* and *R-cadherin* in the developing diencephalon and hypothalamus. At E12.5 there is an extensive overlap of *Pax6* and *R-cadherin* expression in the ventricular and mantle zone of the ventral thalamus, especially in its caudal part (Fig. 6; see also Matsunami and Takeichi, 1995). By E14.5, however, distinct structures in the mantle zone of the ventral thalamus are clearly delineated by virtue of their *R-cadherin* or *Pax6* expression (Fig. 6C,D). We reported previously that the optoeminential zone expresses *Pax6* (Stoykova et al., 1996). This is a forebrain domain, including the caudal ganglionic eminence (CGE), eminentia thalami (EMT), supraoptic paraventricular area (SPV), anterior hypothalamus (AH), posterior preoptic area (POP: see Puelles and Rubenstein, 1993). This optoeminential zone also expresses *R-cadherin* and is therefore another area of co-expression of these two genes (Fig. 6). In contrast, several hypothalamic regions were labelled by *R-cadherin* only; i.e. suprachiasmatic area (SCH), hypothalamic cell cord (HCC), the tuberal hypothalamus (TU) and the retrochiasmatic domain (RCH). In the pretectum (PT), *Pax6* and *R-cadherin* transcripts were detected within its posterior region and in the

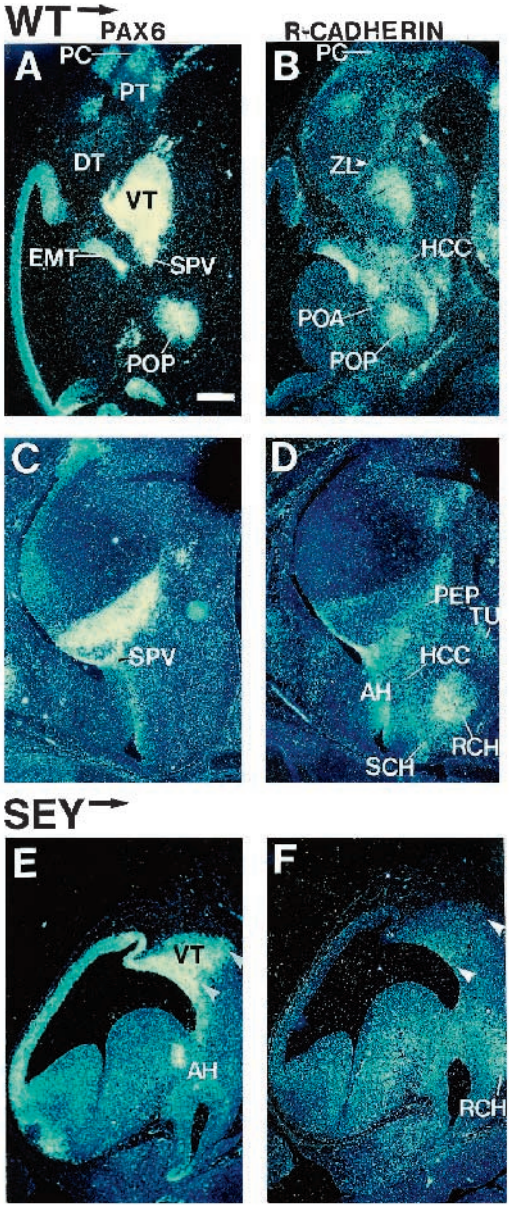


Fig. 6. Expression of *R-cadherin* and *Pax6* in the developing diencephalon and reduced *R-cadherin* expression in the ventral thalamus and optoeminential zone of the *Small eye* brain. (A,B,C,D) Adjacent sagittal sections from E12.5 and E14.5 wild-type brain, hybridised with *Pax6* (A,C) and with *R-cadherin* (B,D) probes. Note the colocalization of the transcripts of the two genes within the ventral thalamus (VT) and the entire optoeminential zone, including the eminentia thalami (EMT), supraoptic paraventricular area (SPV), anterior hypothalamus (AH) and posterior optic area (POP). (E,F) Adjacent sections from E12.5 *Small eye* brain hybridised with *Pax6* (E) and *R-cadherin* (F). The two arrowheads in E and F point to the compartment of the morphologically distorted VT in the mutant brain in which the *R-cadherin* expression is down-regulated to a level only faintly above the background (compare the signal strength in F/B),while the *Pax6 e*xpression is at a comparable level to that in the wild-type brain (compare E/A). AH, anterior hypothalamus, DT, dorsal thalamus; EMT, eminentia thalami; HCC, hypothalamic cell cord; PC, posterior commissure; PEP, posterior entopeduncular area; POA, anterior preoptic area; POP, posterior preoptic area; PT, pretectum; RCH, retrochiasmatic area; SCH, suprachiasmatic area; SPV, supraoptic/paraventricular area; TU, tuberal hypothalamus; VT, ventral thalamus; ZL, zona limitans intrathalamica. Scale bar 300 μm.

posterior commissure, while the zona limitans intrathalamica (ZL) expressed *R-cadherin* only.

In the E18.5 telencephalon (late cortical plate stage), the co-expression *Pax6* and *R-cadherin* remained in the cortical VZ and SVZ (Figs 7A/B, C/E; 8A,B, C,D), and *R-cadherin* remained prominent in the intermediate zone, the deeper layers and the MZ of the lateral olfactory cortex (Fig. 8D,H). The CP expressed moderate levels of *R-cadherin* except for its rostral-most regions (including the insular and perirhinal cortex) where the level of expression appears higher (empty arrow-heads in Figs 7B, 8B). Thus, throughout development cortical *R-cadherin* expression is mainly confined to the lateral regions.

In the forming olfactory bulb, *R-cadherin* and *Pax6* also showed overlapping and specific expression domains. Transcripts of both genes were detected in the rostral VZ/SVZ and the internal granular layer, but the mitral layer only expressed *R-cadherin* and the periglomerular cell layers only expressed *Pax6* (Fig. 8A,B).

In summary, our results show that during brain development *R-cadherin* and *Pax6* transcripts colocalize in the cortical and olfactory proliferating neuroepithelium, in the optoeminential zone, in the pretectum and along two forebrain boundary zones (CTX/LGE, POP/POA). In addition, there are a number of regions where only one or the other of these two genes are expressed.

R-cadherin expression in the *Small eye* mutant

Given that a number of developing brain regions co-express *R-cadherin* and *Pax6*, we wanted to know what happened to *R-cadherin* expression in *Sey* mice. We discovered that *R-cadherin* expression was considerably reduced in areas that in the WT showed co-expression. In E12.5 *Sey/Sey* mice, the prominent *R-cadherin* expression that abutted the two boundary zones, LGE/CTX and POA/POP, was almost completely abolished (Fig. 4J/N, L/P). *R-cadherin* expression in the cortical anlage was severely reduced (Fig. 4C/G), and even the very low level of *R-cadherin* expression in the WT hippocampal anlage was decreased (Fig. 4D/H). In the structures of the basal telencephalon where *R-cadherin* and *Pax6* were not colocalized, however, the expression level of *R-cadherin* was not reduced.

At E14.5, the reduction of *R-cadherin* expression in the *Sey/Sey* cortex was pronounced through the width of the telencephalic wall including the VZ where co-expression had been detected in the WT (compare in Fig. 5E/J, D/I, C/H). However, expression was retained in the MZ where co-expression had not been observed. The strongly labelled cells in the MZ are almost certainly Cajal-Retzius cells given their location, horizontal orientation and their expression of the *reelin* gene (not shown), a specific marker for these cells (D´Arcangelo et al., 1995; Ogawa et al., 1995). The *R-cadherin* expression in the *Sey/Sey* basal ganglia was not substantially changed compared with WT, this not being an area of co-expression (Fig. 5B/G). Interestingly, the vesicle-like structure that is formed in the rostralmost region of the *Small eye* telencephalon (Schmahl et al., 1993) was moderately labelled by the *R-cadherin* (Fig. 5G, arrowhead) and *reelin* probes (not shown).

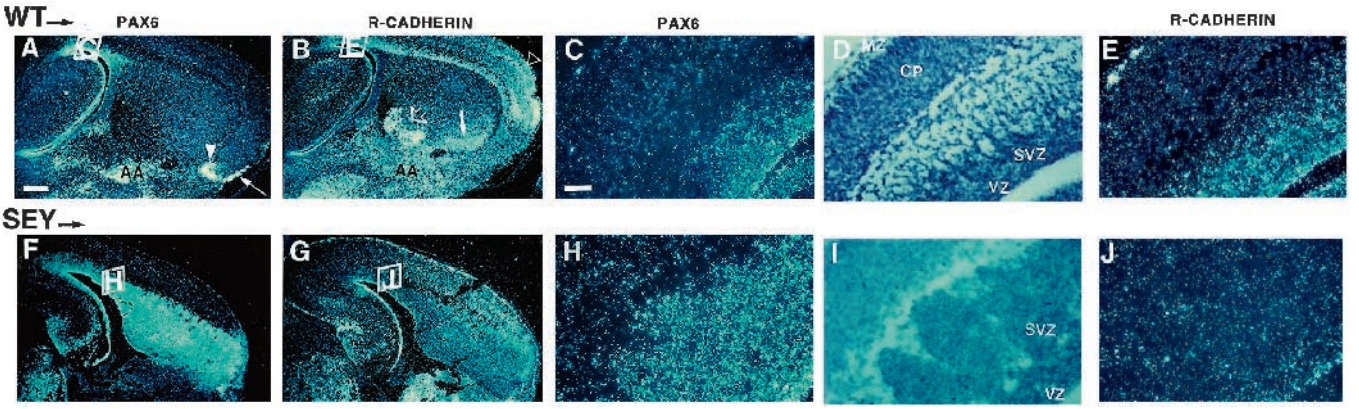


Fig. 7. Decreased level of *R-cadherin* expression in the VZ/SVZ and in the lateral cortex of the *Small eye* brain at stage E18.5. Adjacent sections from wild-type (A,B) and *Sey/Sey* (F,G) brains at approximately corresponding lateral level were hybridised with the *Pax6* (A,F) and with the *R-cadherin* probe (B,G). C,E are dark-field pictures at higher magnification of the dorsolateral cortical region as indicated in A and B showing the comparable level of expression of *Pax6* and *R-cadherin* in the VZ/SVZ in the wild-type brain. H,J are dark-field pictures at higher magnification of the indicated fields in dorsolateral cortical wall of the *Small eye* brain, illustrating the severe decrease of the *R-cadherin* expression in the mutant VZ/SVZ that is much enlarged. D, I are bright-field pictures of E,J. The thin arrow and the arrowhead in A point to the presumable piriform cortex and endopiriform nucleus, respectively, that express *Pax6.* The open arrow and the arrow in B point, respectively, to the presumptive pallidum and to a differentiating field in the basal striatum both of which express *R-cadherin.* Note the diffuse expression for *R-cadherin* in the basal rhinencephalon and the region of the amygdala, and the restricted signal for *Pax6* to differentiating fields and nuclei in these regions. AA, amygdala; CP, cortical plate; MZ, marginal zone; VZ, ventricular zone; SVZ, subventricular zone; Scale bars: in A (B,F,G) 300 μm; in C (D,E,H,I,J) 150 μm.

The decrease in the *R-cadherin* expression persisted until E18.5. The enlarged VZ/SVZ in the mutant brain showed *R-cadherin* expression only slightly above background (Fig. 7J/E), whereas the signal appeared unchanged in the MZ as at earlier stages (compare in Fig. 8L/D,B). An intermediate level of *R-cadherin* expression was detected in the superficial regions of the rostrolateral cortex and the vesicle-like structure (see above) (Figs 5G, 8L). The strongly *R-cadherin* labelled cortical layer, seen in this region in the WT brain, was not detectable in corresponding sections from the mutant brain (Fig. 8D,H/L).

In the diencephalon also, *R-cadherin* expression was substantially decreased only in those forebrain regions where it colocalized with *Pax6*. The expression of *R-cadherin* decreased almost to the background level in the ventral thalamus (prosomere 3) in *Sey/Sey* (Fig. 6E,F). In contrast, no substantial changes in the level of *R-cadherin* expression were detected in the presumptive hypothalamic cell cord and retrochiasmatic area where the two genes are not co-expressed.

To check for the specificity of the observed reduction in *R-cadherin* expression in the mutant brain, we examined the expression of the closely related adhesion molecule *N-cadherin* (Redies and Takeichi, 1993) and Lewis X (Götz et al., 1996). In sagittal and cross sections from E14.5 WT and mutant brains no changes in the level of *N-cadherin* expression could be detected in the forebrain (Fig. 9), as well as in the diencephalon, mesencephalon and myelencephalon (data not shown). Likewise, no obvious changes were observed in Lewis X immunoreactivity (at E14.5, data not shown) and in any of the genes (*Emx2, Otx1, Otx2, Prox1*) whose expression is restricted to the developing cortex (data not shown; Stoykova et al., 1996). These results strengthen the suggestion that the reduced expression of *R-cadherin* in the *Small eye* forebrain regions is a specific consequence of the *Pax6* loss of function condition in the mutant brain.

Disturbances at the cortico-striatal boundary in the *Small eye* brain

We next analysed the cortico-striatal boundary in the WT and *Sey/Sey* mutant. Using the RC2-antiserum as a specific marker for radial glial cells (Misson et al., 1988), we found that radial glial fascicles develop along the cortico-striatal transition zone in the wild-type brain and are most prominent around E16.5 (Fig. 10A, arrows) as previously described (Edwards et al., 1990). In *Sey/Sey* littermates, RC2-immunoreactive cells are present but fascicles could not be detected at the cortico-striatal boundary (Fig. 10B).

We also examined the expression of another marker of the cortico-striatal boundary, the extracellular matrix molecule tenascin-C (TN-C). As shown previously in the rat (Götz et al., 1997), cells at this boundary and the cortical VZ cells show a high level of *TN-C* expression. In the *Sey/Sey* mutant, this expression is abolished (Fig. 10E/F, G/H). Interestingly, no alterations in expression could be detected in the VZ of the medial (hippocampal) telencephalic wall (Fig. 10F/H) or in other regions (mesencephalon, cerebellum) of the mutant brain (data not shown).

Taken together, these observations support the notion that the cortico-striatal boundary in the *Small eye* brain is compromised.

DISCUSSION

Pax6 function and cortico-striatal adhesive behaviour

In this study, we have detected altered cellular adhesion and expression of *R-cadherin* in the developing forebrain of the *Pax6* mutant *Small eye*. These changes are restricted to the *Pax6* expression domains, which fits with the idea that they are caused by the loss of *Pax6* function. These results confirm a

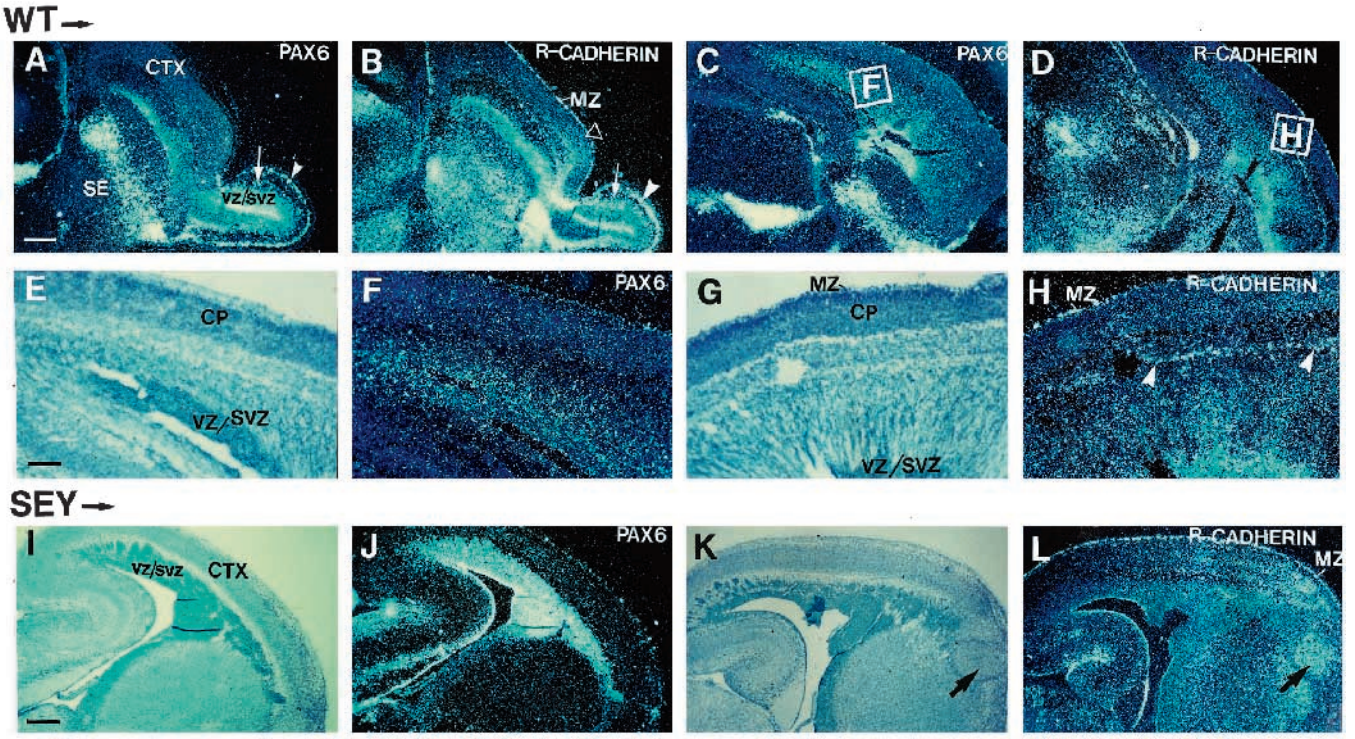
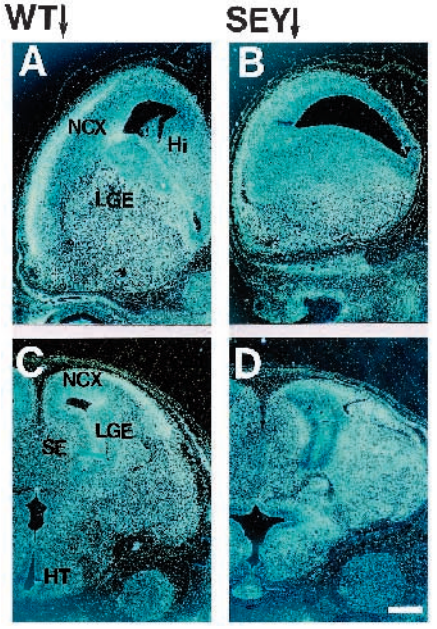


Fig. 8. Comparison of *R-cadherin* and *Pax6* expression in the developing olfactory bulb and olfactory cortex in the wild-type and *Small eye* brain at stage E 18.5. Adjacent sections at two more medial levels (A,B; C,D) of sectioning than those in Fig. 6 from the wild-type brain were hybridised with the *Pax6* and with the *R-cadherin* (B,D) antisense probes, as indicated. In A,B note the colocalization of the *Pax6* and of the *R-cadherin* signal in the olfactory bulb VZ/SVZ and in the presumptive internal granular layer (thin arrows), while the presumptive periglomerular- and mitral cell layer (arrowheads in A and B) are specifically labelled either by *Pax6* or *R-cadherin* probe. E,F and G,H are corresponding bright-/dark-field pictures at higher magnification of areas, shown in C and D respectively, illustrating the overlapping expression of the two genes in the cortical VZ/SVZ also at these more medial levels. (H) Note the strong hybridisation signal with the *R-cadherin* probe in the MZ and a layer of cells that appear to be below the subplate (arrowheads in H); I,K and J,L are bright-/dark-field pictures of adjacent sections from the *Sey/Sey* brain at approximately corresponding level to that of the wild-type brain, hybridised with the probe for *Pax6* and *R-cadherin*. (L) Note the severely decreased expression of *R-cadherin* in the pathologically enlarged VZ/SVZ in the mutant cortex (compare L/D) in contrast to the preserved expression of the gene in the MZ, the superficial layer of the olfactory cortex and the vesicle-like structure (arrows in K,L). However, the *R-cadherin*-positive layer of cells seen in the wild-type brain is not detectable in the mutant olfactory cortex (compare D, H/J). CP, cortical plate; CTX, cortex; MZ, marginal zone; SE, septum; VZ/SVZ, ventricular/subventricular zone. Scale bars: in A (B-D) 50 m; in E (F-H) 100 μm; in I (J-L) 50 μm.



link between patterning of the developing brain and adhesive properties of cells in embryonic brain regions. A similar link has been suggested for patterning by *engrailed* and *Wnt-1* genes and the adhesion molecule E-cadherin (Shimamura and Takeichi, 1992; Shimamura et al., 1994).

In the short-term aggregation assay, the segregation of cortical and striatal cells observed in WT animals during most of neurogenesis is significantly reduced in *Sey/Sey* mutant cells. The low degree of segregation appears to be due to an alteration of the properties of the cortical, but not the striatal cells since *Sey/Sey* and WT striatal cells mix, but *Sey/Sey* and WT cortical cells segregate. This is a satisfying result since it is the cortical not the striatal cells that express *Pax6*. These

Fig. 9. Unchanged level of *N-cadherin* expression in *Small eye* forebrain at stage E14.5. Sagittal (A,B) and cross (C,D) sections from wild-type (A,C) and *Sey/Sey* (B,D) brain were hybridized with a *N-cadherin* probe. The level of the sections in A and B are comparable to A and F respectively in Fig. 5. Hi, hippocampus; LGE, lateral ganglionic eminence; NCX, neocortex; SE, septum. Scale bar, 300 μm.

results accord well with the expression data, which showed a marked reduction in the expression of *R-cadherin* in the cortical but not striatal regions of the *Sey/Sey* telencephalon throughout development. Other adhesion molecules, *N-cadherin* and Lewis X, were not affected by *Pax6* mutation. Since we already know that the segregation of cortical and striatal cells depends on a Ca^{2+}-dependent mechanism (Götz et al, 1996), it seems likely that one component of this mechanism is *R-cadherin*.

The observation that the expression of *N-cadherin* and Lewis X, as well as *Emx2*, *Otx1/2* and *Prox1* (also in Stoykova et al., 1996) were not significantly changed in the mutant brain argues against an overall delay in differentiation of the *Sey/Sey* cortex. This conclusion is further supported by the analysis of differentiating neurons and BrdU-pulse-labelled precursor cells, which showed no gross differences between mutant and wild-type cortex. Nevertheless, we found that the BrdU-labelled cells were more dispersed through the ventricular zone of the *Sey/Sey* cortex (see Fig. 3), so we cannot exclude subtle aberrations in the cell cycle kinetics of *Sey/Sey* precursor cells and further experiments are needed to clarify this issue. Since the failure of the cortico-striatal segregation in the mutant brain cannot be explained by differentiation defects, our results suggest a specific (direct or indirect) link between *Pax6*, the decrease in *R-cadherin* expression and the region-specific segregation in the telencephalon.

Pax6 function and cortico-striatal boundary formation

Our experiments also show that the cortico-striatal boundary is distorted in the *Sey/Sey* mutant brain. RC2-immunostaining revealed that radial glia, whose fasciculated processes normally outline the cortico-striatal border at E16.5, are absent in the *Sey/Sey* mice. These morphological disturbances were preceded by changes in gene expression in this boundary region. In WT animals, *R-cadherin* is particularly strongly expressed in the *Pax6*-expressing, cortical VZ cells that abut the LGE/CTX boundary. The two genes also extensively colocalize in the region of POP at the expression border POP/POA. In *Sey/Sey* mice, *R-cadherin* expression at both of these boundaries was markedly down regulated as early as E12.5. Similarly, the normally high level of *TN-C* expression seen at E14.5 in the neuroepithelial cells at the LGE/CTX border was abolished in the *Sey/Sey* mutant. These results indicate that the cortico-striatal boundary is severely compromised in the *Sey/Sey* mutant. Moreover, the absence of cortico-striatal segregation in vitro and the absence of radial glia fasciculation at this boundary in the mutant brain in vivo raises the possibility that these phenomena are linked. It has been suggested in the developing hindbrain (see e.g. Lumsden and Krumlauf, 1996) that selective adhesion between distinct regions might be a prerequisite for boundary formation. This is at least consistent with

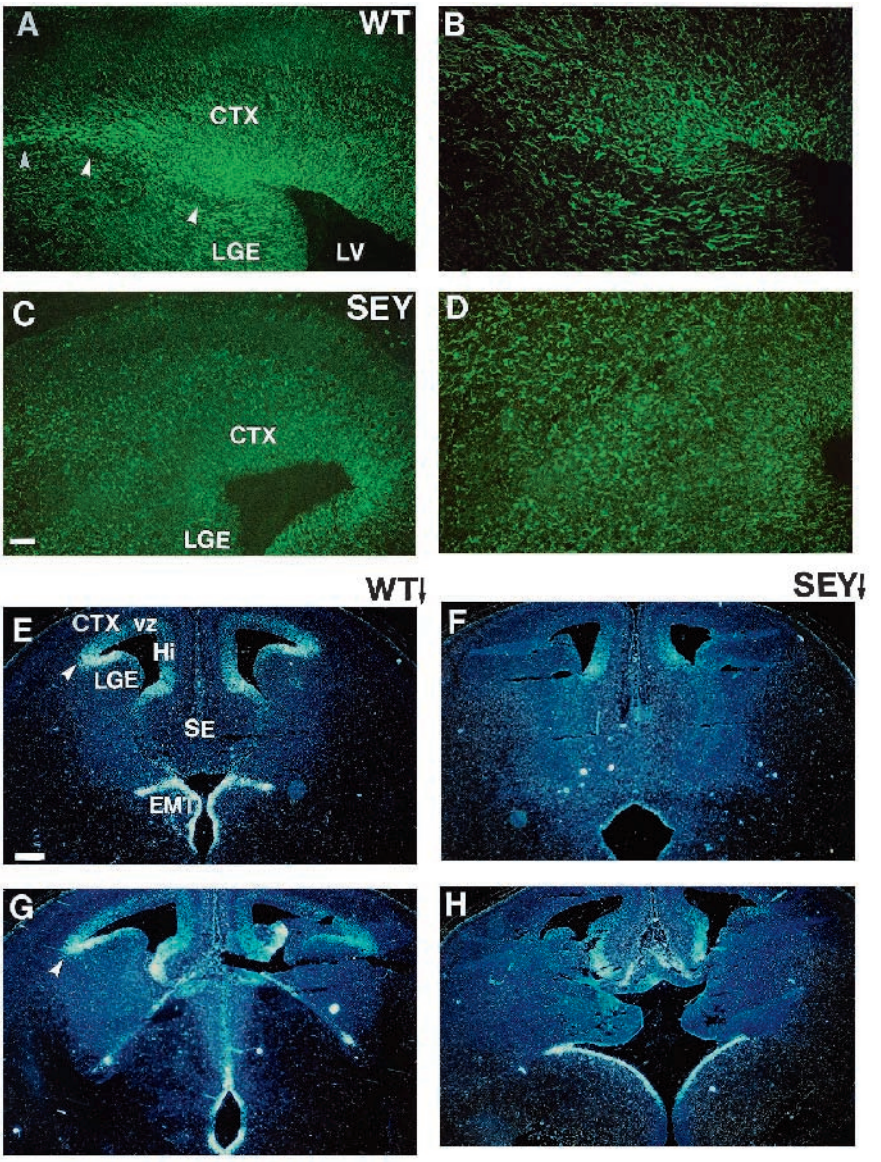


Fig. 10. Immunohistochemical and in situ hybridisation analysis of the border region between cortex and lateral ganglionic eminence in wild-type and *Sey/Sey* forebrain. (A-D) Fluorescent micrographs of RC2-labeled radial glial cells in cross sections from WT (A,B) and *Sey/Sey* (C,D) brains at embryonic day 16.5. B and D are higher magnifications of the boundary zone shown in A and C, respectively. Note the prominent radial glial fascicle between cortex (CTX) and lateral ganglionic eminence (LGE) in WT (indicated by arrowheads), which is missing in *Sey/Sey* mutant brains. Scale bar: 125 μm (A,C); 75 μm (B,D). (E-H) Dark-field micrographs of adjacent cross sections from E14,5 wild-type (E,G) and *Sey/Sey* (F,H) brains, hybridised with antisense in situ probe for *tenascin-C*. Note that, in the mutant brain, the expression of the *tenascin-C* is abolished in the ventricular zone of the dorsolateral telencephalic wall and at the border CTX/LGE, that is strongly labelled in sections at a corresponding level from the wild-type brain (arrowheads in E,G). CTX, cortex; EMT, eminentia thalami; Hi, hippocampus; LGE, lateral ganglionic eminence; LV, lateral ventricle; SE, septum; vz, ventricular zone. Scale bar, 200 μm.

our present results which show that the cortico-striatal segregation precedes the morphological specification of the boundary.

The defects observed at the cortico-striatal boundary in *Sey/Sey* mice are particularly intriguing because the formation of this boundary is one of the earliest regionalisation events within the forebrain VZ. The border neuroepithelium between the cortical and basal ganglia primordia forms a wedge that generates the neurons of the endopiriform nucleus of the basal telencephalon (Bayer and Altman, 1991). This subdivision is accompanied by the confinement of many transcription factors and regulatory molecules to either the cortical or to the striatal compartment (reviewed in Rubenstein and Puelles, 1994). Interestingly, the cortico-striatal expression boundary of many of those genes as well as the radial glial fascicle described above (Edwards et al., 1990) do not coincide with the morphological cortico-striatal sulcus (also Puelles and Rubenstein, 1993; Gänzler and Redies, 1995). Genes expressed within the cortical neuroepithelium such as *AP-2.2* (Chazaud et al., 1996; Fig. 3), *Prox-1* (Oliver et al., 1993; our unpublished data), *Tbr-1* (Bulfone et al., 1995) extend over this border region, whereas genes that are selectively expressed in the striatal anlage – *Dlx1/Dlx2* (Bulfone et al., 1993; Price et al., 1991; Stoykova et al., 1996), *Mash1* (Guillemot and Joyner, 1993; our own unpublished data) and *Six3* (Oliver et al., 1993; our observations) exclude the cortico-striatal wedge from their lateralmost expression domains. It is therefore reasonable to accept this peculiar region of the telencephalic neuroepithelium as a cortical rather than a striatal primordium, a suggestion initially made by Bayer and Altman (1991). If and how the molecular specialisations of the neuroepithelium at the cortico-striatal wedge contribute to the generation of particular neuronal descendants, located in structures of the basal telencephalon (e.g. endopiriform nucleus, piriform cortex; see Bayer and Altman, 1991), and to the formation of specialised radial glial fascicles (De Carlos et al., 1996) remains to be determined.

The alterations that we observed at the forebrain boundaries in *Sey/Sey* mice may also explain our previous results in which heterotopic expression of *Dlx1* was observed in the mutant cortex and POP (Stoykova et al., 1996), precisely at the boundary areas that our current data show are patterned by *Pax6*. The extension of *Dlx1* expression into cortical regions could be explained either by a transcriptional up-regulation of the *Dlx1* gene in the cortical cells due to loss of *Pax6* function, or by a failure in the mutant of the border to restrict cellular migration of *Dlx1*-expressing cells into the cortical domain. A restriction of migration has been reported at this boundary in wild-type mice (Fishell et al., 1993). In light of our results showing altered adhesive properties of *Sey/Sey* mutant cells, we tend to favour the latter explanation.

Pax6-regulated expression of *R-cadherin* and the forebrain phenotypes of the *Small eye* mutant

Several aspects of brain morphology are disrupted in *Sey/Sey* animals: the cortical VZ and SVZ are enlarged (Schmahl et al., 1993; Stoykova et al., 1996); cortical lamination is aberrant at late embryonic stages (Schmahl et al., 1993); the nuclei of the ventral thalamus and the hypothalamo-telencephalic transition zone exhibit abortive growth and differentiation (Stoykova et al., 1996; Glaser et al., 1994) and the olfactory bulb is missing (Hogan et al., 1986). We now know that, in addition, *R-cadherin* expression is disturbed in regions where *Pax6* and *R-cadherin* expression overlap, and where *Sey/Sey* brain is morphologically abnormal. It is not clear how many of the morphological abnormalities are linked to the disturbance of *R-cadherin* expression, but the failure of layer formation in the mutant cortex is one candidate. This hypothesis is supported not only by those cells whose *R-cadherin* expression is disrupted, but also by those whose expression is unaffected. The cells of the VZ and the cortical plate lose *R-cadherin* expression in the *Sey/Sey* cortex, but notably the cells of the marginal zone do not. The MZ is populated by the earliest generated neurons in the cortex (Bayer and Altman, 1991). It has been suggested that these early generated neurons move directly into the telencephalic wall (Smart and Sturrock, 1979; Valverde and Santacana, 1994), whereas the later generated neurons migrate along radial glia processes before settling in their final location (Bayer, 1986; DeCarlos et al., 1996; Rakic, 1971). In *Sey/Sey* animals, the marginal zone cells, including the Cajal-Retzius neurons, seem to achieve their normal position, in contrast to the later neurons that are dependent on radial glial guidance. It seems likely therefore that the radial glial migration is specifically affected in *Sey/Sey* mice because the migratory neurons and/or the radial glial cells have lost their expression of *R-cadherin*.

A similar explanation probably accounts for the abnormal olfactory bulb. *Sey/Sey* mice fail to form olfactory placode and olfactory bulb. Instead, a strange vesicle-like structure begins to form at the rostral-most telencephalic wall at about E14.5 in mice that carry either the *Sey* (Stoykova et al., 1996) or Sey^{neu} (Schmahl et al., 1993) allele. The cells in this structure express high levels of *R-cadherin*, and moderate levels of *reelin* as observed in the mitral cells of the WT olfactory bulb. Therefore this vesicle probably corresponds to the olfactory bulb, and the cells that accumulate in it correspond to mitral cells. Furthermore, they have characteristics (early birthdate and reelin expression) of cortical MZ cells (D'Arcangelo et al., 1995). Like the cortical MZ cells they also express *R-cadherin* but not *Pax6*. So, as in the cortex, the earliest generated neuronal cells express *R-cadherin* independent of *Pax6*, they are born normally, and invade the abnormal olfactory vesicle. The latter-generated granule and periglomerular cells of the olfactory bulb are born in the rostral-most telencephalic VZ and SVZ (reviewed in O´Rourke, 1996). Like the cortical plate cells, these cells normally express *Pax6* and *R-cadherin*, but fail to express *R-cadherin* in the *Sey/Sey* condition, and there is a corresponding failure of these cells to invade the abnormal olfactory vesicle. So, in both cortex and olfactory bulb there is a correlation between *Pax6*-dependant *R-cadherin* expression and migration.

In the diencephalon also, *R-cadherin* expression is reduced in *Sey/Sey* mice only in the regions where it normally colocalizes with *Pax6*. As previously observed (Gänzler and Redies, 1995; Matsunami and Takeichi, 1995), *Pax6* and *R-cadherin* expression overlap extensively in the neuroepithelium and in the mantle zone of the ventral thalamus (VT) at E12.5. Later in development, however, the *R-cadherin* transcripts appear mainly within the presumptive zona incerta and ventral lateral geniculate body in a fashion parallel to the appearance of *Dlx1*, whereas *Pax6* expression is confined mostly to a subpart of the lateral geniculate body, the reticular nucleus and endopeduncular nucleus (Stoykova et al., 1996). In addition, *R-cadherin*

and *Pax6* are coexpressed in the neuroepithelium of the optoeminential zone, a region that can be clearly delineated by its expression of *Pax6* (Stoykova et al., 1996). Both the VT and the optoeminential zone are severely distorted in the *Sey/Sey* mutant brain, as demonstrated by a paucity of tissue within the optoeminential zone, and the abortive growth, the lateral displacement and failure to differentiate of the VT (Stoykova et al., 1996). In these two forebrain regions, the normally high level of *R-cadherin* expression is strongly reduced in the *Sey/Sey* mutant. Thus as elsewhere, *R-cadherin* expression is restricted to regions where it is not normally co-expressed with *Pax6*. As in the telencephalon, the failure of histogenesis correlates with loss of *R-cadherin* expression in the *Sey/Sey* mutant. This alteration of adhesive properties in the region of prosomere 3 and 4 may also underlie the recently described failure of axon guidance in the *Sey/Sey* diencephalon only after entering prosomere 4 and 3 (Mastick et al., 1997).

In conclusion, this study suggests a link between *Pax6*, *R-cadherin* and adhesive properties in the developing forebrain. The selective adhesion of embryonic cortical and striatal cells is disrupted in the *Pax6*-mutant, and there are morphological abnormalities at the cortico-striatal boundary, particularly in the radial glial cells. Meanwhile, there is a loss of expression of *R-cadherin* in areas in which this gene is normally co-expressed with *Pax6*, which shows that *Pax6* regulates *R-cadherin* expression, directly or indirectly. The simplest model consistent with these data would be that the *Small Eye* mutation leads to a disruption of the Ca^{2+}-dependent adhesive mechanisms involving *R-cadherin*, and this leads to the morphological disruption observed in the mutant. Nonetheless, our current data only show a correlation between these phenomena. In particular, a direct link involving R-cadherin has not been proven. Whatever the role of R-cadherin, we have demonstrated here a direct link between region-specific adhesion properties and patterning driven by the homeobox transcription factor, Pax6.

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