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Defects of neuronal migration and the pathogenesis of cortical malformations are associated with Small eye (Sey) in the mouse, a point mutation at the Pax-6-locus*

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Summary. The mouse Small eye (Sey) locus is situated on chromosome 2. Molecular analyses have shown that Sey^{Neu} represents a point mutation leading to a splice site error and loss of the functional gene product. The Sey locus has been shown to be identical with the paired box (Pax)-6 gene, which contains paired-like and homeobox domains and is a developmental control gene. Pax-6 expression occurs in many parts of the central nervous system during embryogenesis. Therefore, we may expect the Sey mutation to result in abnormal development of the central nervous system. The present study shows that Pax-6 mutation has a bimodal effect upon neurogenesis in mouse: it causes a delay of premigratory neurons in a stage-, region-, and gene-dose-dependent manner. Additionally, Sey mutation impairs axonal growth and differentiation. Neurons of the cortical plate cease differentiation on approximately day 16 of gestation and appear to have increased cohesion: their cytoplasm is swollen and vacuolated. These changes coincide both with reduced formation of axons and with the onset of vacuolar degeneration in existing axons, glial cells and radial glial fibers. Consequently, there is an impairment of the peripheral migration of putative neurons so that the neonatal lesion pattern of the neocortical roof becomes dominated by a broad spectrum of neuronal migration disorders.

Key words: Paired box genes – Embryonic mouse – Cerebrum – Neuronal migration disorders

Paired box genes (Pax) are highly conserved sequence motifs in Drosophila, mammals and other vertebrates, which encode gene products with a DNA binding domain [6]. These proteins act as regulators of essential steps in development, e.g., definition of polarity, segmentation and germ-layer induction [18]. In contrast to another class of developmental control genes, the Hox genes, whose expression is region specific [26], the Pax genes are expressed along the entire antero-posterior axis in the neural tube, i.e., Pax-2 [28], Pax-3 [16], Pax-7 [25] and Pax-8 [29] or in the mesoderm-derived vertebral column, i.e., Pax-1 [10]. Recently, a novel Pax gene, Pax-6, was isolated [40]. Tissue-specific expression of Pax-6 during development occurs in the eyes, forebrain, hindbrain, neural tube, pituitary and olfactory epithelium [39]. Recently, it was shown by molecular analysis that the Sey^{Neu} mutation in mouse shows loss of a functional gene product which is identical with the protein normally encoded by the Pax-6 locus [21, 36]. The mouse Small eye (Sey)-Pax-6 locus is located in chromosome 2, in a region homologous to the Wilms tumour/Aniridia complex on chromosome 11 in man [37]. Until now, developmental pathology associated with the Sey mutation has been established only for the eyes and the olfactory organs [22, 23]. We have performed a neuropathological study in Sey^{Neu} mutant mice during fetal development to determine if abnormal neurogenesis in those parts of the CNS expressing the Pax-6 gene [39] was associated with mutation at the Sey locus. Such results may clarify the role of Pax-6 at the cellular level.

Materials and methods

From 25 litters of interspecific matings of $Sey^{Neu}/+$ mice 66 embryos were collected for histological serial sections in transversal and sagittal planes. The embryos represented each embryonal day between day 10 (E10) and day 18 (E18). Day of vaginal plug control was considered as E0. Classification of the presumed genotypes of the embryos was done as described in the text; the criteria are principally based upon earlier descriptions [20, 22, 23]. All embryos were evaluated both by gross inspection and by histology of the nasal and eye regions and the brain. The present findings concentrate mainly upon the observations in 38 fetuses from 15 litters, ranging between E16 and E18. Histological sections were stained with H&E, Giemsa, PAS and by Bodian impregna-

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tion. Additionally, coronal brain sections from 2 wild-type mice and from 4 heterozygous newborn littermates, ranging between 2 and 6 days of age were studied by conventional H&E staining method. For transmission electron microscopy E 16 embryos were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) and embedded in Epon. Thin sections were collected on grids, double stained with uranyl acetate and lead citrate, and viewed and photographed on a Zeiss Elmi.

Results

Using gross morphological evaluation parameters, litters from the interspecific matings of $Sey^{Neu}/+$ mice developed normally until term with the exception of the apparent reduction in the size of the eyes. Three different criteria were used [22, 23] to distinguish the homozygous Sey^{Neu}/Sey^{Neu} embryos from their heterozygous $Sey^{Neu}/+$ and wild-type (+/+) littermates (Table 1): (a) in +/+ embryos, the stage-relevant presence of well-developed eye cups and a smooth, circular iris; (b) in $Sey^{Neu}/+$ embryos the unscheduled and marked reduction of both eye cups and lens diameters; irregular folding of the iris; enlargement of the nasal cavity; well-developed olfactory bulbs. These embryos subsequently had a normal postnatal development [20], and (c) in Sey^{Neu}/Sey^{Neu} embryos aplasia of the eyes, the nasal cavity and of the olfactory bulbs. All such embryos die shortly after birth.

Histologically, the neuropathological effects of the Sey mutation were manifested on the developing eyes, the nasal cavities, the olfactory bulbs and at definite regions of the forebrain and the hindbrain.

The anophthalmia-microphthalmia complex

Although Sey^{Neu}/Sey^{Neu} mutants were consistently affected by anophthalmia, well-developed optic stalks were found in all cases. The stalks did not extend directly toward the ectodermal surface but were considerably distorted. A marked disproportion between abundant neuroblasts and only a faint, irregular rim of differentiated neuronal cells was observable on E14. Upon contact with the ectoderm, which showed normal thickening at the opposition site, the stalks failed to grow to cup-like formations. This lack of eye cup formation was obviously correlated to the improper preponderance of undifferentiated cells at the tip of the stalks.

In heterozygous $Sey^{Neu}/+$ embryos this imbalance between undifferentiated and more differentiated cells within the periphery of the stalks was significantly less marked. Eye cups developed in all instances but with varying diameters. In fact one fetus on E18 with an apparently normal eye development, expressed marked pathological findings in other parts of the CNS, typical for $Sey^{Neu}/+$ heterozygotes.

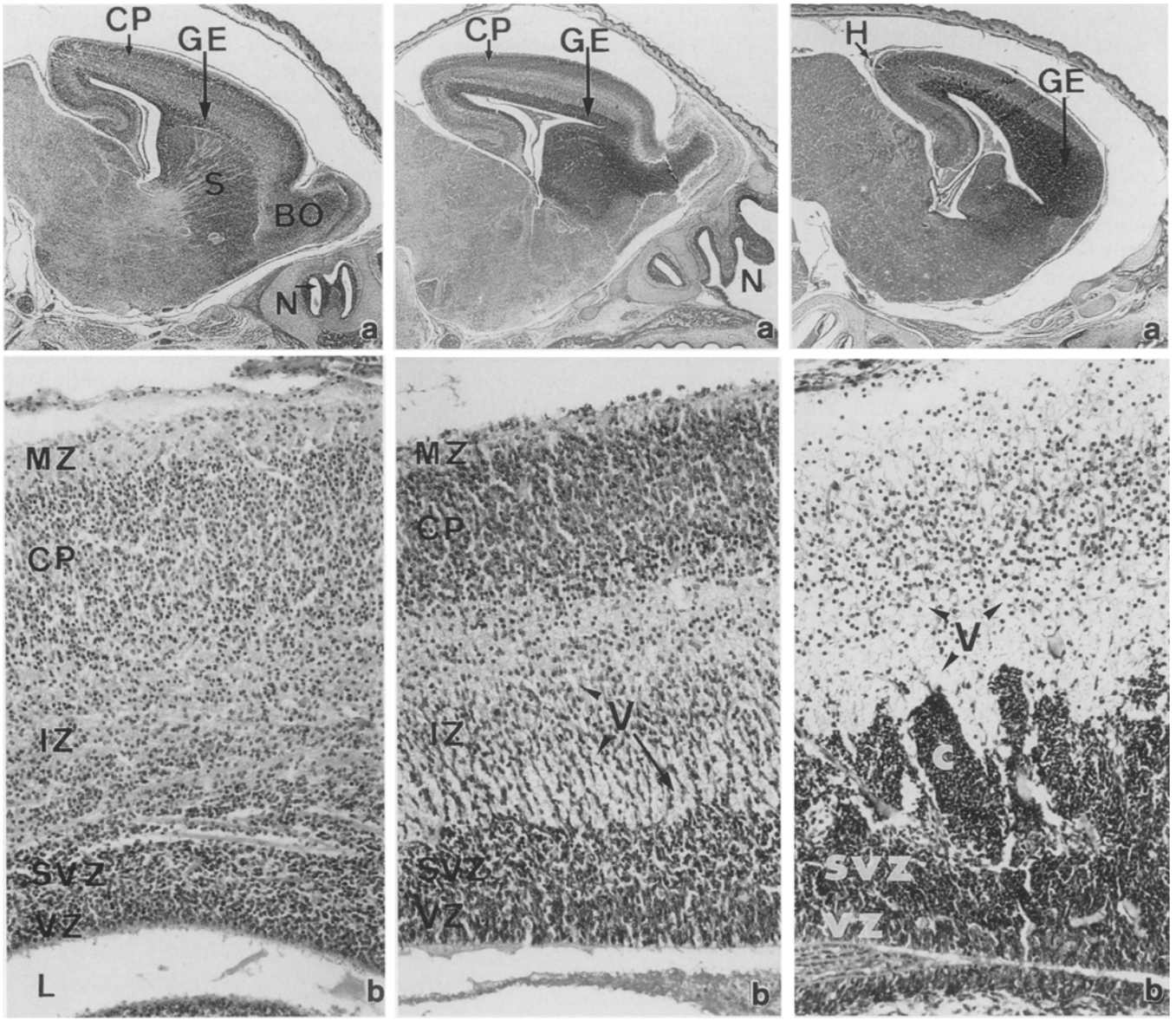
Table 1. Spectrum of developmental anormalies observed in Sey^{Neu} littermates between embryonic day (E)16 and E18

	Wild-type embryos (+/+)	Heterozygous ($Sey^{Neu}/+$)	Homozygous embryos (Sey^{Neu}/Sey^{Neu})
Number of embryos	11	16	11
Reduced diameter of optic group and lens	0	15 (93.7%)	aplasia
Enlarged nasal cavity	0	10 (62.5%)	aplasia
Increased diameter of germinative epithelia:			
olfactory epithelium	0	13 (81.2%)	aplasia
frontobasal forebrain	1 (9.1%)	11 (69.7%)	9 (81.8%)
dorsal forebrain (neocortex)[a]	0	9 (56.3%)	9 (81.8%)
diencephalon	0	9 (56.3%)	10 (90.9%)
cerebellum	0	1 (6.2%)	11 (100%)
Vacuolar degeneration at MZ/CP/IZ	0	13 (81.2%)	11 (100%)
Cortical plate:			
normal appearance	11 (100%)	4 (25.0%)	0
increased compaction	0	10 (62.5%)	0
reduced cell content	0	2 (12.5%)	11 (100%)
failure of lamination and increased compaction	0	0 (0%)	6 (54.6%)
Hypocellularity of the intermediary zone	0	2 (12.5%)	11 (100%)
Agenesis/hypoplasia of the corpus callosum	0	0	9 (81.8%)
Exencephaly	0	0	2 (18.2%)
Schizencephaly	0	0	4 (36.4%)
Cortical heterotopias	0	0	10 (90.9%)
Leptomeningeal ectopias	0	0	4 (36.4%)

[a] Mean diameters and mitotic counts are given in Table 2
MZ, Marginal zone; CP, cortical plate; IZ, intermediary zone

Dysplasia of the olfactory organs

In homozygous embryos (Sey^{Neu}/Sey^{Neu}) development of the nasal cavities was completely absent, with exception of one case of a rudimentary organon vomeronasale within an otherwise undifferentiated mesenchyme of the upper jaw. Olfactory bulbs were not present in any case (Fig. 3a). The potential bulb region was filled with spider web-like extensions of the arachnoid. The forebrain in that region revealed an extension of the germinative epithelium (GE) from the ventricular lumen up to the pial surface (Fig. 3a). The ventricular



Figs. 1–3. Paramedian sagittal sections of the dorsal pallial region of mouse forebrain on embryonal day (E)18. H&E stain, **1a, 2a, 3a** × 17, **1b, 2b, 3b** × 280

Fig.1a,b. Wild-type fetus (eye cups fully developed). Germinal epithelial layer (*GE*) containing a small ventricular zone (*VZ*) and a subventricular zone (*SVZ*)containing 10–15 cell layers. Intermediary zone (*IZ*) with many postmitotic migrating putative neurons. *CP* cortical plate; *MZ*, marginal zone; *L*, lateral ventricle; *S*, striatum; *BO*, olfactory bulb; *N*, nasal cavity

Fig. 2a,b. Heterozygous fetus ($Sey^{Neu}/+$) (reduced eye cups diameter and severe hypoplasia of nasal conchae). Hypoplasia of the telencephalic frontal area. Increased diameter of the VZ (6–10 cell layers) and of the SVZ (about 20–25 cell layers). Dense clustering of emigrating cells within the IZ. Numerous vacuoles (*V*) appearing within the IZ, partly confluencing in a laminar fashion. CP with reduced diameter, but higher cellular package of mostly round to oval neurons. Reduced diameter of the MZ

Fig. 3a,b. Two homozygous fetuses (Sey^{Neu}/Sey^{Neu}) (no eye anlage) with different degrees of cortical malformation. **a** Aplasia of the frontal cortex and of BO. Reduced cortical diameter in median and occipital neocortex. Enlarged, undifferentiated GE, extending to the pial surface. Heterotopias and arachnoidal ectopia (*H*) of cell clusters from the SVZ. **b** Regularily established VZ, but without columnar arrangement of cells. Rather amorphous appearance of the SVZ. Sharp demarcation between SVZ and IZ by axon bundles with excessive vacuolar degeneration (V. Clusters of SVZ cells (*C*). CP with a drastically reduced cell content. Absence of a definite MZ

zone (VZ) was narrow and not definitely delineated from the subventricular zone (SVZ). The SVZ which filled the distance between VZ and pial border revealed no structural pecularities with respect to cell emigration from this compartment, differentiation or strata formation. However, at that topographical position where in wild-type littermates the olfactory bulbs were observable (Fig. 1a) only two tiny nodules of cells within the outmost parts of the fronto-baso-lateral SVZ were present. These resembled aggregates of mitral cells. The undifferentiated SVZ also extended into the fronto-lateral parts of the telencephalic roof. Thus, the lack of GE differentiation was contiguous with the bilateral bulb aplasia. All such fetuses die within maximally 16 h after birth.

The anomalies of the nose and nasal cavities in $Sey^{Neu}/+$ heterozygous mice were described by us recently [20]. The olfactory epithelium was partly arranged in polypous nodules, but showed regular axonal connections with the olfactory bulbs (Fig. 2a).

Dysgenesis of telencephalic stratification

On E14 wild-type embryos already showed formation of a quite distinct cortical plate (CP). Its formation started in both $Sey^{Neu}/+$ and Sey^{Neu}/Sey^{Neu} embryos with a delay of about 12 to 24 h. In homozygous embryos CP formation was subsequently completely absent within the fronto-nasal and fronto-baso-lateral parts of the telencephalon (see above). At all stages, mitotic activity within telencephalic GE was of comparable magnitude in wild-type, heterozygous and in homozygous embryos (Table 2). Irrespective of this, neocortical GE enlargement occurred at the onset of the differentiation of the SVZ from the VZ around E15 to E16. GE diameters showed a posterior to anterior gradient of increase and this was mainly due to hypercellularity of the SVZ in Sey^{Neu}/Sey^{Neu} mutants, whereas in $Sey^{Neu}/+$ mutants the increased GE diameter was a result of an enlarged VZ on E16, while on E18 both the VZ and the SVZ compartments in heterozygotes contributed to an increased width of the GE (Table 2). Since there were simultaneously no differences of the mitotic indices of the GE between +/+, $Sey^{Neu}/+$, and Sey^{Neu}/Sey^{Neu} embryos (Table 2), these observations indicate the postmitotic cells within the GE to be the primary site of Sey gene action.

In Sey^{Neu}/Sey^{Neu} embryos, during the late neurogenesis stage, beginning with E15, putative neurons on their migration route accumulated within the intermediary zone (IZ) shortly after leaving the SVZ. This irregular layer of entrapped cells also contained some mitotic figures (0.34%), in contrast to wild-type embryos showing no mitoses at all within the IZ. During the interval from E16 to E18 apparently no migrating cell penetrated through the IZ. Consequently, the cortical subplate zone was rather poor in cells and the CP largely remained at a developmental stage of about E15. In addition, in 6 out of 11 embryos only two cell layers were discernible, an outer one which was rich in mostly round, densely packed neuroblasts and an inner one containing

Table 2. Growth parameters of the neocortical germinative epithelium (GE) in wild-type, heterozygous and homozygous littermates between E16 and E18[a]

	Wild-type embryos (+/+)	Heterozygous embryos ($Sey^{Neu}/+$)	Homozygous embryos (Sey^{Neu}/Sey^{Neu})
Embryonal day 16			
Frontal VZ	75.8 ± 4.5	92.2 ± 21.3	53.3 ± 9.8**
Frontal SVZ	39.4 ± 2.4	32.8 ± 14.6	374.3 ± 114.8*
Total:	115.2 ± 3.3	125.0 ± 17.2	427.6 ± 94.3*
Occipital VZ	53.3 ± 4.2	74.6 ± 9.8	59.9 ± 7.0
Occipital SVZ	42.6 ± 7.7	44.7 ± 14.3	62.7 ± 15.7
Total:	95.9 ± 5.8	119.3 ± 11.9	122.6 ± 11.2
Embryonal day 18			
Frontal VZ	34.0 ± 3.3	54.1 ± 5.7	20.5 ± 0.8**
Frontal SVZ	68.5 ± 4.5	99.2 ± 7.8*	754.4 ± 127.1*
Total:	102.5 ± 3.9	153.3 ± 6.5*	774.9 ± 122.2*
Occipital VZ	16.4 ± 2.1	61.5 ± 4.9	37.3 ± 0.9 b
Occipital SVZ	55.4 ± 3.6	47.1 ± 4.8	140.6 ± 45.1*
Total:	71.8 ± 2.8	108.6 ± 4.8*	117.9 ± 44.8*
Mean mitotic index on E16	4.83 ± 0.16	5.62 ± 0.21	6.09 ± 0.37
Mean mitotic index on E18	2.88 ± 0.14	2.63 ± 0.19	2.74 ± 0.18

* Significance against +/+ at $P < 0.001$; ** Significance against both +/+ and $Sey^{Neu}/+$ littermates at $P < 0.01$

[a] Values are given as mean diameters with the GE in mmt SD VZ, Ventricular zone; SVZ, subventricular zone

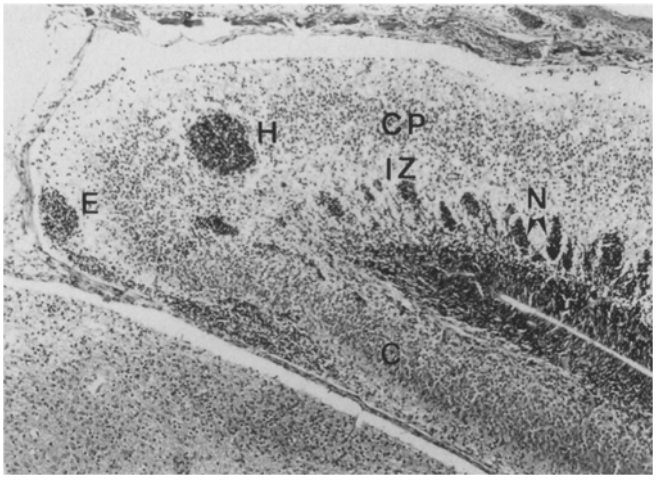


Fig. 4. Displacements of the germinative epithelial tissue: formation of heterotopias (*H*) and of ectopias (*E*) into the arachnoidal space in the occipito-lateral neocortex of a Sey^{Neu}/Sey^{Neu} embryo on E18. Multiple nodules (*N*) of premigratory cells at the lower parts of the intermediary zone (*IZ*). Low cell density of the cortical plate (*CP*) in relation to the cingulate cortex (*C*). H&E stain, × 140

small neuronal cells. There was no vertical orientation of the neurons contained within the CP. Rather, all cells settled down in a rather haphazardous fashion. Until E18, premigratory cells crowded within an entrapment zone which, in its most peripheral parts, was delineated by an imaginal border horizontally traversing the IZ with an abundant rim of vacuoles within its axonal trajectories. The putative migratory cells were clustered in dense nodules which were mostly contiguous with the SVZ (Fig. 3b). Simultaneously, an abundant vascularization pattern of the SVZ was detectable. All homozygous embryos revealed a complete lack of the marginal zone (MZ), leading to an extension of the most peripheral CP cells up to the pial border of the neocortex.

In $Sey^{Neu}/+$ embryos migration of putative neurons was significantly hampered only in 2 out of 16 cases (Table 1) which subsequently showed a reduced cell content of the CP. In all other cases the IZ appeared to contain a normal set of radially migrating cells. However, when leaving the IZ, most of the migrating neuronal cells apparently went directly into the CP compartment, rather than to the normal establishment of a regular

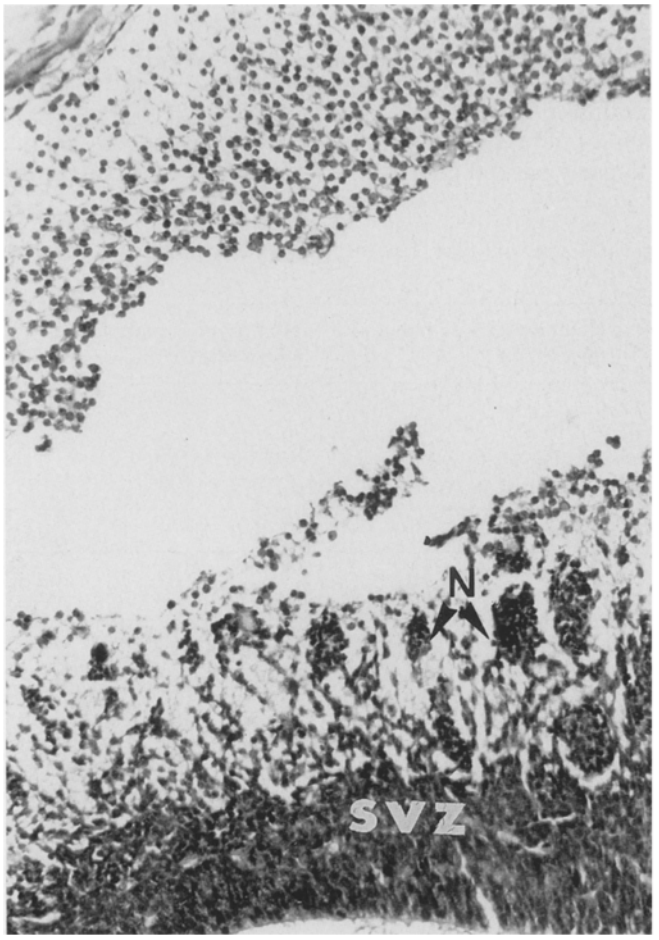


Fig. 5. Schizencephalic clefts in the occipito-parietal cortex of a Sey^{Neu}/Sey^{Neu} embryo on E18. Multiple nodular aggregates (*N*) of premigratory cells, already departed from the subventricular zone (*SVZ*). Cortical periphery is unlayered with low cell density. H&E stain, × 350

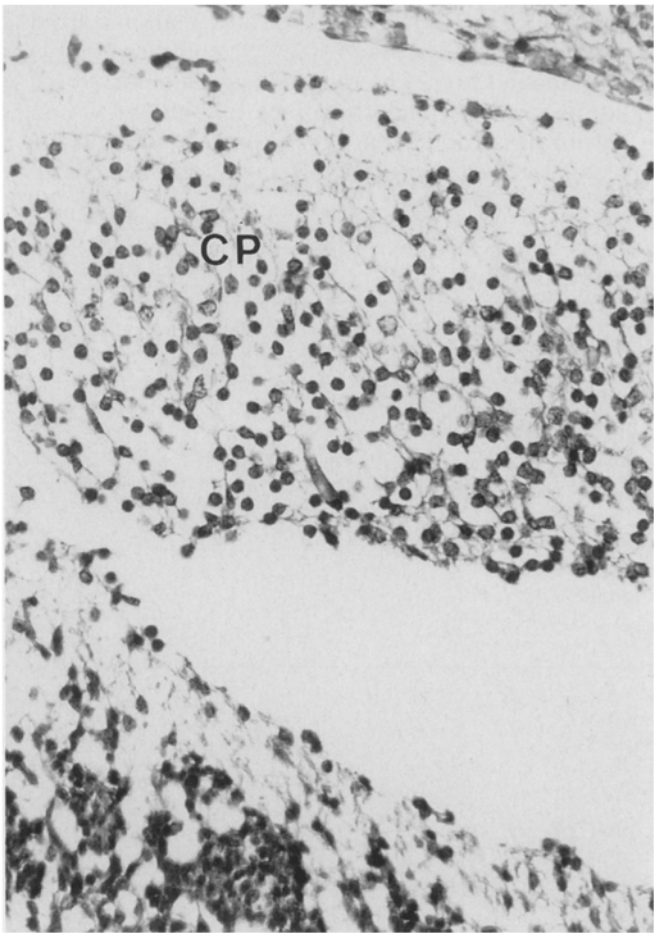
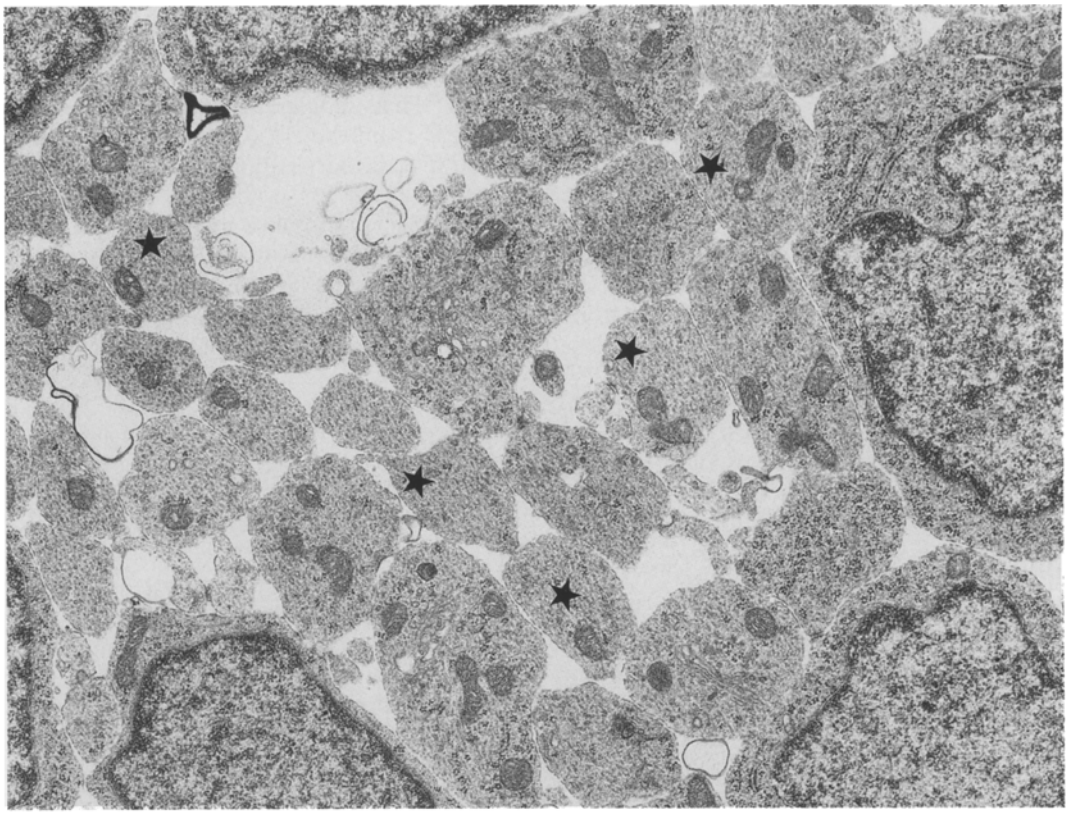


Fig. 6. Schizencephalic cleft with distinct confinement to the hypoplastic neocortex. Honeycomb-like appearance of the cortical plate region (*CP*) due to widespread cell loss and lack of structural fibers. Paramedian sagittal section of a Sey^{Neu}/Sey^{Neu} embryo on E18. H&E stain, × 560



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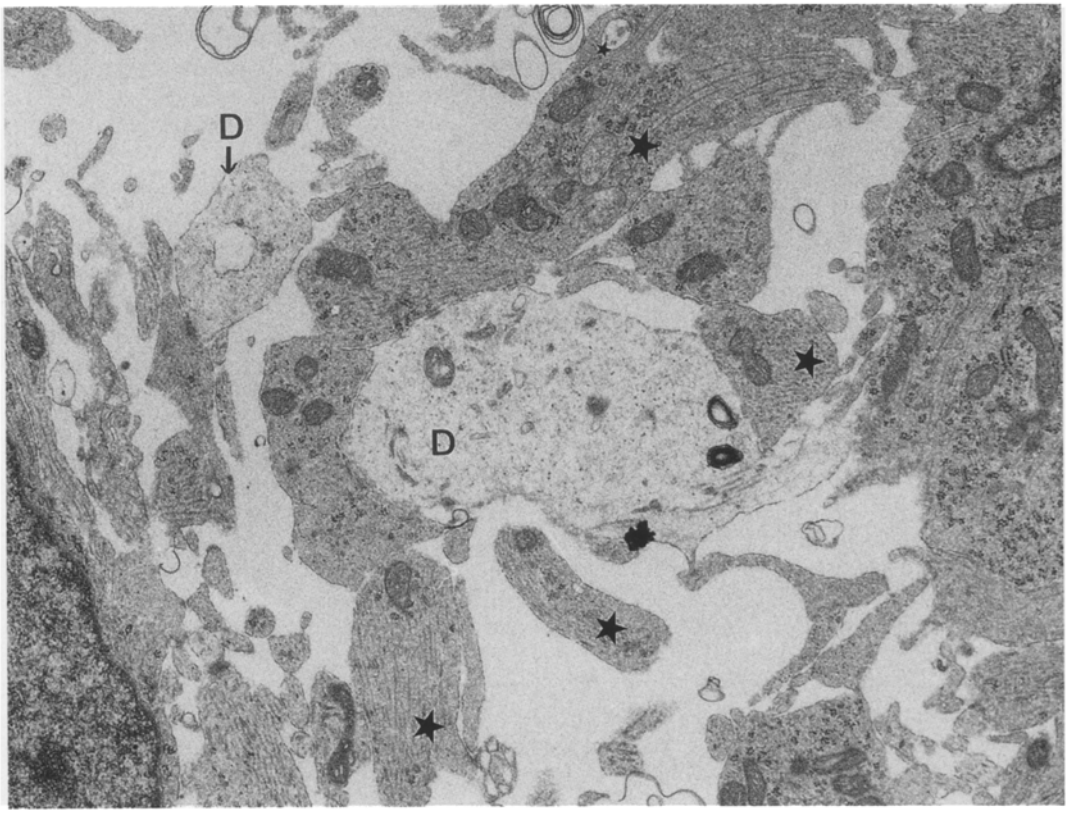

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Fig. 7. Transverse section through the cortical plate (*CP*) of a wild-type embryo on E16. Neuronal cells are moderately elongated and interspersed with numerous axonal bundles (*stars*). × 16000

Fig. 8. Cortical plate region of a heterozygous $Sey^{Neu}/+$ embryo on E16. Loose packing of neuronal cell bodies without distinct alignment axes. Loose, irregular arrangement of axons of largely varying diameters. There are both fibers of rather normal appearance (*stars*) and some with pale swelling and of dystrophic appearance (*D*). × 16000

cortical subplate zone (Fig. 2b). Another remarkable finding in 10 embryos (Table 1) was a profound alteration of the CP towards an increased compaction. No neuron revealed a bipolar shape. Instead, they rounded up and frequently revealed vacuoles within the cytoplasm. Cell nuclei were round and mostly euchromatic. Consequently, the CP revealed a quite compacted appearance with sticky adhesions of neurons and no columnar alignments (Fig. 2b). The MZ was irregularily defined, of smaller diameter than in wild-type littermates, and even contained numerous cells normally belonging to the outer CP. Fibers within the MZ mostly appeared clumsy. Additionally, electron microscopical studies (Figs. 7, 8) revealed that the radial glial fibers were of comparable magnitude in the different types of embryos. However, broad, swollen and pale axons of dystrophic appearance were consistently present in heterozygous and homozygous embryos (Fig. 8) amongst many axonal processes without any alterations. Concomitantly, a marked loosening of package of fibers indicates the lack of intact fiber barriers at the CP-MZ border in homozygous and in heterozygous mutant mice. These barriers normally result from the horizontal to tangential ingrowth of axonal strata from the earliest neurons settled within the thalamus and the neocortex.

Dysplasia of the basal ganglia

Sey^{Neu}/Sey^{Neu} embryos on E18 revealed a significantly lower content of motor fiber bundles within the striatum. Lack of fiber ingrowth into this area was paralleled by loss of structural compartmentation within this area as early as on E14. The GE extended up to the pial border at the brain basis with a rather uniform distribution of dividing neuroblasts. The mitotic rate was, however, unchanged in comparison to wild-type embryos. As a result of no restriction of the GE in only the dorsal parts of the ganglionic eminences, the latter structures were enlarged to about a twofold area diameter of the wild-type littermates on E14. However, irrespective of this, already on E18 the basal ganglia were severely hypoplastic in all homozygous Sey mutants (Fig. 3a). This seems not only to result from a decreased fiber content, but also apparently from the abundance of ectopically dividing neuroblasts producing an inappropriate amount of definite neuronal cells. Thus, the basal ganglia were of a significantly decreased cellular content at term. Many of the Sey^{Neu}/+ embryos also showed a decreased volume of the basal ganglia (Fig. 2a).

Formation of gray matter heterotopias and ectopias within the subarachnoid space

In Sey^{Neu}/Sey^{Neu} embryos establishment of fiber bundles within the IZ was quite visible at the diagonally running border to the frontally expanded VZ and SVZ (this situation is seen in Fig. 3a). Fiber density diminished gradually in fronto-occipital direction. Consequently, laminar confinements between SVZ, IZ, CP and MZ were quite poor in the most occipital parts of the neocortical roof. This enabled a number of nodular aggregates of cells from the SVZ to separate from the latter and to be dislocated peripherally. These nodules were partly settled at the presumed CP-MZ-border (10 cases, 90.9 %; Table 1), but also penetrated the pial border and imposed as ectopias (4 cases, 36.4 %) within the subarachnoid space (Fig. 4). There was neither mitotic activity nor any differentiation potency observable within the heterotopias and the ectopias. Two of the Sey^{Neu}/Sey^{Neu} embryos (18.2 %) revealed exencephaly which may be interpreted as a more extended breakdown of the superficial glial barrier than in the pathogenesis of ectopias.

Development of schizencephaly

Between E16 and E18 the subcortical area of +/+ embryos (mainly defined as IZ) is filled with a significant amount of thalamo-cortical and cortico-cortical axon bundles (Fig. 1b). However, in Sey^{Neu}/Sey^{Neu} embryos at this age only tiny bundles of fibers crossed the IZ (Fig. 3b). Both in homozygous and in heterozygous mutants these fibers contained multiple vacuoles. In four Sey^{Neu}/Sey^{Neu} embryos (36.4 %) many vacuoles came to confluency which ultimately led to a broad-spaced disruption of the subplate region in the parieto-occipito-lateral districts of the neocortical roof (Figs. 5 and 6), leading to typical "schizencephaly". No continuity between the bilateral schizencephalic clefts and the subarachnoid space was observed. There was also no inverse relationship between the presence of schizencephaly and the vascularization density neither within the SVZ nor within the bordering CP. Both laminae were well supplied with vessels. Especially the enlargement of the SVZ was paralleled by a high degree of vascularization. Schizencephaly was not observed in Sey^{Neu}/+ embryos.

Dysgenesis of the diencephalon and of the metencephalon

In Sey^{Neu}/Sey^{Neu} embryos the brain aqueduct extended up to the mesdiencephalic border and concomitantly the luminal space of the third ventricle was rather irregularly shaped. The GE of the diencephalon was enlarged in 9/16 heterozygous embryos (= 56.3 %) and in 10/11 Sey^{Neu}/Sey^{Neu} embryos (= 90.9 %; Table 1). There were no abnormalities of the thalamic and hypothalamic areas, also the pituitary was well developed in all cases. The cerebellar anlage was of comparable size in 15/16 Sey^{Neu}/+ embryos, whereas in 1/16 heterozygous embryos, as well as in all Sey^{Neu}/Sey^{Neu} embyros a marked enlargement of the external granular layer was observed. In addition, cells of GE were highly packed and separated from the underlying parts of the cerebellum by a band of highly vacuolated fibers.

Discussion

The pattern of malformation observed in the CNS correlates with the topographical and developmental specific pattern of Pax-6 gene expression [39]. The main signals of Pax-6 gene expression are found in the dividing brain neuroepithelium and at the border to the SVZ, indicating the presence of Pax-6 during and after mitosis. The Sey malformation pattern is also consistent with the expression of Pax-6 expression in the GE of the lateral and dorsal telencephalic wall, the diencephalic wall, in the olfactory bulb and in the external granular layer of the cerebellum. Interestingly, the study of Walther and Gruß [39] indicates no direct relationship between Pax-6 expression and organogenesis of the striatum and of the olfactory epithelium. This would suggest that normal development of these regions is dependent upon the proper development of neighboring cell populations whose differentiation is directly dependent upon normal Pax-6 expression.

One prominent neuropathological finding in Sey mutant mice is presented by the increased volumes of the GE within the forebrain (tel- and diencephalon) and the hindbrain (met-encephalon). Since there were simultaneously no differences of the mitotic percentages of the GE between +/+, $Sey^{Neu}/+$, and Sey^{Neu}/Sey^{Neu} embryos, there is substantial evidence to point to the postmitotic cells within the GE as a primary site of the Sey gene action. Recent neurodevelopmental evaluations by Altman and Bayer [1] gave strong indication to the fact that "some (possibly all) neurons interrupt their radial migration within the SVZ for 1–2 days". Thus, the prevailing view of the direct and continuous radial path of putative neuroblasts leaving the GE [30] should be modified based on the present results to include the concept of an accumulation and temporary sojourn of these cells within the SVZ [3]. Apparently, in Sey mutants the postmitotic cells have an impeded developmental program which extends their resting phase within the SVZ.

Concomitantly with the increase of the GE compartments in mutant embryos, a further dramatic alteration was seen mainly in Sey^{Neu}/Sey^{Neu} embryos and at markedly milder degree also in $Sey^{Neu}/+$ embryos on E16. The IZ revealed polycystic vacuoles and dystrophic degeneration of the fibers within. The severity of these changes apparently correlated with the enlargement of the underlying SVZ, but did not reveal any influence upon cell death rate within the SVZ compartment. Obviously there exists a temporal (possibly also a causal) link between fixation of cells within the SVZ and dystrophic degeneration of the bordering fibers of the IZ. Dystrophic changes occurred largely as a dispersion of very small cystic vacuoles within the lower IZ parts in $Sey^{Neu}/+$ embryos. Dystrophy was present in all fibers of the lower IZ, resulting mostly in loss of axon bundles from the external sagittal stratum (ESS) [8]. In all genotypes of embryos, establishment of these horizontally sprouting axons within the IZ occurred during the normal developmental schedule about from E15 onward. However, this axonal stratum underwent dystrophic degeneration on E16, with largely varying severity depending on genotype.

An important function of ascending axons departing from the horizontally oriented parts of the ES is the organization of an orderly radial alignment of neurons within the CP [12, 15, 35]. Marked loss of this function was evident in most of the $Sey^{Neu}/+$ embryos on E18 by lack of vertical columnar organization of the CP and by the abnormal shape and cohesions of these neurons. Similar lamination disturbances were described for human agyria-pachygyria [11]. In addition, in some embryos even overlapping histological findings of pachygyria [24] and of microgyria [2, 33] were present.

Polycystic vacuolating lesions within the IZ in Sey^{Neu}/Sey^{Neu} embryos on E18 underwent confluency in 4 from 11 cases. As a result, widely extending bilateral clefts within the parieto-dorso-occipital cortex were observable. These alterations are identical to the histological picture of schizencephaly in man [41]. No continuity between the intraparenchymal clefts and the leptomeningeal space was present. Irrespective of schizencephaly arising within the IZ, the underlying areas of enlarged SVZ revealed an abundant vascularization pattern, which obviously correlated with the number of premigratory neuro-glial stem cells [4]. Thus, in Sey^{Neu}/Sey^{Neu} mutants no inverse correlation between blood supply of the neocortex and the severity of schizencephaly (porencephaly) could be confirmed [2]. Otherwise, this would have argued for an encephaloclastic pathogenesis of schizencephaly which was postulated either for ischemic [9] or infectious processes [14] occurring after the migration processes have finished. Sey^{Neu}/Sey^{Neu} embryos also developed cortical heterotopias and leptomeningeal ectopias. These observations give strong affirmation to the crucial role of functional defects at the glia limitans [7] as well as at the MZ [5] for pathogenesis of all forms of brain ectopias.

There are some paralleles between Sey mutant mice and the neurological mouse mutant "Weaver" with respect to the normal mitotic rate of the GE [32, 34], and the target of gene action within the premigratory neurons [34] as well as the disturbance of contacts between axons and radial glial cells [19, 31]. However, in contrast, the main pathological event in Sey mutant embryos is represented by a late and sudden onset of degenerative processes of fibers running within the subcortical layers. This schedule is apparently in contrast to the finding of a continous expression of Pax-6 within neocortical roof of mice from E11 onwards [39]. This may be explainable, however, if we consider a late-appearing axonal differentiation disorder from early established neurons as a key event in pathogenesis. Such neurons of possibly thalamic origin [8] may have developed completely normally up to the time of axon sprouting. Only at the period of ESS establishment, when the thalamocortical and corticocortical axons come into contact with the radial glial fibers and the migrating pre-neurons, is there triggered in Sey mutants a haphazardous cascade of still unknown events, ultimately leading to fiber dystrophy and to secondary

migration disorders. Spongy fiber dystrophy in Sey mutant mice has close similarities with spongy leukodystrophies in man (Canavan's disease, dystrophy of van Bogaert-Betrand type) which show a prevalent affect on glial cells [38]. These conditions are described to occur either during early infancy [27] or even prenatally [38]. The underlying cause may be linked to the developmental neurobiology of cortical astroglia, as was recently analyzed [17]. Accordingly, neurons and cells of the astroglial lineage are not produced concomitantly throughout neurogenesis. The main bulk of astrocytic precursors destined ultimately for the supragranular neocortical layers are produced after the migration of infracortical neurons and migrate to the cortex between E16 and the first postnatal days. Consequently, during this period migration disturbances in Sey mice may lead to a gene-dose-related deprivation of the astrocytic equipment for the cortex layers. If we consider that appropriate astrocytic equpiment is a necessary condition for both survival and differentiation of the cortical neurons [13] we may expect serious alterations in cortical and subcortical structures in Sey mutant mice, similar to those described here. Conclusively, Sey mutant mice are regarded as a good model for pathogenesis of periventricular leukomalacia in premature infants.

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References

1. Altman J, Bayer A (1990) Horizontal compartmentation in the germinal matrices and intermediate zone of the embryonic rat cerebral cortex. Exp Neurol 107:36–47
2. Barth PG (1987) Disorders of neuronal migration. Can J Neurol Sci 14:1–16
3. Bayer SA, Altman J, Russo RJ, Dai X, Simmons JA (1991) Cell migration in the rat embryonic neocortex. J Comp Neurol 307:499–516
4. Breier G, Albrecht U, Sterrer S, Risau W (1992) Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 114:521–532
5. Caviness VS, Evrard P, Lyon G (1978) Radial neuronal assemblies, ectopia and necrosis of developing cortex: a case analysis. Acta Neuropathol (Berl) 41:67–72
6. Chalepakis G, Fritsch R, Fickenscher H, Deutsch O, Goulding M, Gruss P (1991) The molecular basis of the undulated Pax-1 mutation. Cell 66:873–884
7. Choi BH, Matthias SC (1987) Cortical dysplasia associated with massive ectopia of neurons and glial cells within the subarachnoid space. Acta Neuropathol (Berl) 73:105–109
8. Crandall JE, Caviness VS Jr (1984) Axon strata of the cerebral wall in embryonic mice. Dev Brain Res 14:185–195
9. Dekaban A (1965) Large defects in cerebral hemispheres associated with cortical dysgenesis. J Neuropathol Exp Neurol 24:512–540
10. Deutsch U, Dressler GR, Gruss P (1988) Pax-1, a member of a paired box homologous murine gene family, is expressed in segmented structures during development. Cell 53:617–625
11. Dobyns WB (1989) The neurogenetics of lissencephaly. Neurol Clin 7:89–105
12. Edwards MA, Yamamoto M, Caviness VS (1990) Organization of radial glia and related cells in the developing murine CNS. An analysis based upon a new monoclonal antibody marker. Neuroscience 36:121–144
13. Evrard P, Gressens P, Volpe JJ (1992) New concepts to understand the neurological consequences of subcortical lesions in the premature brain. Biol Neonate 61:1–3
14. Friede RL, Mikolasek J (1978) Postencephalitic porencephaly, hydranencephaly or polymicrogyria. A review. Acta Neuropathol (Berl) 43:161–168
15. Gadisseux JF, Evrard P, Misson JP, Caviness VS (1989) Dynamic structure of the radial glial fiber system of the developing murine cerebral wall. An immunocytochemical analysis. Dev Brain Res 50:55–67
16. Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P (1991) Pax-3, a novel murine DNA-binding protein expressed during early neurogenesis. EMBO J 10:1135–1147
17. Gressens P, Richelme C, Kadhim HJ, Gadisseux J-F, Evrard P (1992) The germinative zone produces the most cortical astrocytes after neuronal migration in the developing mammalian brain. Biol Neonate 61:4–24
18. Gruss P, Walther C (1992) Pax in development. Cell 69:719–722
19. Hatten ME, Liem RKH, Mason CA (1986) Weaver mouse cerebellar granule neurons fail to migrate on wild-type astroglial processes in vitro. J Neurosci 6:2676–2683
20. Heinzmann U, Favor J, Plendl J, Grevers G (1991) Entwicklungsstörung des olfaktorischen Organs. Ein Beitrag zur kausalen Genese bei einer Mausmutante. Anat Anz [Suppl] 170:511–512
21. Hill RE, Favor J, Hogan BLM, Ton CCT, Saunders GF, Hanson IM, Prosser J, Jordan T, Hastie N, van Heyningen U (1991) Mouse Small eye results from mutations in a paired-like homeobox-containing gene. Nature 354:522–525
22. Hogan BLM, Horsburgh G, Cohen J, Hetherington CM, Fisher G, Lyon MF (1986) Small eye (Sey): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse. J Embryol Exp Morphol 97:95–110
23. Hogan BLM, Hirst EMA, Horsburgh G, Hetherington CM (1988) Small eye (Sey): a mouse model for the genetic analysis of craniofacial abnormalities. Development 103 [Suppl]:115–119
24. Jellinger K, Rett A (1976) Agyria-Pachygyria (Lissencephaly syndrome). Neuropädiatrie 7:66–91
25. Jostes B, Walther C, Gruss P (1991) The murine paired box gene, Pax-7, is expressed specifically during the development of the nervous and muscular system. Mech Aging Dev 33:27–38
26. Kessel M, Gruss P (1990) Murine developmental control genes. Science 249:374–379
27. Kolkmann F-W, Völzke E (1966) Über die spongiösen Dystrophien des Nervensystems im frühen Kindesalter. Z Kinderheilk 97:222–239
28. Nornes H, Dressler GR, Knapik E, Deutsch U, Gruss P (1990) Spatially and temporally restricted expression of Pax-2 during murine neurogenesis. Development 109:797–809
29. Plachov D, Chowkury K, Walther C, Simon D, Guenet JL, Gruss P (1990) Pax-8, a murine paired box gene expressed in the developing excretory system and thyroid gland. Development 110:643–651
30. Rakic P (1988) Defects of neuronal migration and the pathogenesis of cortical malformations. Prog Brain Res 73:15–37
31. Rakic P, Sidman RL (1973) Weaver mutant mouse cerebellum: defective neuronal migration secondary to abnormality of Bergmann glia. Proc Natl Acad Sci USA 70:240–244
32. Rezai Z, Yoon CH (1972) Abnormal rate of granule cell migration in the cerebellum of "Weaver" mutant mice. Dev Biol 29:17–26

33. Richmann DP, Stewart RM (1974) Cerebral microgyria in a 27-week fetus: an architectonic and topographic analysis. J Neuropathol Exp Neurol 33:374–384
34. Smeyne RJ, Goldowitz D (1989) Development and death of external granular layer cells in the Weaver mouse cerebellum: a quantitative study. J Neurosci 9:1608–1620
35. Takahashi T, Misson J-P, Caviness VS (1990) Glial process elongation and branching in the developing murine neocortex: a qualitative and quantitative immunohistochemical analysis. J Comp Neurol 302:15–28
36. Ton CCT, Miwa H, Saunders GF (1992) Small eye (Sey): cloning and characterization of the murine homolog of the human aniridia gene. Genomics 13:251–256
37. van der Meer de Jong R, Dickinson ME, Woychik RP, Stubbs L, Hetherington C, Hogan BLM (1990) Location of the gene involving the Small Eye mutation on mouse chromosome 2 suggests homology with human Aniridia 2 (AN2). Genomics 7:270–275
38. Vuia O (1977) Congenital spongy degeneration of the brain (van Bogaert-Bertrand) associated with micrencephaly and ponto-cerebellar atrophy (Contributions to the pathology of glial dystrophy of intrauterine origin). Neuropädiatrie 8:73–88
39. Walther C, Gruss P (1991) Pax-6, a murine paired box gene, is expressed in the developing CNS. Development 113:1435–1449
40. Walther C, Guenet JL, Simon D, Deutsch U, Jostes B, Goulding MD, Plachov D, Balling R, Gruss P (1991) Pax: a murine multigene family of paired box-containing genes. Genomics 11:424–434
41. Yakovlev PI, Wadsworth RC (1946) Schizencephalies – A study of the congenital clefts in the cerebral mantle. J Neuropathol Exp Neurol 5:169–206