Persistent reelin-expressing Cajal–Retzius cells in polymicrogyria


Summary
Cajal–Retzius (CR) cells are early-developing cells important in mammalian corticogenesis. Reelin, a protein secreted by CR cells, is essential for completion of neuronal migration and cortical lamination. Lack of reelin causes the ‘reeler’ phenotype in mice and autosomal recessive lissencephaly with cerebellar hypoplasia in man. Focal increases in reelin and CR cells are associated with thickening and local invaginations of the marginal zone and microgyria in animal studies. It has been suggested that abnormalities of reelin expression may be involved in human polymicrogyria. We have studied CR cells and reelin expression in pathological sections of human polymicrogyria to explore this possibility. Occurrence, distribution, morphology and reelin expression in CR cells were studied in 12 cases of human polymicrogyria, ranging from 21 gestational weeks to 10 years of age. Findings were compared with age-matched controls. Large, reelin-positive CR-like cells were more numerous in the majority of the polymicrogyria cases and persisted for longer than usual, up to 10 years of age. The CR-like cells tended to cluster and were most frequent in fused molecular layers in the polymicrogyria. Reelin-expressing CR-like cells were also found in bridges between the molecular layer and overlying leptomeningeal heterotopia and within the heterotopia itself. Clusters of CR-like cells were also found in adjacent non-polymicrogyric cortex. No clusters were seen in the control subjects. Increased numbers of CR-like cells were seen in both familial and acquired cases. In contrast to previous reports, the findings show that large CR-like cells persisted for longer than usual, up to 10 years of age, and that they may continue to express reelin. Their maximal aggregation in regions of polymicrogyria and overlying leptomeningeal heterotopia suggest an association between the presence of these cells and polymicrogyria, which we interpret in the light of recent findings concerning the roles of reelin and its downstream signalling pathway in neuronal and glial developmental dynamics and post-developmental function.

Keywords: Cajal–Retzius cells; reelin; polymicrogyria

Abbreviations: CR = Cajal–Retzius; GW = gestational week; GFAP = glial fibrillary acidic protein

Introduction
Developmental pathology is important in several neurological disorders including refractory epilepsy (Spreafico et al., 1999) and developmental delay (Sarnat, 1992). Cajal–Retzius (CR) cells are a set of primitive cells important in mammalian cortical development (Meyer et al., 1999). Though identified more than a century ago, only recently have their roles in development begun to be understood. The CR cell population is dynamic, derives from a variety of origins and is thought to become far less numerous by term in the normal human brain (Meyer and Goffinet, 1998; Meyer et al., 2000). CR cells establish early neuronal circuitry in the developing brain (Aguiló et al., 1999), and express a number of genes known to be important in human cerebral development: LIS1 (Clark et al., 1997), EMX2 (Mallamaci et al., 1998), fukutin (Saito et al., 2000) and RELN (Meyer and Goffinet, 1998). The gene RELN encodes the protein reelin, which is secreted extracellularly by layer I neurones through a constitutive, nonvesicular mechanism (Lacor et al., 2000).

Manipulation of CR cells and reelin secretion affects cerebral development in animal models. Mutation of RELN...
Table 1 Polymicrogyria and control cases studied

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Aetiology (if known)</th>
<th>Region studied</th>
<th>Other changes</th>
<th>Matched control age</th>
<th>Region studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21 GW</td>
<td>Familial</td>
<td>NK</td>
<td>U</td>
<td>20 GW</td>
<td>Entire brain hemisphere</td>
</tr>
<tr>
<td>2</td>
<td>30 GW</td>
<td>Ischaemic</td>
<td>Frontal</td>
<td>U; LMH</td>
<td>33 GW</td>
<td>NK</td>
</tr>
<tr>
<td>3</td>
<td>37 GW</td>
<td></td>
<td>Temporal</td>
<td>U; LMH</td>
<td>37 GW</td>
<td>Temporal</td>
</tr>
<tr>
<td>4</td>
<td>39 GW</td>
<td></td>
<td>Entire hemisphere</td>
<td>Microcephaly; L; LMH</td>
<td>38 GW</td>
<td>Frontal</td>
</tr>
<tr>
<td>5</td>
<td>1 day pn</td>
<td>NK</td>
<td>Frontal</td>
<td>L</td>
<td>1 day pn</td>
<td>NK</td>
</tr>
<tr>
<td>6</td>
<td>7 days pn</td>
<td>NK</td>
<td>NK</td>
<td>?</td>
<td>9 days pn</td>
<td>NK</td>
</tr>
<tr>
<td>7</td>
<td>8 days pn</td>
<td>CMV infection</td>
<td>Frontal and insular</td>
<td>U</td>
<td>8 days pn</td>
<td>Frontal and temporal</td>
</tr>
<tr>
<td>8</td>
<td>6 months</td>
<td>Familial</td>
<td>Temporal and parietal</td>
<td>?; LMH</td>
<td>7 months</td>
<td>Temporal and insular</td>
</tr>
<tr>
<td>9</td>
<td>9 months</td>
<td>NK</td>
<td>NK</td>
<td>L</td>
<td>9 months</td>
<td>Frontal</td>
</tr>
<tr>
<td>10</td>
<td>11 months</td>
<td>Familial</td>
<td>Temporal and insular</td>
<td>U</td>
<td>11 months</td>
<td>Frontal, temporal and insular</td>
</tr>
<tr>
<td>11</td>
<td>4 years</td>
<td>NK</td>
<td>NK</td>
<td>U; LMH</td>
<td>4 years</td>
<td>NK</td>
</tr>
<tr>
<td>12</td>
<td>10 years</td>
<td>NK</td>
<td>Entire hemisphere</td>
<td>L; Refractory epilepsy</td>
<td>10 years</td>
<td>NK</td>
</tr>
</tbody>
</table>

GW = gestational weeks; pn = postnatal; CMV = cytomegalovirus; U = unlayered polymicrogyria; L = layered polymicrogyria; ? = distinction not possible; LMH = leptomeningeal heterotopia; NK = not known. Case 7 has been described previously (Harding and Baumer, 1988).

in mice causes the ‘reeler’ phenotype, with absence of the marginal zone and inversion of the normal pattern of cortical lamination (Caviness and Rakic, 1978; D’Arcangelo et al., 1995). Ablation of CR cells causes premature differentiation of radial gliia into maturing glial cells and arrest of late-migrating neurones (Supér et al., 2000). Increased numbers of CR cells and increased reelin expression are associated with thickening and local invagination of the marginal zone (Ringstedt et al., 1998). Local cortical injury, that is eventually associated with the development of a microgyrus, causes an initial local reduction and subsequent increase in the number and longevity of CR cells (Supér et al., 1997). Reelin deficiency causes one form of human lissencephaly (Hong et al., 2000). Abnormalities of reelin expression may influence other human cerebral developmental malformations. In particular, it has been suggested that overexpression may be associated with polymicrogyria in humans (Meyer and Goffinet, 1998; Ringstedt et al., 1998). We sought to examine this possibility by studying human polymicrogyria.

Methods
The study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee. The material used was anonymized and surplus to diagnostic requirements. Twelve cases of polymicrogyria were identified, ranging in age from 21 gestational weeks (GW) to 10 years. Twelve controls were matched for age. For comparison, two cases with lissencephaly (20 and 22 GW) were also studied. One polymicrogyria specimen was from a hemispherectomy performed because of drug-resistant epilepsy (in a 10-year-old); in all other cases, the specimens were from post-mortem examinations. Case details are given in Table 1. Cases with affected siblings (familial) were considered likely to have a genetic aetiology, whilst in three other cases there was histological proof of an acquired aetiology (two with porencephalic cystic change and distribution in the territory of the middle cerebral artery, and one with evidence of cytomegalovirus infection).

Sections were cut at 10 µm and mounted onto 3-aminopropyltriethoxysilane-coated slides (Sigma, Poole, UK). Sections were dewaxed in xylene, rehydrated in a graded series of alcohols (100%, 95%, 70%), washed in distilled water and placed in PBS (phosphate-buffered saline). Some sections were stained routinely with cresyl violet and reticulin preparations and immunohistochemistry was performed for glial fibrillary acidic protein (GFAP), calretinin and reelin.

Monoclonal antibodies for reelin immunohistochemistry (clone 142) were a generous gift from Dr Goffinet (Neurobiology Unit, University of Namur Medical School, 61 Rue de Bruxelles, 5000 Namur, Belgium). The specificity of this antibody has been demonstrated (de Bergeyck et al., 1998). To block endogenous peroxidase activity, sections were incubated in methanol and 0.6% hydrogen peroxide for 30 min. Sections were then microwaved in 0.01 M citrate buffer for 10 min, followed by blocking of non-specific staining by incubation in 1 : 5 normal goat serum in a humidity chamber for 30 min. The primary antibody was applied at dilution 1 : 200 in PBS buffer and slides incubated overnight at 4°C. The secondary antibody, 1 : 100 biotinylated goat anti-mouse diluted in PBS, was incubated for 1 h at room temperature. Sections were washed in PBS, incubated for 1 h at room temperature with Vectastain ABC reagent (ABC kit; Vector Laboratories, Peterborough, UK) then washed in PBS, incubated for 10 min in 0.025% diamino-benzidine with 0.02% hydrogen peroxide, washed, dehydrated, cleared and mounted.

For calretinin immunochemistry (monoclonal anti-calretinin antibodies; Swant, Bellinzona, Switzerland), neither a separate blocking step nor antigen retrieval were required. The primary antibody was applied at 1 : 2500 dilution in PBS containing 0.3% Triton and 10% NGS and 0.01%
sodium azide, and incubated for 3 days at 4°C. Incubation with the secondary antibody, diluted in PBS with 1% NGS, was for 4 h at room temperature. ABC was applied in PBS with 1% NGS and incubated for 2 h at room temperature. Immunoreaction was developed as for reelin; sections were counterstained with 1% light green.

The sections were examined by five workers separately, including three neuropathologists. It was not possible to blind for underlying pathology. The pathological types of polymicrogyria were determined from the GFAP-stained sections.

Occurrence, distribution and morphology of immunoreactive cells were studied in the GFAP-, calretinin- and reelin-stained sections and the distribution and localization of the immunoreactive cells were compared between sections. In this report we have concentrated on the larger cells in the marginal zone/molecular layer that express reelin and calretinin: these characteristics identify CR cells (Meyer et al., 1999). We have not separated ‘Retzius cells’ from ‘Cajal cells’ (Meyer et al., 1999). As the morphologies of the larger cells vary, we have considered them all to be members of a loosely defined ‘reelin-producing Cajal–Retzius family’ (Meyer et al., 1999) and used the term ‘Cajal–Retzius-like cells’ (CR-like cells). The presence, distribution and degree of gliosis were determined from the GFAP-immunostained sections. Findings from the polymicrogyria cases were compared with age-matched control subjects.

Following Meyer and Goffinet (Meyer and Goffinet, 1998), estimates of CR-like cell numbers were made on reelin-immunostained sections. Cells were counted over a length of 10 000 µm of layer I using an eyepiece graticule ×10 objective and Leica DM RB microscope (Leica Microsystems, Heerbrugg, Switzerland). In the control cases, counting was commenced in the sulcus of a gyrus, moving the eyepiece graticule systematically along the length of the layer towards the gyral surface. Only large positively labelled cells in layer I with the morphology of CR-like cells were counted; smaller reelin-positive cells were not included. In polymicrogyria cases, a similar length of superficial cortex was counted both within the area of malformation and in the adjacent normal-appearing cortex where available. The counts are estimates and not stereologically based, being limited by the material available.

Results

Twelve cases of polymicrogyria were studied. There were four cases of layered and six cases of unlayered polymicrogyria and in two cases definite categorization could not be made. Five cases had additional leptomeningeal heterotopia. The calretinin- and reelin-positive cells observed were most commonly large bipolar horizontal neurones, some with large ‘globoid’ perikarya. Double immunolabelling was not performed, but similar immunostaining patterns with calretinin and reelin suggest labelling of the same population of cells.

At mid-gestation (20–21 GW), frequent large calretinin- and reelin-positive CR-like cells were seen in the superficial part of the marginal zone in control subjects. These were evenly distributed (Fig. 1A). Smaller, rounded reelin-positive cells, a feature of more mature cortex, were not seen. In the polymicrogyria cases, large CR-like cells were found in the superficial part of the marginal zone. These cells were more unevenly distributed than in the control subject. In one case the number of CR-like cells appeared reduced compared with the control subject (Fig. 1C). In specimens from two subjects with lissencephaly (aged 20 and 22 GW) there were also numerous CR-like cells in the molecular layer across the whole extent of the cerebral hemispheres (Fig. 1D).

At 30–33 GW, few large CR-like cells were seen in the superficial part of the marginal zone in control subjects (Fig. 2A). There were no smaller, reelin-positive cells in deeper parts of the marginal zone or other parts of the cortex. Large reelin-positive CR-like cells were more numerous in the superficial part of the marginal zone in the polymicrogyria case. These cells were more unevenly distributed (Fig. 2B–D).

At 37 GW to term only rare, large, superficial CR-like cells were seen in control subjects (Fig. 3A). No clusters of reelin-positive cells were seen in control cases; smaller reelin-positive cells were found in deeper parts of the marginal zone. In the polymicrogyria cases there were more frequent larger CR-like cells in the superficial part of the marginal zone. These cells were more common in the fusions of the polymicrogyric marginal zone and also tended to cluster. Increased numbers of CR-like cells could also be seen in the adjacent non-polymicrogyral sulcal marginal zone (Fig. 3B–D). Smaller reelin-positive cells were found in the deeper parts of the marginal zone in the polymicrogyria cases, similar to the pattern found in control specimens.

In controls from term to 10 years of age, there were only rare single reelin-positive, large CR-like cells in the molecular layer (Fig. 4A). There were smaller positive cells in deeper parts of the molecular layer. In five of seven polymicrogyria cases aged from 7 days post-natal to 10 years, there were still numerous large calretinin- and reelin-positive cells (Fig. 4B and C). These larger, CR-like cells were found in the superficial part of the molecular layer. In normal cortex adjacent to polymicrogyric cortex there was an excess of reelin-positive CR-like cells in the molecular layer compared with controls. There were also smaller reelin-positive cells in the deeper parts of the molecular layer, similar to the pattern found in controls. In two post-mortem postnatal cases (Cases 8 and 11) reelin-positive cells were also seen in deeper cortical layers (Fig. 4D); immunoreaction was less intense on these cells compared with CR-like cells.

There were five cases with leptomeningeal glioneuronal heterotopia (aged 30 GW to 4 years). Reticulin staining revealed breakage in the basement membrane. There were increased numbers of CR-like cells. Calretinin- and reelin-positive CR-like cells were most frequent around the bridges between the molecular layer and the leptomeningeal...
Fig. 1 (A) Cortex from a post-mortem control of 20 gestational weeks (GW). Reelin immunohistochemistry shows frequent, relatively evenly spaced CR-like cells, mainly in the more superficial aspects of the marginal zone (bar = 80 µm). (B) Case 1: polymicrogyria in a foetus of age 21 GW showing abnormal convolutions of the superficial cortical plate (haematoxylin stain; bar = 80 µm) and adjacent section (C) immunostained for reelin which shows scattered immunoreactive cells present in the marginal zone overlying the polymicrogyric cortex which, in this case, appeared in reduced numbers compared with the age-matched control (bar = 80 µm). (D) Lissencephalic cortex (21 GW) demonstrating numerous reelin immunoreactive cells in the marginal zone (bar = 80 µm).
Fig. 2 (A) Control of 30 weeks shows fewer reelin immunoreactive cells (arrowed), even in a sulcus where more CR-like cells tended to be observed (bar = 100 µm). (B) Polymicrogyric cortex (Case 2) of 30 GW (Nissl; bar = 280 µm) and adjacent reelin immunostained section (C) (bar = 280 µm) which show abnormal distribution and more frequent immunoreactive CR-like cells of varying size in the marginal zone. A grouping of reelin-positive cells is marked by arrows in (D) (bar = 30 µm).
Fig. 3 (A) Age-matched control shows few CR-like reelin-positive cells (bar = 80 µm) whereas in polymicrogyria at 1 day (Case 5), in a sulcus adjacent to the polymicrogyric zone, there are prominent and increased numbers of reelin immunopositive cells (B) forming ill-defined clusters (arrowheads) (bar = 80 µm); numerous CR-like calretinin immunopositive cells were also seen in the same sulcus (C) (bar = 80 µm). (D) Persistent clusters of reelin immunopositive cells in the marginal zone of the abnormal cortex (arrowhead) (bar = 80 µm). In both control cases and polymicrogyria, smaller reelin immunopositive cells are present in deeper cortical layers.
heterotopia (Fig. 5). There were also CR-like cells within the heterotopia (Fig. 5).

GFAP immunostaining demonstrated subcortical and subpial gliosis to a variable degree in regions of polymicrogyria in all cases except one (Case 5) and labelled heterotopic astrocytes in all cases with leptomeningeal heterotopia. In one case only (Case 8) marked cortical gliosis was evident (see Fig. 6A). Residual radial glial fibres were not demonstrated with our immunohistochemistry.

Polymicrogyria had a known acquired aetiology in three cases (Cases 2 and 4, ischaemic; Case 7, infective), and was familial in three cases (Cases 1, 8 and 10, all with affected siblings). The possible genetic basis for familial occurrence was not known. An excess of reelin-expressing CR-like cells was seen both in cases known to have an acquired aetiology (Cases 2 and 4), and in one thought to have a genetic aetiology (Case 8; Fig. 6). Case 1 was also familial, but was the youngest case studied (20 GW) and showed fewer rather than more CR-like cells (Fig. 1). Case 10 was familial; though reelin-positive CR-like cells were seen, the numbers of CR-like cells were not increased. CR-like cell number and distribution in Case 7, with proven CMV infection, did not differ significantly from the control.

CR-like cell counts in Cases 2–12 and respective controls showed the following mean number (range, standard deviation) of CR-like cells in 10 000 µm of layer I: in polymicrogyric cortex 25.5 (4–83, 22); in normal adjacent cortex (available in eight cases) 25 (1–98, 32.5); and in controls 8.6 (2–19, 5.6). Given the small number of cases, statistical analysis is limited; the difference between polymicrogyric cortex and control cortex was significant (\( P = 0.017 \), Wilcoxon signed ranks test, two-tailed). Case 1 was excluded from analysis as we noted fewer CR-like cells in this case, as stated above.

There was preservation of reelin expression or ‘overexpression’ in most of the polymicrogyria cases compared with control cases. The provenance of the study cases and the control cases differed: all controls were hospital post-mortems fixed soon after death, and embedded within a week, whilst the brain from polymicrogyria cases usually
Fig. 5 (A) A case of polymicrogyria (Case 3) with overlying leptomeningeal glioneuronal heterotopia at 37 GW (Nissl; bar = 285 µm). Reticulin staining (B) demonstrates breaks in the basement membrane and the bridges between the marginal zone and the heterotopia (bar = 80 µm). Large reelin-positive cells (C) and calretinin-positive cells (D) are noted in the vicinity of the bridges and within the heterotopic element (indicated with an asterisk; in both, bar = 40 µm). In another area, more numerous CR-like cells are concentrated in the heterotopic element with some cells remaining in the marginal zone on reelin (E) and calretinin (F) immunohistochemistry (in both, bar = 80 µm).

incurred delay to post-mortem and longer fixation interval, as the polymicrogyria was usually unsuspected and found incidentally. In the surgical case, antigen preservation was ideal, with no significant hypoxia prior to resection, immediate immersion in fixative and processing within a week; Case 7 also had prompt preservation and processing. Our findings were similar in polymicrogyria specimens with the best and the worst preservation. We did find reelin expression in rare large CR-like cells, but without any clustering, in some control cases which would have had
intermediate prefixation post-mortem delay and length of fixation equivalent to that for the surgical cases. Thus, whilst autolysis and length of fixation are important issues in neuropathology generally, they cannot explain our findings.

Discussion
In the normal human brain, large CR-like cells have been thought to be rare in adult cortex (Cajal in DeFelipe and Jones, 1988; Belichenko et al., 1995; Fonseca and Soriano, 1995; Martin et al., 1999) and reelin expression in large CR cells has been considered to represent a transient phenotype, restricted to the developmental period (Meyer et al., 1998). Our observational studies show that large CR-like cells overlying polymicrogyric cortex may persist well into postnatal life, in greater than expected numbers, and continue to express reelin. We have also shown that these cells tend to cluster abnormally. In adjacent normal-appearing cortex, there was also a persistent excess of reelin-expressing CR-like cells. The findings parallel the excess of CR-like cells seen in the molecular layer of the apparently normal hemisphere contralateral to the injured malformed hemisphere in an animal model of polymicrogyria (Supér et al., 1997). Changes in CR-like cells were demonstrated in some cases known to have an acquired polymicrogyria and in some known to have familial polymicrogyria. In contrast to previous reports suggesting CR-like cells are not found in leptomeningeal heterotopia (Marin-Padilla, 1999), we have shown reelin-expressing polymorphic CR-like cells in such heterotopia overlying polymicrogyria.

Human neuropathological studies are necessarily limited by ethical and practical considerations, especially in terms of age-matched control tissue. We were able to study control cases, but were not always able to match for brain region. However, a recent study has shown little variation in the distribution of large CR-like cells in 13 different areas from three normal cases aged 38–39 GW (Ding et al., 2000), and another autopsy study reported no difference between prefrontal and visual cortex in eight older controls (Martin et al., 1999). The original works of Cajal and Retzius remain important data sources for the cells these pioneers described.
Retzius was unable to stain large CR-like cells in his postnatal material, whilst Cajal, who was able to stain these cells postnatally but found them to be rare, did not report any significant regional variation in his detailed studies of a limited number of cases (Cajal in DeFelipe and Jones, 1988). In three polymicrogyria cases with excess CR-like cells and one control case in which it was possible to study regional distribution, no obvious difference was found in our study. No published studies in controls have illustrated clustering of CR-like cells. We do not feel, therefore, that a relative lack of regionalized control tissue is a valid alternative explanation of our findings.

Reelin has multiple receptors and multiple effects in the normally developing brain (Senzaki et al., 1999; Trommsdorff et al., 1999; Dulabon et al., 2000); its roles in the abnormally developing, and in the postnatal, brain are yet to be fully explored. There is peculiar aggregation of reelin-expressing CR-like cells on both sides of the tissue bridges connecting polymicrogyric cortex and overlying leptomeningeal heterotopia, through the disrupted basement membrane. Recent studies show that reelin enables detachment of neurones from radial glia and inhibits neuronal migration (Dulabon et al., 2000). Breaks in the basement membrane overlying the marginal zone are known to lead to leptomeningeal heterotopia (Blackshear et al., 1997). Neurotrophins, generated in response to cortical injury (Mudó et al., 1993), can influence CR cells and reelin expression (Brunstrom et al., 1997; Ringstedt et al., 1998), and may mediate aggregation of CR-like cells around breaks in the basement membrane, possibly limiting neuronal egress.

Abnormal reelin activity in development may be associated with abnormal cortical folding. Neurotrophin-4 applied to cortical slices from embryonic mice increases the number of neurones in the molecular layer (Brunstrom et al., 1997). These neurones are CR-like and calretinin-positive and presumably also reelin-expressing, though this was not shown. Most of the CR-like cells were found in heterotopic collections and the illustrations in this report show thickening of the marginal zone and polymicrogyria-like invaginations of underlying cortex, though this is not mentioned in the text (Brunstrom et al., 1997) In transgenic mice overexpressing another neurotrophin, brain-derived neurotrophic factor, overall levels of reelin were decreased (Ringstedt et al., 1998) However, these transgenic mice still developed microgyria, with overfolding, in areas with unexplained aggregations of CR cells and such microgyria was attributed to a local increase in reelin secretion (Ringstedt et al., 1998). Meyer and Goffinet also proposed that reelin may be involved, albeit indirectly, in excess cortical folding after their studies of foetal CR cells in humans (Meyer and Goffinet, 1998).

Both polymicrogyria and leptomeningeal heterotopia may have genetic or environmental causes (Tamagawa et al., 1989; Iida et al., 1994; Guerrini et al., 1999). We have shown an excess of CR-like cells in some, but not all, cases of both acquired and familial polymicrogyria. We presume that the latter cases are likely to be genetically mediated. We cannot determine from our observational findings whether polymicrogyria leads to the aggregation and persistence of CR-like cells, or whether brain injury or malprogramming leads to influx of CR-like cells which then cause, or contribute to the development of, polymicrogyria. The observations that CR-like cells may also be numerous in non-polymicrogyric cortex adjacent to polymicrogyric cortex and in lissencephalic cortex suggest, however, that their aggregation need not lead to cortical overfolding. It is, incidentally, notable that despite the increase in cortical surface area associated with polymicrogyria (Richman et al., 1975; Sisodiya and Free, 1997), and the enhancement of ‘developmental dilution’ of CR-like cells this should cause (Belichenko et al., 1995), we find in most cases increased numbers of CR-like cells overlying polymicrogyria, further supporting an association between CR-like cells, reelin overexpression and polymicrogyria.

Epilepsy is the most common clinical manifestation of polymicrogyria in humans (Guerrini, 1999). Animal models of microgyria have been well studied. Hyperexcitability is most marked in the surrounding, normal-appearing, non-microgyric tissue, the paramicrogyral zone (Jacobs et al., 1996; Redecker et al., 1998), possibly due to extensive functional reorganization (Redecker et al., 1998; Redecker et al., 2000). Thus, separation of the paramicrogyral zone from the microgyria does not influence evoked epileptiform activity (Jacobs et al., 1999). In humans, polymicrogyria has been much less well studied. However, the cortex around the polymicrogyria is also most likely to be the cause of associated epilepsy (Brodtkorb et al., 1998; Sisodiya, 2000). We found a persistent excess of reelin-expressing CR-like cells in the molecular layer of normal-appearing cortex adjacent to the polymicrogyria. In the adult human brain, extracellular reelin, secreted by neurones in the molecular layer, associates with dendritic spines on deeper pyramidal neurones (Rodriguez et al., 2000). We also found positivity for reelin around neurones in deeper laminae in postnatal polymicrogyric brain (Fig. 4D). Pyramidal neurones have never been found to express reelin in mammalian brain (Pesold et al., 1999), suggesting persistent CR-like cells may secrete reelin even after migration has ceased. Lack of reelin in the adult human brain has been associated with neuropil attenuation and dendritic spine changes in subjects with psychosis (Impagnatiello et al., 1998; Guidotti et al., 2000). We cannot determine what, if any, effects increased or persistent reelin expression in polymicrogyria and perilesional cortex might have, though a recent review has suggested that the signalling pathway activated by reelin may affect neurtogenesis in the post-developmental human brain (Bothwell and Giniger, 2000).

Acknowledgements
We wish to thank Dr A. M. Goffinet for the generous gift of the reelin antibody and Lilian Martinian, Nick Win and Steve Durr for excellent technical assistance. The work was
supported by grants from the Epilepsy Research Foundation, Glaxo-Wellcome plc, University of London Central Research Fund, Institute of Neurology and Patrick Berthoud Trust. S.H.E. is supported by the National Society for Epilepsy, UK and by grants from Sahlgrenska University Hospital and the Medical Faculty of University of Göteborg, Sweden.

References


Guidotti A, Auta J, Davis JM, Gerevini VD, Dwivedi Y, Grayson DR, et al. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatry 2000; 57: 1061–9.


Received January 15, 2001. Revised March 8, 2001
Accepted March 15, 2001