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# Transplantations of olygodendrocytes in the CNS

*F. Lachapelle, A. Gansmuller, M. Baulac*, Baron A. van Evercooren, N. Baumann, M. Gumpel*

*U 134 INSERM Hôpital de la Salpêtrière, 47 Bd de l'hôpital, Paris*
** U 106 INSERM Hôpital de la Salpêtrière, 47 Bd de l'hôpital, Paris*

The intracerebral transplantation in the newborn mouse central nervous system (CNS) is so far the only method allowing long-term investigations of identified populations of olygodendrocytes in the very complex spatial environment on the developing CNS *in situ*. This technique, coupled with immunohistochemical and ultrastructural studies provides a dynamic system which allowed the autors to study the myelination process *in situ*.

*Key words*: intracerebral transplantation, olygodendrocytes, myelination, homochronic grafts, heterochronic grafts.

From the first studies on myelination, most often performed in man, it has been clearly established that it does not proceed throughout the CNS at one and the same time, but seems to follow in some way the caudo-rostral gradient of the phylogenetic development of this system. The same gradient is observed in rodents which for practical reasons have been widely used as models to study the myelination process. In mouse, myelination starts at birth and is almost completely achieved at P35, allowing the study of the entire process in a very short period of time.

Three types of classical approaches have contributed to the knowledge of glial cell maturation and myelination:

— First, the biochemical analysis of myelin components and the sequential study of their deposition in the myelin sheath have been performed on normal and dysmyelinating mutant mice and provided much information to aid our understanding of myelin formation [3].

— More recently, the production of poly- or monoclonal antisera allowed to detect *in situ* the presence and localization of specific antigenic components. These immunohistochemical studies proved very fruitful in identifying the sequential synthesis of these components in the myelin-forming cells and in analysing their deposition during the myelin formation. Moreover, these techniques have allowed to identify the myelin-forming cells early in development [13] and to trace the different glial cell lines [1, 16, 17, 18].

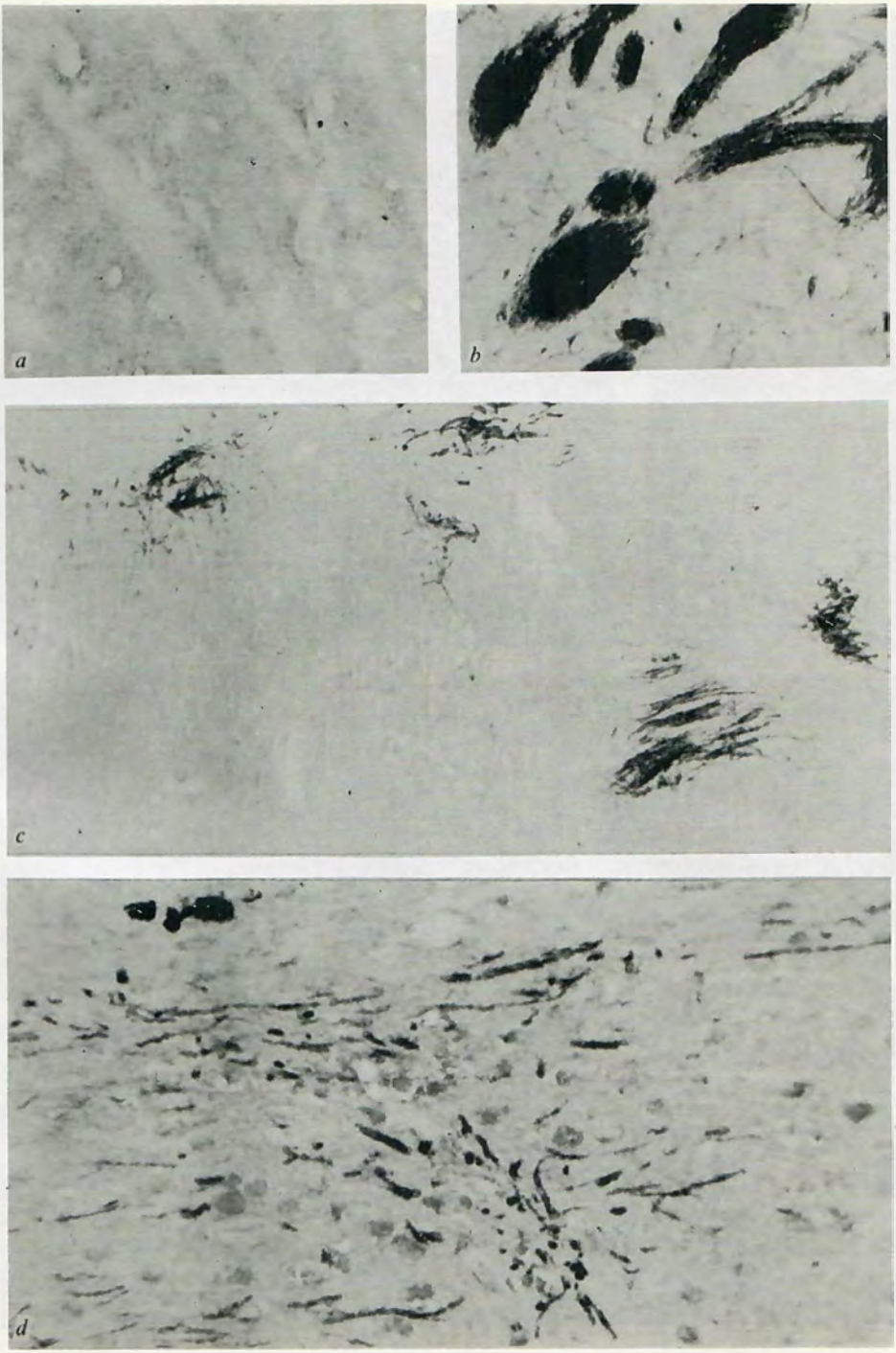


Fig. 1. Immunohistochemical study of the MBP-positive myelin synthesized by transplanted oligodendrocytes (PAP technique)

*a* — absence of immunostaining with anti-MBP anti-serum in the striatum of an adult (2 months) shi/shi homozygote × 150); *b* — strong immunostaining with the same anti-MBP anti-serum in the striatum of a control normal mouse of the same age. (PAP technique) × 150); *c* — dispersion of the MBP-positive myelin patches in the shi/shi brain 70 days after graft. (Fragment of olfactory bulb from newborn control mouse into the newborn shi/shi brain) (× 300); *d* — high magnification of a MBP-positive myelin patch: axons from different fascicles are myelinated by both normal myelin and shiverer myelin (× 700)

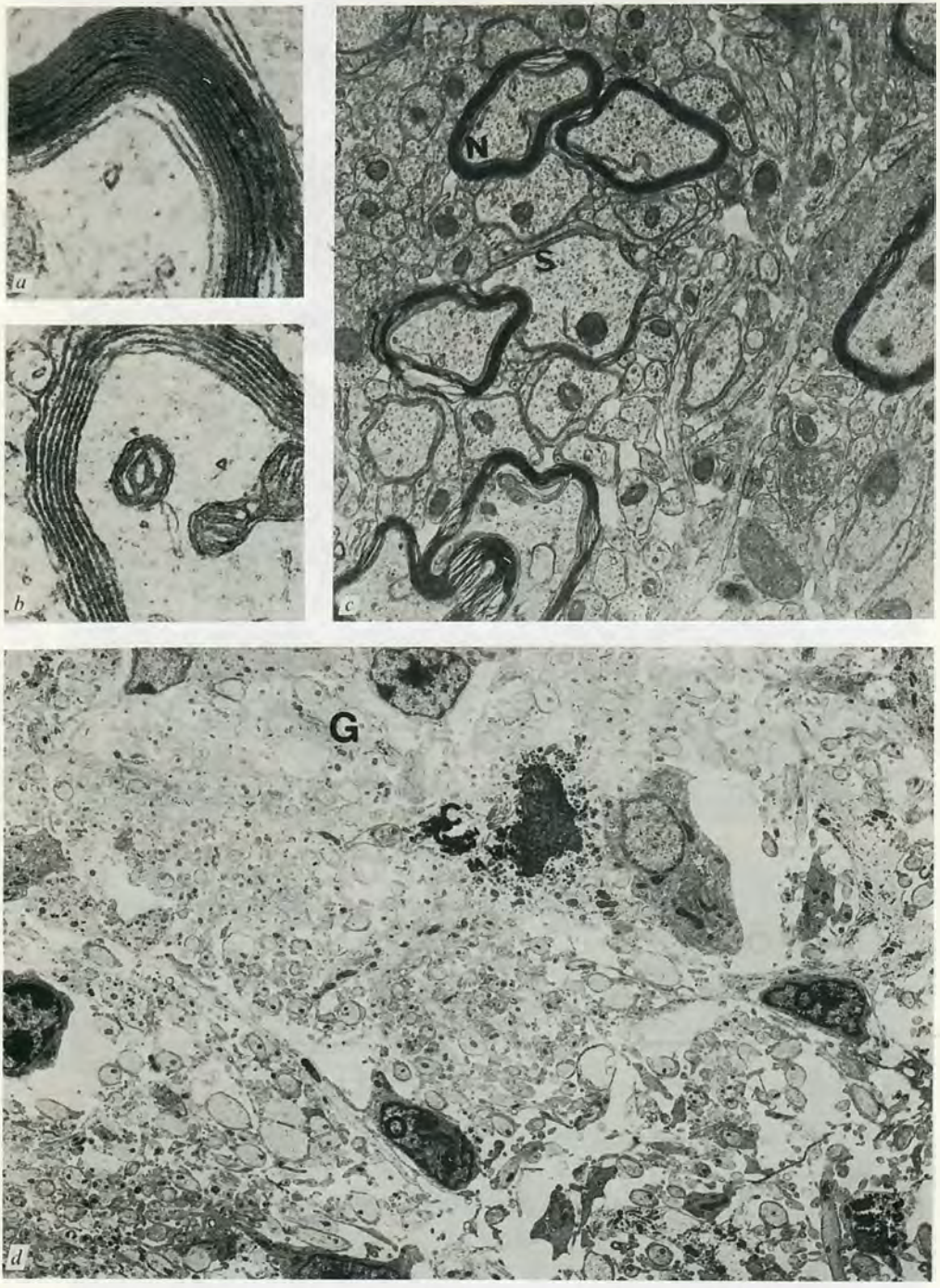


Fig. 2. Ultrastructural study

*a* the normal myelin, well compacted, presents a continuous major dense line (× 1000 000); *b* — the shiverer myelin is poorly compacted and devoid of the major dense line (× 100 000); *c* — normal myelinated axons (N) among shiverer-myelinated axons (S) in the same fascicles (× 20 000); *d* — aspect of the ring of cell processes surrounding the graft (G): one can observe charcoal (C) and oligodendrocytes (O) leaving the graft 12 days after transplantation (× 5 000)

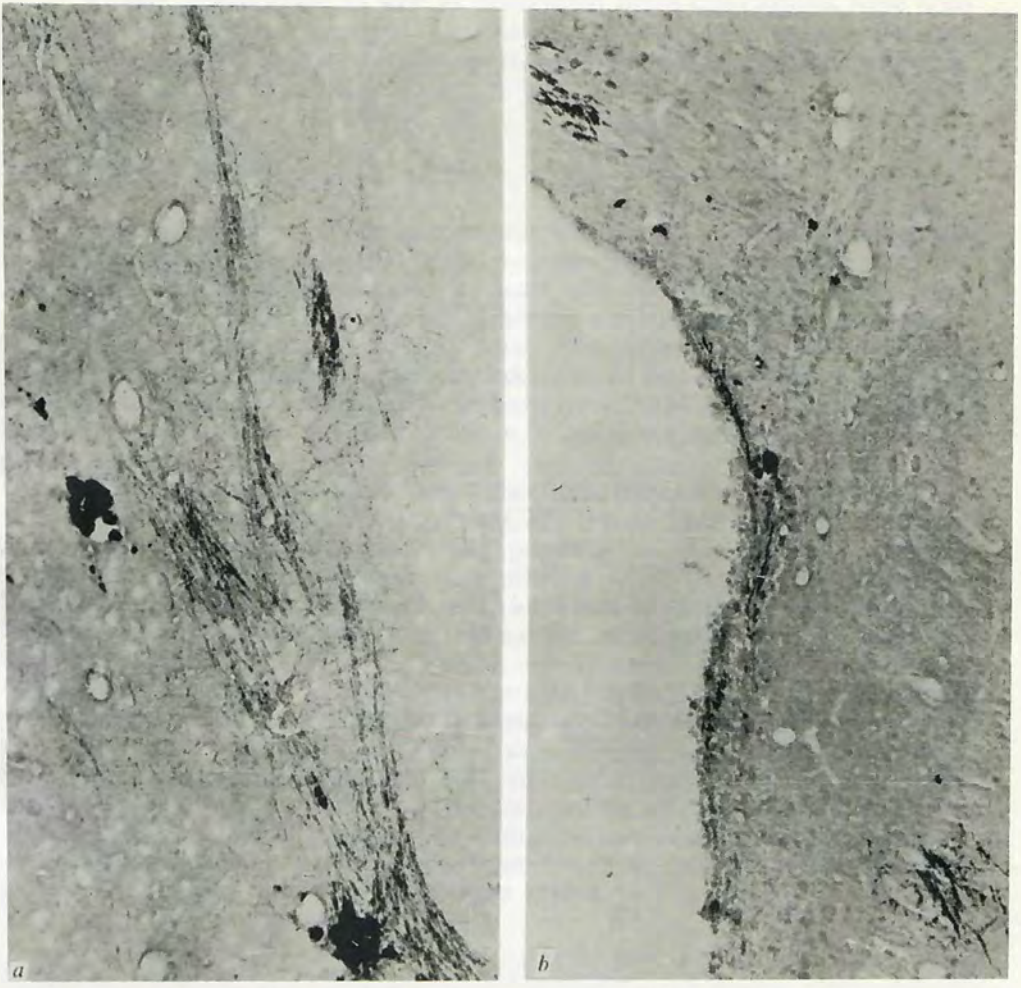


Fig. 3

*a* — oligodendrocytes from spinal cord of normal mouse synthesize MBP-positive myelin in the striatum of the shi/shi host brain (newborn in newborn 60 days after grafting) (× 190); *b* — normal oligodendrocytes from cerebellum synthesize MBP-positive myelin in the lateral ventricle region of the shi/shi host brain (newborn 70 days after grafting) (× 190)

— At last, organotypic as well as dissociated brain cell cultures have been widely used to study the neuroglial relationships. They are very useful in defining the role of intrinsic factors regulating the differentiation and behaviour of myelinating cells [4, 21, 22].

## The Shiverer model

This type of work can only be carried out with a clear identification of the implanted cells or their products in the host parenchyma. In order to identify the transplanted oligodendrocytes or at least the myelin they form post-transplantation, we have developed the "Shiverer model" using two phenotypical traits of the Shiverer mutation of the mouse. The autosomal recessive mutation Shiverer (shi) [14] has been identified to a deletion of five out of the seven axons of the structural gene encoding of myelin basic protein (MBP) [19] in chr. 18 [20]. Homozygotes are fertile but their lifespan is considerably reduced (90 to 150 days).

Biochemical as well as immunohistochemical studies have previously shown a total absence of MBP in the homozygote CNS [11, 5]. Thus, using an anti-MBP antiserum it is possible to detect the presence of the myelin formed by transplanted oligodendrocytes able to synthetize MBP [7, 12] (Fig. 1*a, b, c*). Moreover, this defect is correlated with an absence of the major dense line (MDL), a reduced number of sheaths, and a defect of compaction of the myelin in the homozygotes CNS [15] (Fig. 2 *a, b*).

Electron microscopy (EM) allows the identification of the normal myelin 'hesized by the transplanted oligodendrocytes. This myelin is well compacted presents a well defined major dense line [6] (Fig. 1 *b*). Different types of linating cells (oligodendrocytes, Schwann cells) have been grafted under arious conditions (fragments of CNS tissue, pellets of reaggregated cells) into Shiverer mice CNS at different age. The examples discussed here will be limited to results obtained after implantation of fragments of neural tissue form different species into newborn shi/shi homozygote brains.

## Grafting technique

The grafting technique used in all types of experiments has been extensively described in previous papers [7, 12]. Briefly, tissue from defined regions of the donor's CNS are dissected out and placed in saline on ice. The meninges are carefully removed and the tissues are cut up into 1/2 $mm^3$ fragments. Then, the fragments are rolled in charcoal used as a marker to localize the implant in the host parenchyma. After cold anaesthesia, the receiver's skull is incised with an iridectomy scissor and one or several fragments are introduced into the host's brain using a thin glass pipet connected to a peristaltic micropump.

## Homochronic grafts of normal mouse CNS tissue into the newborn Shiverer CNS

### *Myelinating behaviour*

In the first series of experiments, fragments of olfactory bulb from newborn normal mice were implanted into the rostral thalamus of newborn shi/shi homozygotes.

The myelinating behaviour of the grafted oligodendrocytes was evaluated by staining the MBP-positive myelin synthesized by these cells in the host mutant brain. The immunodetection was performed by classical immunohistological procedure on frozen sections of the CNS tissue of the host as described elsewhere [7, 12]. Sagittal sections were cut at the level where charcoal indicated the presence of the graft. The study was performed every 5 days from day 10th to day 30th after transplantation, and then at day 50th, 70th, 90th, 120th and 150th. In these homochronic conditions (newborn transplant into newborn host brain) normal oligodendrocytes were able to survive, migrate and synthesize MBP-positive myelin in the host parenchyma (Fig. 1 *c*). Simultaneously, "Sham experiments" were performed in which fragments of olfactory bulb from newborn shi/shi homozygotes were injected into the brain of newborn shi/shi homozygotes. In no case MBP-positive myelin was observed, whatever the age of the examined animals [2, 7, 8, 9, 12]. Chronological studies showed that the first MBP-positive patches were observed from day 15th after implantation. The amount of myelin formed and its dispersion in the host brain were maximum at day 20-25th and did not increase or decrease after this time during the host's lifespan.

*Distribution of the MBP-positive myelin*

In order to study the distribution of the MBP-positive myelin patches, semiserial horizontal sections of the entire grafted brain were performed 50 to 150 days after grafting [2]. This study showed that myelin patches were widely distributed throughout the brain in both hemispheres and in the commissures. They were generally isolated from each other and one could never reconstitute a continuum of MBP-positive myelin from the region of the graft up to the more distant patches. At last, whatever the extension and the dispersion of the patches, MBP-positive fibers were always visible at the periphery of the graft. When immunoreactive myelin segments were tightly aligned in the same fascicle, patches appeared rather dense, but in no case were all axons in one fascicle wrapped by normal myelin as proved by EM (Fig. 1 *d*).

*Migration behaviour of the transplanted cells*

The location of MBP-positive patches at long distances from the site of grafting suggests significant migration of transplanted cells. In several cases we found MBP-positivity in the rostral spinal cord even when the graft was placed in the thalamus [2]. In order to analyse the conditions of this migration, we designed two types of experiments. First, in order to evaluate the influence of the cellular environment, fragments of olfactory bulb were injected into different regions of the CNS [9]. Second, fragments from different regions of the CNS (spinal cord, cerebellum, roof of the lateral ventricle, olfactory bulb) were injected into the rostral thalamus. We thus tried to determine: a) if a regional heterogeneity of the oligodendrocyte population exists, and b) if privileged targets exist for oligodendrocytes from defined anatomical regions [2].

In both types of experiments the transplanted oligodendrocytes were able to survive, migrate and synthetise MBP-positive myelin after transplantation, and no behavioural trait could be correlated with the origin of the graft (Fig. 3 *a, b*). However, the migration appeared to be correlated in some way with the anatomical environment. More precisely, the dispersion of the oligodendrocytes was favoured by the proximity of large axonal pathways [2].

*Host-graft interactions*

This study has been performed on shi/shi homozygotes implanted at birth with fragments of olfactory bulb from normal mice.

The graft region was dissected and processed for EM, following standard procedures [6]. An ultrastructural examination of the graft showed an intensive membrane activity between graft and host tissue from 6 to 15 days. A ring of cell processes arising from all types of neural cells surrounded the graft. Cells from both graft and host tissue contributed to the formation of this ring. Moreover, isolated oligodendrocytes have been observed between the host and the graft, suggesting that these cells are leaving the graft (Fig. 2 *d*).

Healthy astrocytes, oligodendrocytes and neurons could be observed in the graft even 130 days after transplantation. At this stage the graft was surrounded by gliotic astrocytes and isolated from the host tissue by a basal lamina. No sign of acute rejection was observed.

*Ultrastructural study of newly-formed myelin*

The EM examination of the implanted region showed that in transverse sections axons myelinated by normal oligodendrocytes were dispersed among bundles of nonmyelinated axons or axons surrounded by Shiverer myelin. The normal myelin, more compacted and more electron-dense could be easily distinguished from the Shiverer myelin (Fig. 2 *d*). Longitudinal sections showed that the same axon might be myelinated by both types of myelin. In this case the node of Ranvier seemed normal, although the number of oligodendroglial loops formed at the node was greater on the normally myelinated side than on the abnormal side.

At higher magnification, the Shiverer myelin was typically noncompacted, while the normal myelin was thicker and presented a well-defined continuous MDL.

Because of the very extensive dispersion of the normal myelin patches observed after transplantation, it was necessary to design a procedure allowing the immunohistochemical prelocalization of the MBP-positive regions in the host brain before their ultrastructural observation. We used in this case thick vibratome sections (60μ). One of two sections was treated for immunohistolocalization of the MBP-positive patches and the corresponding region of the adjacent section was dissected and prepared for classical EM [6].

After coloration with toluidine blue, the normal myelin which was thicker and more contrasted, could be distinguished from the Shiverer myelin on semithin sections. Their EM examination showed that they could be recognized ultrastructurally as normal myelin and one similar to the myelin observed at short distances from the graft. No sign of rejection (inflammatory reaction, reactive gliosis) was observed in the vicinity of the normal myelin even 150 days after implantation.

## Heterochronic grafts: transplantation of human embryonic CNS tissue into the newborn mouse brain

In order to investigate the chronological aspects of myelination, homochronic transplantations — newborn into newborn — were carried out.

These studies demonstrate that the timing of myelination by transplanted oligodendrocytes is similar whatever the origin of the graft and wherever the site

of implantation. However, the first appearance of myelin is delayed (15 days after transplantation), when compared with myelination *in situ* [10].

In order to demonstrate an inductive or modulating effect of the cell environment on the differentiation and the myelinating behaviour of the transplanted cells, we designed an experiment in which human embryonic CNS tissues were grafted into the newborn shi/shi brain. Indeed, in man, according to Yakovlev and Lecours [23], myelination starts at E 150 in the motor roots of the spinal cord and at E 180 in the brain itself. By contrast, it starts at E 20 and is almost completely achieved at P 35 in the mouse. The timing of myelin differentiation in these two species is so different that interspecies grafts may provide a useful model to demonstrate the effect of environment on oligodendrocyte differentiation.

The human MBP is cross-reactive with the antisera used in our homospecific experiments, and the human myelin can be recognized by EM because of its high degree of compaction and the presence of MDL. It was thus possible to study the myelinating behaviour of the transplanted oligodendrocytes [10].

In a preliminary series, fragments of human embryonic CNS tissue from different regions were grafted into newborn shi/shi rostral thalamus. Under these conditions human oligodendrocytes from all parts of the CNS were able to differentiate, migrate, and form MBP-positive myelin at distance from the graft. The ultrastructural examination confirmed the normal phenotype of MBP-positive myelinated fascicle. Transverse ultrathin sections through major fascicles showed axons with normal myelin adjacent to axons with Shiverer myelin [10].

The chronological study of the MBP-positive myelin was performed on a series of shi/shi brains implanted at birth with human embryonic CNS (fragments of the roof of the lateral ventricles). Tissue from 6, 10, 17 and 18-week-embryos was used for this study.

In these series, MBP-positive myelin was observed 20-30 days after transplantation. Under these conditions human cell lines differentiated and matured much more rapidly than *in situ*. These results showed a strong modulating influence of the cell environment on the timing of differentiation of the transplanted cells.

## Conclusions

Intracerebral transplantations in the newborn mouse CNS is so far the only method allowing a long-term study of identified populations of oligodendrocytes in the very complex spatial environment of the developing CNS *in situ*.

The fact that normal (mouse or human) and Shiverer oligodendrocytes compete to myelinate axons in the same fascicle suggests a recognition of similar myelination signals in different species. This lends support to the use of this model in the study of the behaviour of myelinating cells in vivo. This may be especially true in the case of oligodendrocytes, which seem to be sensitive to the biological environment for the expression of their sophisticated behaviour.

The long-distance dispersion of MBP-positive myelin patches attributed to migratory behaviour of the transplanted oligodendrocytes after birth reflects their usual behaviour. This can only be studied under in vivo conditions.

The study of the first appearance of MBP-positive myelin after transplantation suggests a strict timing of myelin expression under these conditions regardless of oligodendrocyte origin. Mouse and human implanted oligodendrocytes express MBP 15 to 20 days after transplantation. This is very

surprising in case of human oligodendrocytes which would not be expected to myelinate for 10 weeks after transplantation. Therefore, there may be a strong modulating effect of the host environment on the differentiation chronology of grafted cells.

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