Acta neuropath. (Berl.) 42, 205–209 (1978)

Acta Neuropathologica


# Radio-Autographic Study of Cell Proliferation Secondary to Wallerian Degeneration in the Postnatal Rat Optic Nerve

J. Valat, J. Fulcrand, A. Privat[1], and R. Marty

Laboratoire de Neuropathologie Expérimentale Université des Sciences et Techniques du Languedoc F-34060 Montpellier Cédex, and

[1] Laboratoire de Culture de Tissu Nerveux INSERM U. 106 Hôpital de Port Royal, 123 bd. de Port-Royal, F-75014 Paris, France

**Summary.** In the course of our study on Wallerian degeneration in the young rat optic nerve after unilateral enucleation, cell proliferation was measured by quantitative radio-autography. Ninety-two newborn rats were separated into four groups which were operated at 2, 5, 8 and 20 days postnatal (key stages) respectively. In each of these groups, the animals were sacrificed after progressive delays ranging from 3 h to 30 days (DPO). They received, 3 h before the sacrifice, an intraperitoneal injection of 2 μCi/g weight of tritiated thymidine. After the radio-autographic procedure, semi-thin sections were examined, and labeled as well as unlabeled cells were counted on the whole cross section of the operated nerve, as well as on the contralateral nerve. In the operated nerve, the four key stages may be separated into two reactive patterns, depending on whether the enucleation is performed before or after the myelination gliosis. The two first key stages (2 and 5 DPN) show leveling down of the curve of their proliferative indices, when compared with the control, whereas the two other key stages (8 and 20 DPN) showed a leveling up of the curve. The comparison of these data with those of previous work (Fulcrand and Privat, 1977; Valat et al., 1978) permitted the evaluation of cell death, which is specially evident at the key stage 8 DPN. The proliferative ability of neuroglial cells thus appear no to be closely dependent upon an intrinsic genetic program, but rather to be modulated by epigenetic events. The early absence of the axonic signal induces a decrease of this proliferation, whereas the more or less belated interruption of the same signal induces a reactive gliosis, with a net increase of proliferative indices over the control.

**Key words:** Quantitative radio-autography – Wallerian degeneration – Cell proliferation – Optic nerve – Postnatal rat.

Offprints requests to: Dr. J. Fulcrand (address see above)

A series of recent publications (Privat and Fulcrand, 1976; Fulcrand and Privat, 1977; Valat et al., 1978) has been devoted to several aspects of cell reactivity in the optic nerve after enucleation. The latter was carried out during the early life of the animal at the so-called key stages of 2, 5, 8, and 20 days, chosen in accordance with the chronology of myelination in the nerve.

The ultrastructural study (Fulcrand and Privat, 1977) has evidenced the nature and the function of the various cell lines affected by the Wallerian degeneration. The quantitative analysis carried on semi-thin sections (Valat et al., 1978) has measured several parameters such as the total number of cells per cross section, the cell density and the percentages of the different cell lines (Fulcrand and Privat, 1977). In this view, the use of the radio-autographic technique, after labeling with 3H thymidine, allows on the one hand the measure of cell proliferation and on the other hand the evaluation of some previously hypothetized parameters such as cell death, in the above cited experimental conditions.

Most, if not all, radio-autographic work in this context deals with adult animals (Sjöstrand, 1965; Kreutzberg, 1966; Schultze and Kleihues, 1967; Adrian, 1968; Skoff and Vaughn, 1971; Hattori, 1973; Kerns and Hinsman, 1973; Reznikov, 1975; Skoff, 1975; Herndon et al., 1976).

The aim of the present work is, therefore, to apply this dynamic criterion to the chronologic study of the interference in between myelination gliosis and reaction gliosis which testify for the combination of genetic and epigenetic factors.

## Materials and Methods

Ninety-two rats of the Sprague-Dawley strain have been used, with two animals for each stage.

Each of the four key-stages, defined as the age at which the animals are operated, comprises 11 progressive post-operation delays: 0, 1, 2, 3, 4, 6, 7, 8, 12, 16, and 30 days.

0001-6322/78/0042/0205/$ 1.00

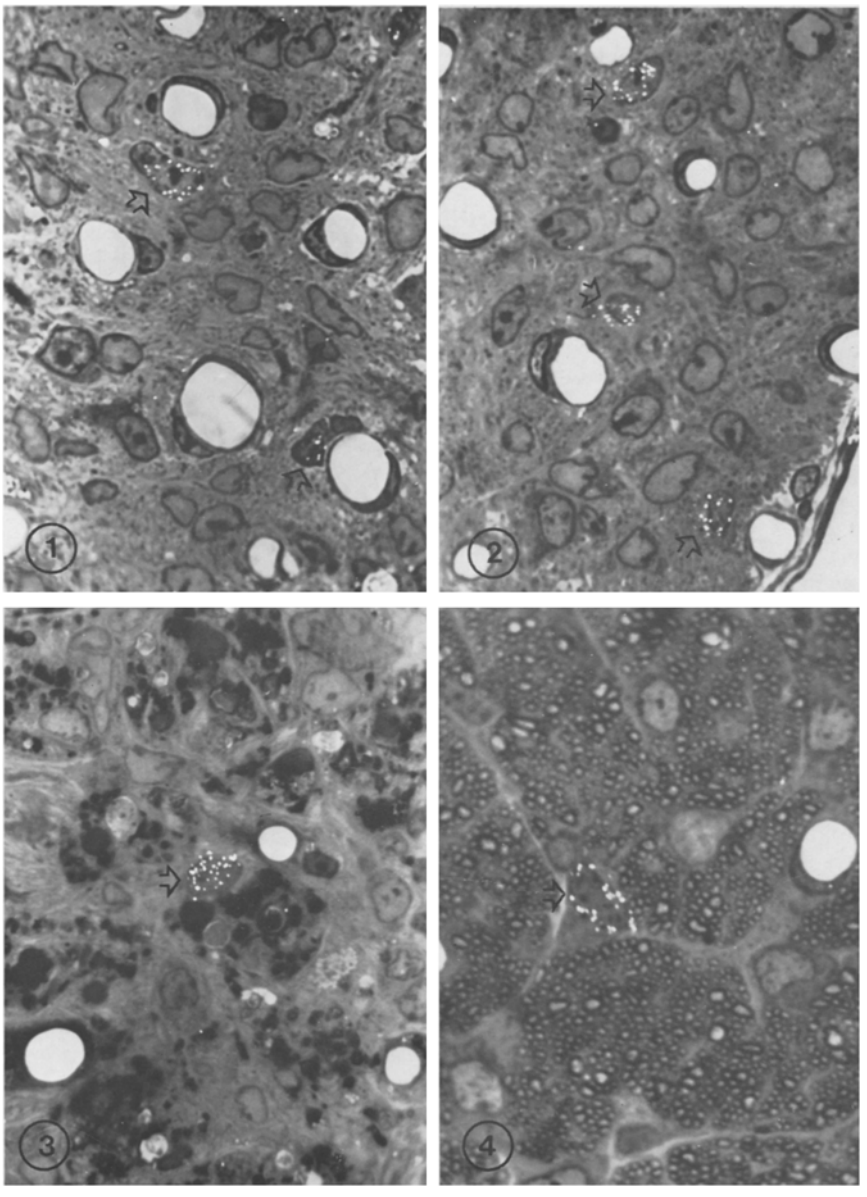

**Plate I**
Light micrographs of semi-thin section stained with toluidine blue after radio-autography. Pictures have been taken with a composite illumination, so that the silver grains appear as bright points on a gray background. The background labeling appears especially low. Arrows point to labeled cells.
**Fig. 1:** key stage 2 DPN – 12 DPO × 1065.
**Fig. 2:** key stage 5 DPN – 7 DPO × 1065.
**Fig. 3:** key stage 20 DPN – 6 DPO × 866.
**Fig. 4:** control – 20 DPN × 1619

The animals received a simple intraperitoneal injection of 3H methyl-thymidine (specific activity 25 Ci/m Mole) 2 μ Ci/g body weight, at the same time in the afternoon, to eliminate nycthemeral variations. They were sacrificed three hours after the injection, this delay being neglected in the chart of DPO's.

Surgical and histological techniques have been detailed elsewhere (Fulcrand and Privat, 1977).

For the radio-autography, 1μm thick sections were coated with Ilford L4 photographic emulsion. After one month of exposure, they were revealed in the usual conditions (Fulcrand and Marty, 1971), and stained with toluidine blue.

Counts were performed on cross sections of the medial segment of the operated optic nerve, the contralateral nerve being used as a control. Several non-serial sections were examined to compensate for individual variations.

The sections were observed and photographed with transmited light, reflected light, and mixture of the two (Pl. I, Fig. 1–4). As the background was particularly low, cells with at least three grains were counted. The number of grains per nucleus, i.e., the labeling intensity, depends upon the time of injection in the S phase of the cell cycle. The labeling index was defined as the ratio of labeled cells to the total number of cells. The numbers obtained were subjected to the usual statistical treatment (Student's *t*-test).

## Results

The chronological evolution of labeling indices for the four key-stages have been graphically expressed in comparison with the corresponding values of the control nerve, whereas the evolution of the total number of labeled cells per cross section was plotted in histograms (Pl. II, Figs. A, B, C, D, and Pl. III).

*In the operated nerve, at the key stage 2. DPN.* (Pl. II, Fig. A).

The comparison of the curves of indices shows two different phases: a first rapid raise up to the 4th day, with similar slopes for the two curves, the experimental nerve being at a distinctly lower level, second, a progressive decrease from the 4th to the 30th DPO, the curves being superposed from the 5th day onward.

The comparison of histograms shows the higher absolute number of labeled cells in the control nerve at all the stages studied

*At the key stage 5 DPN* (Pl. II, Fig. B), three successive phases are evident, the first being characterized in the operated nerve by a rapid decrease from 3 h to 4 DPO, the initial level being higher than at the preceding key stage. The decrease is more progressive in the control. The second phase consists of a plateau in the two nerves from the 4th to the 8th day, and the third of very slow decrease, the two curves being superimposed. The histograms show generally higher values in the control nerve, especially at the 2nd DPO; only the initial value is higher in the experimental nerve.

*At the key stage 8 DPN* (Pl. II, Fig. C), two phases are evident. In the first one, the profiles of the two curves are very distinct. The experimental nerve shows higher indices than those of the control, with a peak at one DPO. After 4 DPO, the two curves show an irregular decrease.

The histograms confirm the inversion in favor of the experimental nerve, from 1 to 3 DPO.

*At the key stage of 20 D.P.N.* (Pl. II, Fig. D), the values for the operated nerve are always higher than in the control. After an initial raise, the curve shows a peak which is delayed when compared with that of the preceding key stage. Then occurs a slow and irregular decrease.

The histogram confirms the higher level of proliferation in the operated nerve.

Plate III presents the variations of the labeling indices for the four key stages and the control with the postnatal age of the animal (DPN) in the abscissa. They show clearly the characteristic leveling down of the curve for the premyelination period (2 and 5 DPN), and the leveling up for the periods of myelination (8 DPN) and post-myelination (20 DPN), when compared with the control.

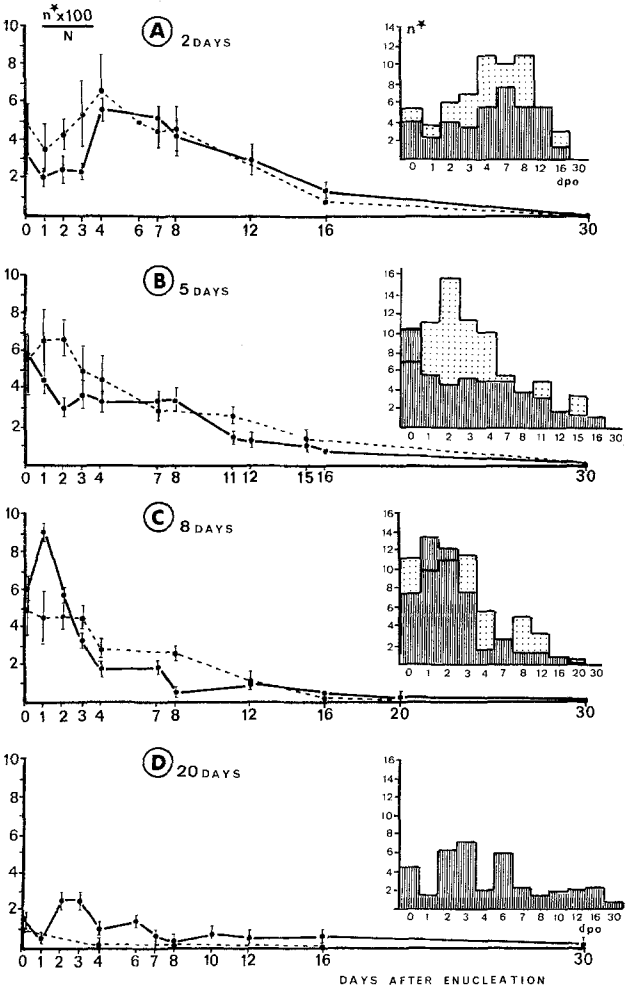


**Plate II.** Labeling indices have been figured on the left diagram per each key stage, and absolute numbers of labeled cells per cross section on the right histogram. ------ control; ▨ control; —— experimental; ▥ experimental

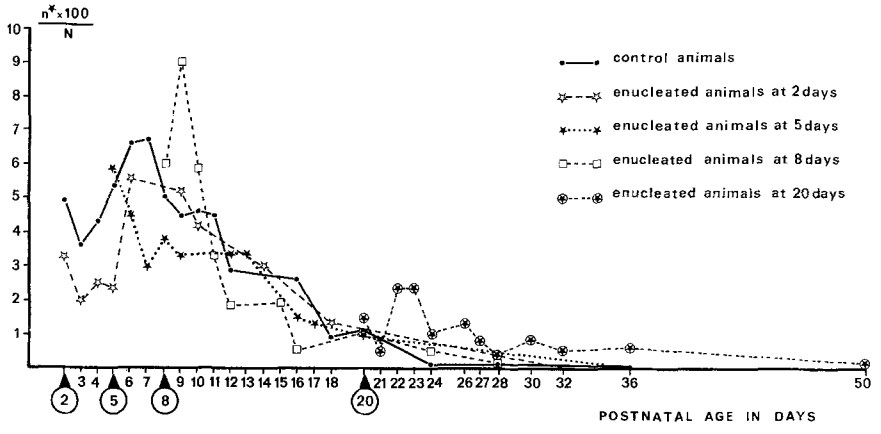


**Plate III**
The labeling indices for the four different key stages have been figured on the same diagram, the age of the animal being plotted in abscissa

## Discussion

The results of the present work bring a dynamic element in to the knowledge of the interactions between gliogenesis and reactive gliosis, but they must be compared with the conclusions of the other papers of the series (Fulcrand and Privat, 1977; Valat et al., 1978) in order to be fully understandable. Three points arise:

*The proliferation* is visualized by two parameters: the absolute number of labeled cells per cross section and the labeling index.

*In the control* the graphical representation of the labeling index and of the number of labeled cells point to the presence of a period of intense proliferation in between the 5th and 8th days postnatal, due to the onset of myelination (Fulcrand et al., 1975).

*In the experimental animal*, the comparison of the curves of the four key stages (Pl. III) shows two symetrical variations from the curve of the control nerve. The first one is a leveling down, whereas the second one is a leveling up. The chronological border between the two corresponds to the critical period located between 5 and 8 DPN, corresponding to the onset of myelination.

*At the key stage 2 DPN* the proliferation expressed either in absolute or in relative number is always lower than in the control. Toward the end of the 1st week post operation, the proliferation is reduced by about one third. It appears then that the enucleation and its consequence, the Wallerian degeneration, disturbs appreciably the proliferative program of the glial cells. This results apparently from the lack of an adequate signal, corresponding to the destruction of the axons.

*At the key stage 5 DPN*, the enucleation has a similar effect with, however, an initial high labeling index, as a consequence of the beginning of the onset of the myelination program, with the maturation of the axons and the differentiation of the oligodendrocyte line (Fulcrand and Privat, 1977). Later on, the mitotic capabilities are reduced when compared with the control, especially versus the peak of myelination gliosis (2 DPO). This decrease is likely to result from the absence of axonal contact.

*At the key stage 8 DPN*, the second reactive modality is already present with an increased proliferation over the control, especially for 1 and 2 DPO. The active myelination has triggered the corresponding program of sustained proliferation. In this way, reactive gliosis is added to myelination gliosis.

*At the key stage 20 DPN*, now, the second reactive pattern, characterized by a leveling up of the curve, is fully realized as seen on Plate II D and Plate III. The integrated surface between the two curves, from 3 h to 30 DPO, corresponds to the reactive gliosis, already evidenced by the absolute numbers per cross section (Valat et al., 1978). It is now evident that the active phase of myelination is almost completed. The slight delay of the peak of reactive gliosis when compared with the key stage 8 DPN may be explained by the lengthening of the reaction of the nerve. This key stage, quoted above as post-myelination, shows similar phenomena to those found in the adult by Skoff (1975).

*Cell Death.* As suggested in previous publications (Valat et al., 1978), cell death is hardly appreciable by direct means, though, in the normal animal Lewis (1975) was able to estimate the turnover of cells in the rat subependymal layer from the count of pycnotic nuclei.

However, the present study allows an indirect estimation of cell death by comparison of the total number of cells per cross section, the number of labeled cells, and the cell densities (Valat et al., 1978). In order to eliminate the influence of the elongation of the nerve in the early postnatal period and the correlative dispersion of the cells, the evaluations in the severed nerve have been compared with those of the control of the same age. In these conditions it appears clearly that cell death is an important event at the key stage 8 DPN, since, after five DPO with an almost similar labeling index during this period, the total number of cell per section (Valat et al., 1978) is reduced by 25 %. The latter is most likely due to the lack of adequate stimulus for the differentiation of oligodendrocytes, with the degeneration of axons (Fulcrand and Privat, 1977).

*Interference between Genetic and Epigenetic Factors.* The present results finally bring some insight on this issue, as they point to the proliferative capacity of the cells of the optic nerve; this, together with the ability to differentiate, which is the subject of an other publication, constitute the program of a cell. Indeed, it appears that the proliferative ability of these cells is not a genetically programmed character, as it may be reduced or enhanced, depending of the period of enucleation and hence of the status of the axons.

In conclusion, the present work undertaken as a part of a study of neuroglial maturation and reactivity in the optic nerve of the rat allowed the quantitative evaluation of the neuroglial proliferation, in absolute numbers per cross section and labeling index, and the indirect estimation of the cell death as well as the discrimination between the intrinsic program of the cells and the influence of the tissular surrounding.

*Acknowledgements.* The autors acknowledge the help and advice of Dr. J. E. Gruner and the technical collaboration of Mrs. J. Boyer and M. J. Drian, and MM. D. Le Cren and P. Sibleyras. The resarch was supported by grants from INSERM n° 77.1.119.6, C.E.A. Saclay, E. R.A.-C.N.R.S. n° 187 and D.G.R.S.T.

## References

Adrian, E. K., Jr.: Cell division in injured spinal cord. Am. J. Anat. **123**, 501–520 (1968)

Fulcrand, J., Marty, R.: Migrations névrogliques dans le tractus optique chez le Rat. Radio-autographie quantitative au cours du développement postnatal. Exp. Brain Res. **14**, 38–47 (1971)

Fulcrand, J., Privat, A.: Neuroglial reactions secondary to Wallerian degeneration in the optic nerve of the postnatal rat: ultrastructural and quantitative study. J. Comp. Neurol. **176**, 189–224 (1977)

Fulcrand, J., Valat, J., Marty, R.: Maturation et réactions névrogliques dans les voies optiques primaires: étude postnatale chez le Rat. VIIè Congrès International de Neuropathologie, Budapest, pp. 733–735, Amsterdam: Excerpta Medica 1975

Hattori, H.: Electron microscopic autoradiographic studies on the origin, nature and fate of reactive cells in the central nervous system of mouse after stab-wounding. J. Kyoto Pref. Univ. Med. **82**, 403–426 (1973)

Herndon, R. M., Weiner, L. P., Price, D. L.: Thymidine labeling of glia during remyelination after J. H. M. injection. Abstract 51th Meet. Amer. Ass. Neuropath. 1978, 108

Kerns, J. M., Hinsman, E. J.: Neuroglial response to sciatic neurectomy. I: light microscopy and autoradiography. J. comp. Neurol. **151**, 237–254 (1973)

Kreutzberg, G. W.: Autoradiographische Untersuchung über die Beteiligung von Gliazellen an der axonalen Reaktion im Facialiskern der Ratte. Acta neuropath. (Berl.) **7**, 149–161 (1966)

Lewis, P. D.: Cell death in the germinal layers of the postnatal rat brain. Neuropath. Applied Neurobiol. **1**, 21–29 (1975)

Privat, A., Fulcrand, J.: Glial reaction in the optic nerve of the young rat after unilateral enucleation. Anat. Rec. **184**, 505 (1976)

Reznikov, K. Y.: Incorporation de 3H thymidine dans les cellules de la névroglie du cortex temporal et dans les cellules de la zone sous-épendymaire chez des souris de deux semaines ou adultes à l'état normal ou après traumatisme cérébral (en russe). Ontogenez **6**, 169–176 (1975)

Schultze, B., Kleihues, P.: D.N.S. Synthese der Neuroglia im experimentellen Hirnödem. Experientia **23**, 941–942 (1967)

Sjöstrand, J.: Proliferative changes in glial cells during nerve regeneration. Z. Zellforsch. **68**, 481–493 (1965)

Skoff, R. P.: The fine structure of pulse labeled (3H thymidine cells) in degenerating rat optic nerve. J. Comp. Neurol. **161**, 595–611 (1975)

Skoff, R. P., Vaughn, J. E.: An autoradiographic study of cellular proliferation in degenerating rat optic nerve. J. Comp. Neurol. **141**, 133–156 (1971)

Valat, J., Fulcrand, J., Marty, R.: Réactivité cellulaire postnatale du nerf optique après énucléation chez le Rat. I. Aspects quantitatifs en relation avec la myelinisation. Acta Anat. **100**, 5–16 (1978)

Received February 2, 1978/Accepted March 16, 1978