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# Developmental expression of major myelin proteins in hypomyelinated *pt* mutant rabbit

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**Abstract.** A term "paralytic tremor" (*pt*) is attributed to a neurological mutation of *Chinchilla* rabbits, affecting the development of the central nervous system (CNS). A quantification of myelin protein content indicates the strong CNS hypomyelination during the development (1-120 postnatal days). SDS-PAGE electrophoresis of total brain homogenates, followed by immunoblotting, shows a reduced concentration of major myelin-connected proteins. MBP deficiency coressponds approximately to the level of the hypomyelination, whereas PLP expression is drastically reduced.

**Key words:** development, *pt* rabbit mutant, myelin proteins, CNS hypomyelination

Paralytic tremor (*pt*) is an X-linked recessive mutation which appeared spontaneously in 1964 within a *Chinchilla* rabbit lane in the Department of Comparative Neurology PAN (Mińsk Maz., Poland). The mutation is characterized by: a coarse body tremor, a spastic limb paresis, an increased muscular tone and an exaggeration of tendon reflexes. The neurological symptoms appear during the second week of life and partially regress at the later stage of development, although a complete recovery is never achieved. A lifespan of the affected animals is normal or slightly reduced (Osetowska and Leszowski 1975). As previous studies have shown, CNS myelination is deficient. Morphological studies confirmed the presence of numerous hypomyelinated axons wrapped in thin and uncompacted myelin sheaths (Taraszewska 1984, Taraszewska and Zelman 1985, 1986). Biochemical studies showed reduced concentrations of myelin-connected lipids (Domańska-Janik at al. 1986) and specific changes in enzyme activities (Domańska-Janik at al. 1988, 1992).

Considering the fact that the myelin formation process could be delayed and/or prolonged in *pt* rabbits, we performed a developmental studies for animals aged from 1 up to 120 days. The animals were supplied by the Department of Comparative Neurobiology PAN; mutants were strictly control-

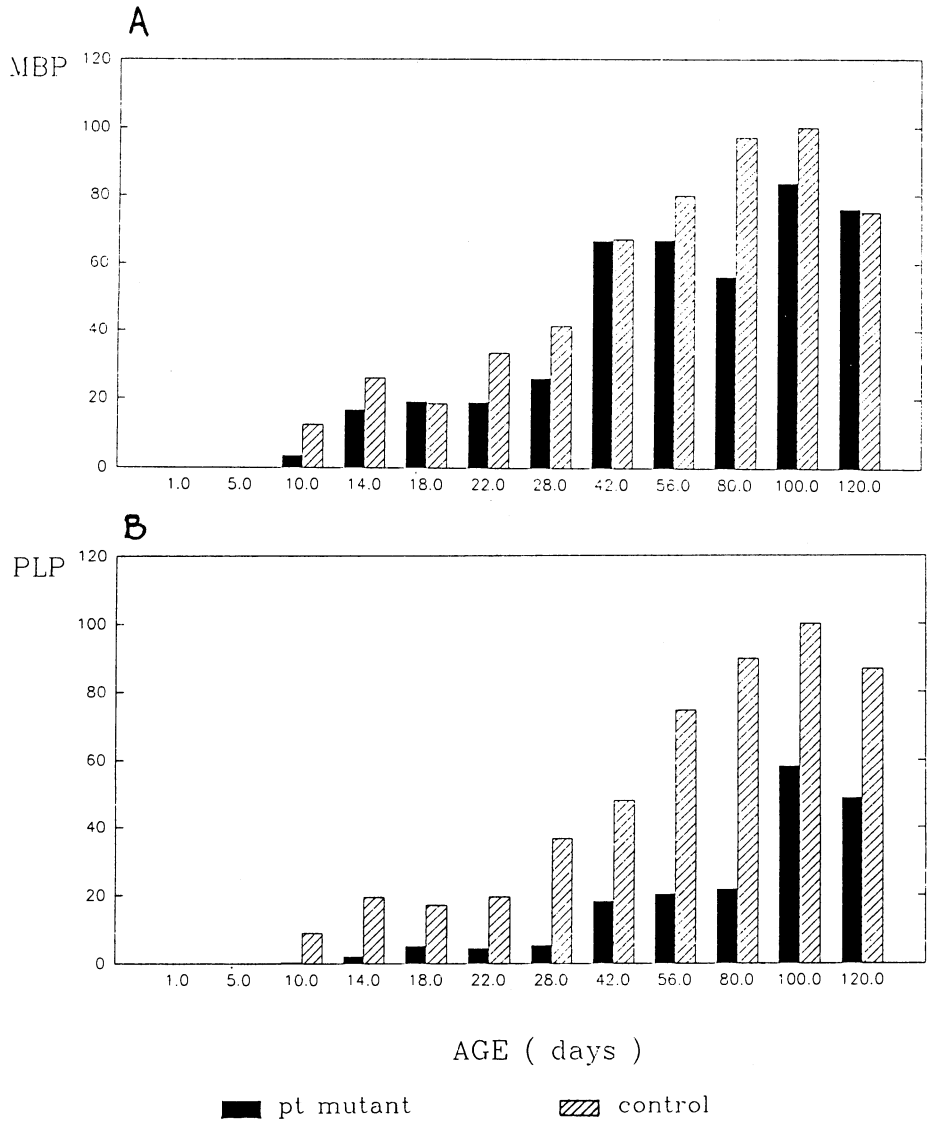


Fig. 1. MBP (A) and PLP (B) concentrations in *pt* mutant and control brain homogenates: densitometric analysis of the representative immunoblot after incubation with specific antisera followed by incubation with 125-I Protein A and subsequently detected by autoradiography.

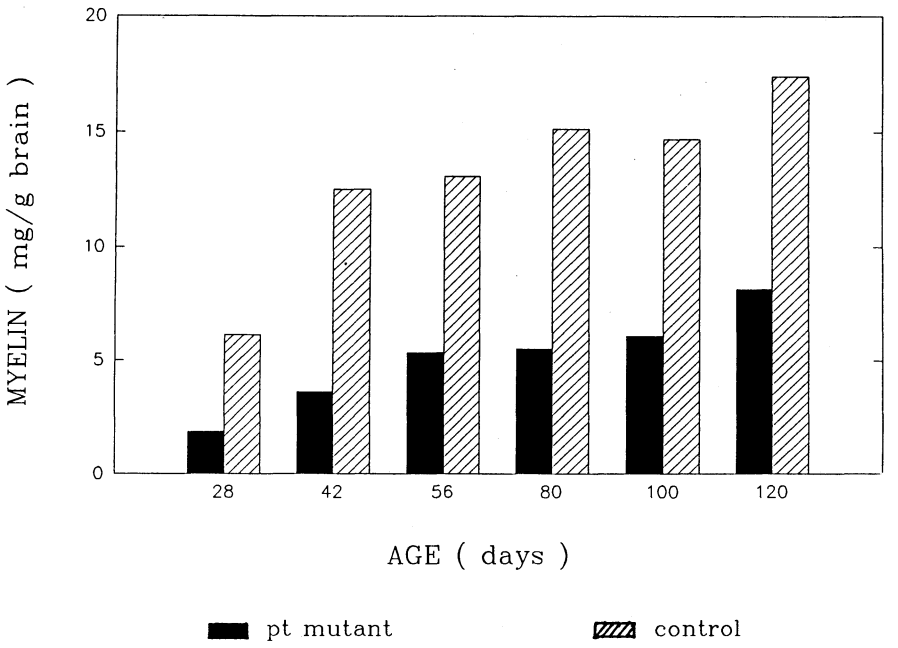


Fig. 2. A total protein content determined by the method of Lowry (1951) in the myelin fraction isolated according to the method of Norton and Poduslo (1973) from *pt* mutant and control animal brains. The values are means of two myelin preparation experiments.

led for their *pt* trait and agematched control rabbits derived from the same *Chinchilla* lane. They were killed by an intravenous air injection and a decapitation. Brains, without a cerebellum, were homogenized in 0.32M sucrose and thereafter used for myelin preparation according to the method of Norton and Poduslo (1973). The proteins in homogenate samples were analyzed by SDS-PAGE technique and subsequently electrophoretically transferred to polyvinylidene difluoride membranes (Milipore,Bedford) by the method of Towbin et al. (1979). Western blotting with polyclonal antibodies (made at Prof.J.- M.Matthieu laboratory) against proteolipid protein (PLP) and myelin basic protein (MBP), respectively, was carried out on them; immune complexes were detected with $^{125}I$ protein A (NEN,Boston) and an autoradiography. Immunoblots were subject to a densitometric analysis (Pharmacia LKB UltroScan XL). Protein concentration in partially delipidated (diethyl ether/ethanol) myelin samples were estimated according to Lowry et al. (1951).

The densitometric analysis of the immunoblots (Fig. 1) indicates that an expression of PLP and MBP-the most abundant myelin proteins-begins about tenth day of life and is significantly reduced in *pt* rabbits. PLP expression level is reduced to about 30% of control, whereas MBP deficiency is not so strong. A protein concentration measured in the myelin isolated from animals aged 28 to 120 days (Fig. 2) shows the significant decrease during the entire investigated developmental stage. The comparison between PLP and MBP expression level observed in the homogenates and a degree of hypomyelination, as to myelin content, indicates that MBP deficiency may reflect the extent of myelin deficiency in *pt* rabbits. And conversely, a drastic reduction of PLP expression characteristic for the entire investigated development is probably caused by a mutation in PLP gene. The PLP gene is localized on chromosome X (Duncan et al. 1990) and this is in agreement with a genetic background of the mutation which is inherited in the sex-linked manner. A question arises if this is really the PLP gene mutation which is responsible for *pt* syndrome and how this mutation is possibly suppressed during development. This problem is currently investigated.

Our studies show that the amount of *pt* myelin never reaches the level present in control animals but is reduced to about 30%. The myelin content in 120-days old *pt* rabbits corresponds approximately

to that one of 28 days old normal animals. In spite of the neurological symptoms regress in the later stages of development (included in our studies), hypomyelination is approximately constant and doesn't undergo regression. This proves that the process of myelin formation is delayed, deficient and prolonged in *pt* mutant rabbits.

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