ning densitometer. The amount of vasopressin mRNA detected was corrected for the amount of total RNA loaded by quantitating the amount of 18S ribosomal RNA on photographs of ethidium bromide–stained gels.
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# Long Lives for Homozygous Trembler Mutant Mice Despite Virtual Absence of Peripheral Nerve Myelin

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**Nervous system functions are dependent on point-to-point communication of signals along neuronal axons, and axonal insulation by myelin is thought to speed such conduction. Loss of previously formed myelin or lack of myelin formation can have serious, even fatal, consequences. Mice homozygous for the trembler mutation make virtually no peripheral nervous system myelin, yet have long and functional lives. This result calls into question the view that peripheral nervous system myelin plays a vital role, at least in this species.**

AXONS OF CENTRAL AND PERIPHERAL neurons are accompanied along their lengths by supporting glial cells in vertebrates and in many invertebrates, but specialization of those glial cells to form compact myelin is a predominantly vertebrate phenomenon (*1*). Although conduction over axons with glial specializations other than myelin can be quite rapid (*2*), for any given axonal diameter the addition of a myelin sheath, with resultant saltatory conduction, is thought to increase conduction speed markedly (*3, 4*). Loss of previously formed central nervous system (CNS) or peripheral nervous system (PNS) myelin can alter conduction, with devastating consequences, as in the human demyelinating disorders multiple sclerosis (*5*) and the Guillain-Barré syndrome (*6*), respectively. The failure to make an adequate amount of central or peripheral myelin during development is also associated with severe behavioral dysfunction. For example, mice hemizygous for the jimpy mutation, in which CNS but not PNS myelin formation is very deficient, have weakness and seizures and usually die before 1 month of age (*7*). Mice homozygous for the autosomal semidominant trembler-J ($Tr^J$) mutation have a severe maturational defect such that almost no peripheral myelin sheaths are ever made, although central myelination appears normal (*8*). Such mice have very severe quadriparesis and die before 3 weeks of age. We now report the unexpected observation that mice homozygous for the original dominant trembler (*Tr*) allele (*9*), *Tr/Tr*, also have virtually no PNS myelin, and yet have long and functional lives.

Trembler arose in a noninbred stock (*9*) and is maintained noninbred by ourselves and others. We have placed the *Tr* mutation and the closely linked (1 cM) marker vestigial tail (*vt*) on the same chromosome 11 (*10*). The short tail phenotype expressed by *vt/vt* mice (*11*) serves to distinguish *Tr/Tr* from *Tr/+* mice, as we have established previously for $Tr^J$ mutants (*12*). With matings of the form *Tr vt*/+ + × *Tr vt*/+ +, short-tailed trembling offspring were *Tr vt/Tr vt* and long-tailed trembling offspring were *Tr vt*/+ + with greater than 95% probability (*12*). Nontrembling offspring were of + +/+ ? genotype. We examined pathological material from 12 *Tr vt/Tr vt* mice ranging in age from 22 days to 10 months, as well as 10 *Tr vt*/+ + and 7 + +/+ ? littermates. Peripheral nerves were examined in all cases; in two littermate trios (*Tr vt/Tr vt*, *Tr vt*/+ +, and + +/+ ?), very extensive sampling was performed, including specimens from proximal and distal hindlimb nerves, autonomic nerves, dorsal and ventral spinal roots, cranial nerves, spinal cord segments and dorsal root ganglia from cervical, thoracic, and lumbar levels, and various parts of the brain. A total of 30 separate PNS sites were examined in the two *Tr vt/Tr vt* mice from these two extensively studied trios. We determined the percentage of large-enough fibers that were myelinated in whole sciatic or sciatic branch sections from all *Tr vt*/+ + mice in samples of at least 200 fibers at ×1000. Because there were so few myelinated fibers in nerves from *Tr vt/Tr vt* mice (see below), we counted the total number of myelinated fibers per nerve cross section in whole sciatic or sciatic branch nerves from all *Tr vt/Tr vt* mice.

We confirmed the findings of Falconer (*9*), with the added confidence offered by use of the *vt* marker, that *Tr* homozygotes and heterozygotes are behaviorally indistinguishable, both having a coarse action tremor and moderate quadriparesis with a waddling gait. The mild behavioral heterogeneity within each of the genotypic classes *Tr vt/Tr vt* and *Tr vt*/+ + in our outcrossed stock did not obscure the classification. Both groups of mice were long-lived. Our *Tr vt*/+ + mice often survived for more than 2 years, and our oldest *Tr vt/Tr vt* mice seemed quite healthy at more than 1 year of age. However, the quadriparesis of some *Tr vt/Tr vt* mice of more than 1 year of age became noticeably worse than that of their *Tr vt*/+ + littermates. Females of each genotype mated and bore progeny easily, but males of both genotypes mated poorly. To make *Tr vt*/+ + × *Tr vt*/+ + matings fruitful, we usually had to give the *Tr vt*/+ + males prior breeding experience with + +/+ + females.

At all ages studied, nearly all nerves of *Tr vt/Tr vt* mice were virtually completely devoid of myelinated fibers (Fig. 1A). In

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contrast, all nerves from *Tr vt*/+ + mice contained thinly myelinated fibers, with some variation among individuals (Fig. 1, B and C), while nerves from normal mice contained abundant well-myelinated fibers (Fig. 1D).

These qualitative observations were borne out by counts of myelinated fibers in nerves from *Tr vt*/+ + and *Tr vt*/*Tr vt* mice. The percentage of large-enough fibers that were myelinated in nerves from different *Tr vt*/+ + mice ranged from 13.2 to 41.5% with a mean of 28.4% and a standard deviation of 8.9%. Since a sciatic nerve from a normal mouse contains about 3850 myelinated fibers (*13*), the number of myelinated fibers in *Tr vt*/+ + sciatic nerves was about 1100 on average and about 500 in the least well myelinated *Tr vt*/+ + mouse. In contrast, in the *Tr vt*/*Tr vt* mice the greatest total number of myelinated fibers present in any whole sciatic or sciatic branch cross section was six. Thus PNS myelination in the most heavily myelinated *Tr vt*/*Tr vt* mouse was about 1% of that in the least heavily myelinated *Tr vt*/+ + mouse. The number of myelinated fibers in *Tr vt*/*Tr vt* sciatic nerve cross sections differed at times at different levels along the same nerve.

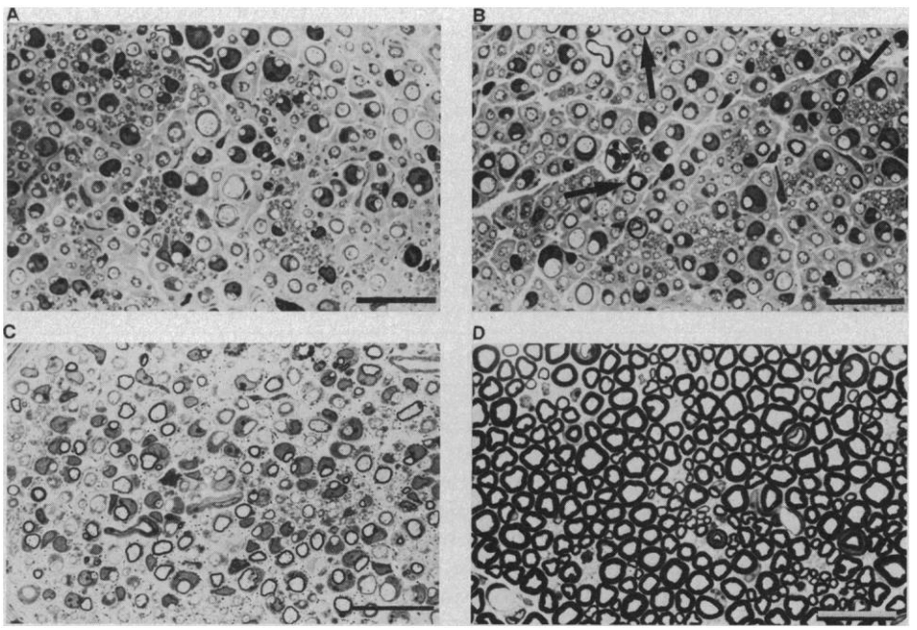


**Fig. 1.** Transverse sections (1 μm) of sciatic nerves from *Tr vt*/*Tr vt* (**A**), *Tr vt*/+ + (**B** and **C**) and + +/+ ? (**D**) mice. (A), (B), and (D) are from 4-month-old littermates, while (C) is from a 1-month-old *Tr vt*/+ + mouse. Myelin sheaths are absent in (A), while (B) and (C) illustrate the range of myelination found in nerves from different *Tr vt*/+ + individuals. Nerves from most *Tr vt*/+ + animals were in the midportion of this range. The arrows in (B) point to some of the remaining myelin sheaths within the field. Bars, 20 μm. Mice were systemically perfused under general anesthesia with 1% paraformaldehyde and 3% glutaraldehyde in 0.1*M* Sorenson's phosphate buffer, *p*H 7.4. Tissue samples were then processed for epoxy plastic embedding. Subsequently, 1-μm sections were cut and stained with alkaline toluidine blue for examination by light microscopy.

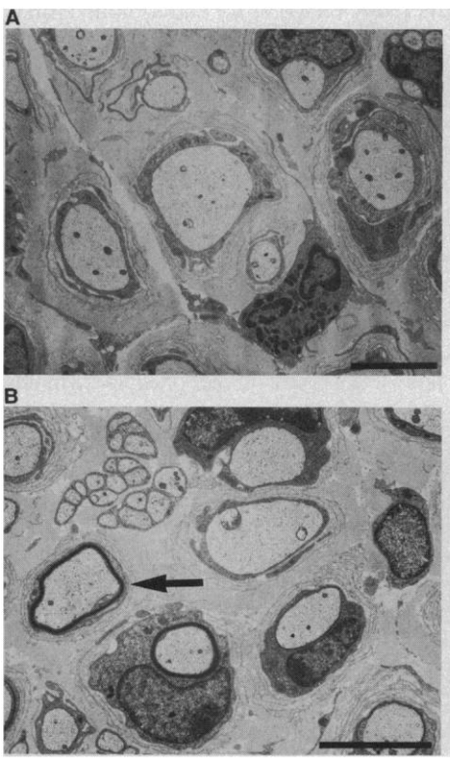


**Fig. 2.** Electron micrographs of transverse sections from sciatic nerves from 7-month-old *Tr vt*/*Tr vt* (**A**), *Tr vt*/+ + (**B**) and + +/+ ? (**C**) littermates. Some fibers are myelinated in (B) (arrow), but none are in (A). Individual axons have been ensheathed but not myelinated (promyelin fibers) in nerves from both mice. No such promyelin fibers are present in the nerve from the + +/+ ? littermate (C). Bars, 4 μm. After animal perfusion and tissue processing as in Fig. 1, ultrathin sections were cut and stained with lead citrate and uranyl acetate.

The marked difference in the number of myelinated fibers in nerves from *Tr vt*/*Tr vt* and *Tr vt*/+ + mice contrasted with their identical behavior. In the reverse relationship, the mild behavioral heterogeneity within the *Tr vt*/*Tr vt* class was not matched pathologically; the myelin deficit was strikingly uniform, such that Fig. 1A is representative of nerves from *Tr vt*/*Tr vt* mice at all ages examined.

Occasional PNS regions in *Tr vt*/*Tr vt* mice contained small patches of contiguous or nearly contiguous myelinated fibers. These regions were proximal segments of spinal roots and cranial nerves, almost always within 100 μm of the brainstem or spinal cord.

Qualitatively, the myelination defect was the same in mice of both *Tr vt*/*Tr vt* and *Tr vt*/+ + genotypes (Fig. 2). Defective fibers were blocked in their maturation at the "promyelin" (*14*) stage, at which Schwann cells have singled out and ensheathed individual axons, but have failed to proceed to the next step of forming myelin (Fig. 2, A and B). This qualitative defect has been described previously in *Tr*/+ mice (*15, 16*). Spinal cord and brain sections were normal in mice of all genotypes.

The fundamental question addressed by this study is whether myelin is essential in the PNS of the mouse. The ability of the trembler homozygotes to function fairly well and have a long life despite the virtual absence of myelin in the PNS casts doubt on the view that PNS myelin plays a vital role, at least in this species. Either of two interpretations would explain this seeming discrepancy between structure and function: PNS myelin may be a "luxury" item that is dispensable given the short conduction distances in mice or, alternatively, PNS myelin might be necessary in mice unless some robust form of compensation occurs.

The available evidence does not suggest a clear answer to the above question. The *Tr vt*/*Tr vt* data certainly suggest that PNS

myelination is superfluous in the mouse. Severe (but not total) CNS hypomyelination in quaking mutants allows long life (7). Rapid conduction along PNS pathways is not necessary in mice, since motor conduction velocity in *Tr/+* mice is less than 10% of normal (*17*). However, the early death of trembler-J homozygotes (*8*) and jimpy hemizygotes (7), which have extreme PNS and severe CNS hypomyelination, respectively, may be seen as evidence of myelin's utility. Either view of myelin's utility is compatible with the very different functional capacities of *Tr vt/Tr vt* and $Tr^J$ *vt*/$Tr^J$ *vt* mice in spite of their identical PNS appearances. Myelin could be dispensable but $Tr^J$ *vt*/$Tr^J$ *vt* mice might have a fatal lesion outside of the PNS; myelin could be necessary but *Tr vt/Tr vt* mice could have compensated for its lack, perhaps by redistribution of axonal membrane sodium channels (*18*). The results of failure to ever form myelin may be quite different from the effects of acute demyelination (*19*).

The few myelinated fibers in some cross sections of nerves from *Tr vt/Tr vt* mice are also of interest. It is unlikely that such myelinated fibers make any significant functional contribution, because they were less than 1% of the large-enough fibers in any nerve from any *Tr vt/Tr vt* mouse. Further, because different levels of the same sciatic nerves contained different numbers of myelinated fibers, it is unlikely that any one peripheral nerve fiber in a *Tr vt/Tr vt* mouse's nerve was myelinated completely along its length. It seems more likely that any single nerve fiber in a *Tr vt/Tr vt* mouse contained very few (if any) widely scattered myelin segments, each such segment originating from a single Schwann cell and corresponding to a single internode in a normal nerve. The small numbers of myelinated fibers in cross sections of very proximal spinal roots and cranial nerves, though extending such a short distance as to be of no functional importance, were a curiosity. That such fibers always occurred close to the spinal cord or brainstem and always occurred in clumps of contiguous or nearly contiguous fibers suggest, but do not prove, that this myelin was not of peripheral (Schwann cell) origin but was rather of central (oligodendrocyte) origin, as an irregular extension of spinal cord or brainstem myelin into the periphery.


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# Naturally Occurring Auxin Transport Regulators


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**The process of polar auxin transport, central to a plant's auxin relations, can be inhibited by a group of synthetic compounds that apparently act by binding to a plasma membrane protein known as the naphthylphthalamic acid (NPA) receptor. No endogenous ligand to the NPA receptor, capable of affecting polar auxin transport in plants, has yet been found. It is now shown that a group of flavonoids—including quercetin, apigenin, and kaempferol—can specifically compete with [$^3$H]NPA for binding to its receptor and can perturb auxin transport in a variety of plant tissues and transport systems in a manner closely paralleling the action of synthetic transport inhibitors. Because the active flavonoids are widely distributed in the plant kingdom and exert their effects at micromolar concentrations approximating likely endogenous levels, they may act as natural auxin transport regulators in plants.**


THE PLANT GROWTH SUBSTANCE indole-3-acetic acid (IAA; "auxin") must be transported basipetally from its sites of synthesis in shoot apices and young leaves to the subapical target tissues in which it exerts its many developmental effects. The current hypothesis describing the mechanism of polar auxin transport (PAT) [the "chemiosmotic hypothesis" (*1*)] includes $H^+$ gradient–driven cytoplasmic auxin accumulation by diffusion of lipophilic undissociated IAA molecules ($pK = 4.7$) (*1*) and by carrier-mediated cotransport of IAA anions and $H^+$ ions (*2*), and a transmembrane efflux of IAA anions on a carrier preferentially localized at the basal end of cells in the transport pathway (*1, 3, 4*). A group of synthetic compounds, exemplified by naphthylphthalmic acid (NPA), can inhibit PAT apparently by blocking the polar efflux step and causing a net IAA accumulation in transporting cells (*5*). These compounds (polar auxin transport inhibitors, or PATIS) do not directly compete with IAA but act through their own receptor, the NPA receptor, in plant cell plasma membranes [dissociation constant ($K_d$) values for NPA reported from 2 to 500 $\mu M$ (*6*)]. The NPA receptor and PATIS binding to it have been characterized (*7*), and the receptor's conformation inferred from structure-activity studies (*8*). Many PATIS compete for NPA binding with a specificity parallel to their effects on auxin transport. Yet despite the probable physiological significance of the NPA receptor, no endogenous ligands have been established. We now report that certain commonly occurring flavonoids, with specific structural requirements for activity, both inhibit auxin transport and compete for binding to the NPA receptor in the same manner as NPA in etiolated *Cucurbita pepo* L. hypocotyls and other plant shoot tissue.

We investigated phenolic compounds as PATIS because of earlier work (*9*) that recorded an increase in phenolics in tomato plants root-fed with sodium quinate, a carbohydrate precursor of phenylpropanoids and flavonoids. Such plants were dwarfed, with high levels of phenolics and reduced polar auxin movement from apical buds to roots. Their high IAA content was attributed to phenolic inhibition of both PAT and of IAA oxidase, which is antagonized in vitro by *o*-dihydric phenols, but stimulated by monohydric phenols (*10*).


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