Dendritic and axonal distribution of the microtubule-associated proteins MAP$_2$ and tau in the cerebellum of the nervous mutant mouse

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The fate of the different types of axons and dendrites in the nervous mutant mouse has been studied with antibodies raised against the two major microtubule-associated proteins, MAP$_2$ and tau. These proteins are specific markers of dendrites and axons, respectively. (1) Immunoblot analysis of cerebellar extracts showed that MAP$_2$ concentration is markedly reduced (by approximately 90%) in the adult mutant. A 60% decrease was already noticed at day 20 postnatal, i.e., when all the Purkinje cells are present in their normal location and in apparent normal number. (2) Immunohistochemical studies performed at an adult stage with anti MAP$_2$ antibodies showed marked alterations in the shape of the dendrites of the rare surviving Purkinje cells present in the lateral sections of the cerebellum of the mutant. In the vermis, where 50% of the cells survive in adulthood, the MAP$_2$ antibody revealed both clusters of cells with a normal density and an intricated and extensive pattern of dendritic arborization and isolated cells showing either an apparently normal or an altered dendritic tree. (3) At day 20 postnatal the same antibody revealed, in the lateral sections severe abnormalities of the dendrites of the Purkinje cells which were different from those seen in adulthood in the vermis. Thus, although few or any Purkinje cells are dead at this stage, a large proportion of them have already profound dendritic alterations. In contrast, in the vermis the Purkinje cells and their dendritic tree are undistinguishable at this stage from those of the unaffected normal mice. (4) Immunoblot and immunohistochemical studies performed with the anti Tau antibody suggested that the majority of the axonal fibers of the cerebellum were present both at day 20 postnatal and at later adult stages. This suggests that, although deprived of their postsynaptic targets these axons can survive for a long time after Purkinje cell death. However, an anti-neurofilament monoclonal antibody which stains specifically the axons of the basket cells, revealed an altered morphology of the basket cell nest in the regions devoid of Purkinje cells. (5) In conclusion the alterations in the morphology of dendrites seem to represent an early event of Purkinje cell degeneration and to be correlated with a marked decrease in expression of MAP$_2$. It remains unclear, however, whether such changes in expression of MAP$_2$ represent a primary effect of the mutation or if it is only a precocious result of Purkinje cell degeneration.

INTRODUCTION

The ‘nervous’ mouse mutant is characterized by the death of the majority of the Purkinje cells of its cerebellum: 90% of these macroneurons in the lateral hemispheres and more than 50% in the vermis disappear progressively from the third to the sixth postnatal week. Until day 23 postnatal it has been reported that the Purkinje cells are present in their normal location and in an apparent normal number. However, all the major classes of postsynaptic contacts have developed before the massive and selective degeneration of the Purkinje cells. For instance electron microscopic studies have shown that this was the case for both the parallel and the basket cell fibers. The conclusion was that, although deprived of their postsynaptic targets, the axons of these interneurons can survive for a long time after Purkinje cell death.

MAP$_2$ and tau, which are specific markers of the dendrites and the axons respectively, have been used in this work to label presynaptic and postsynaptic fibers of the nervous mutant mouse. MAP$_2$ is a high molecular weight microtubule-associated protein...
associated protein (280 kDa) which copolymerizes with the microtubules present both in the growing and adult brain dendrites. At immature stages MAP2 is composed both of a single high-molecular-weight component (MAP2b) and of a small immunologically related polypeptide of 65–70 kDa (MAP2c small MAP2). At mature stages small MAP2 disappears whereas two ‘big MAP2’, MAP2a and b are expressed. Differential expression of the microtubule-associated proteins tau has also been described; in adulthood tau is composed of several proteins with molecular weights ranging from 50 to 70 kDa whereas at immature stages only one component of 48 kDa is present. Juvenile and adult taus are specific of growing and stabilized axonal microtubules respectively.

In this work we have therefore used these two markers to study the fate of the different types of axons and dendrites in the cerebellum of the nervous mouse mutant. This was done at a stage, 20 days, at which both all the Purkinje cells and their axonal counterparts are considered to be present, and, at later stages characterized by massive Purkinje cell loss.

MATERIALS AND METHODS

Antigens, antisera and immunological detection of antigens using protein blotting

Adult rat brain microtubules were prepared by two cycles of polymerization–depolymerization. Thermostable MAPs were obtained by heating these microtubules 5 min at 100 °C in the presence of 0.75 M NaCl and 2 mM dithiothreitol, followed by centrifugation for 20 min at 20,000 g. Thermostable MAPs were purified by SDS polyacrylamide gel (4–15%) electrophoresis in the conditions previously described. Gel slices corresponding to the mature MAP2 and tau were homogenized in phosphate buffer, dialyzed and concentrated by the Amicon procedure. Controls of the purity of the eluted proteins were assayed by gel electrophoresis followed by silver staining.

The different purified antigens were injected initially subcutaneously to rabbits in complete Freund’s adjuvant with 3–4 booster injections made in complete adjuvant monthly. Bleedings were tested by immunoblotting according to Towbin et al. with several modifications. 125I-labeled donkey anti-rabbit Ig were used as second antibody. The blot was then autoradiographed on Amersham, Hyperfilm βMax. In most cases the antisera were affinity purified according to Krohne et al. The best results were obtained by transferring onto a nitrocellulose filter the contaminating antigen; for instance with the anti-tau sera, which slightly cross-reacted with MAP2, the diluted serum was incubated for 2–3 h at room temperature in the presence of a strip of nitrocellulose containing MAP2 transferred from a preparative gel. After this period of contact the serum was tested for its monospecificity towards tau protein and, if necessary, treated in the same conditions with another strip of MAP2-bound nitrocellulose. Preimmune sera were used in all experiments as controls and gave negative results when tested by immunoblot or immunocytochemistry.

Homogenates of mutant and control brain hemispheres and cerebellum were prepared as previously described and then centrifuged at 105,000 g for 1 h. Sodium dodecyl sulfate (2.3% final), beta mercaptoethanol (5% final) and sucrose (10% final) were added to the samples which were boiled for 5 min at 100 °C. Aliquots containing the same amount of protein (5 or 20 μg) were applied to SDS acrylamide gels and electrophoresis was carried out in the conditions described previously. Immunoblot analysis of the gels was then performed as indicated above. Relative densitometric quantitation was performed as previously described.

Tissue preparation and immunohistochemistry

Brains were removed from 20 days old and adult nervous mutant mice, kindly provided by Dr. Guenet and J. Formentin (Institut Pasteur, Paris). They were sagittally sliced and fixed overnight in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.6. Vibratome sections were washed in 0.5 M Tris-buffered saline, pH 7.6 and incubated for 30 min in 0.005% H2O2 in methanol to inhibit endogenous peroxidase. They were labeled using the peroxidase-antiperoxidase method. Antibodies were diluted in Tris-buffered saline with 1% normal goat serum and 1% Triton X-100. The peroxidase reaction was de-
veloped by 30 min of preincubation in 0.04% di-
aminobenzidine in 0.01 M imidazole in 0.05 M Tris,
pH 7.4 followed by incubation in the diaminobenzi-
dine solution with 0.015%
H2O2. The anti-neurofila-
ment monoclonal antibody35 (RT 97) was a gift of Dr
B.H. Anderton (Saint George's Hospital Medical
School, London); this antibody is spécifie of a phos-
phorylated epitope of the 200 kDa neurofilament
subunit.

RESULTS

Immunoblots of nervous brain extracts with anti-
MAP2 and anti-tau antibodies

Immunoblot analysis clearly show that prépara-
tions obtained from the adult (44 days) cerebellum
mutant (nr/nr) contain much less MAP2 immuno-
reactive material (Fig. 1A, lane 4) than the extracts
prepared from the heterozygote (+/nr) (Fig. 1, lane
3) or the wild (not shown) controls. In contrast céré-
bral extracts obtained from both the adult control
and the mutant seem to contain similar levels of
MAP2 immunoreactivity (Fig. 1, lanes 1,2).

Anti-tau antibodies detect in the cerebral extracts
3 closely spaced bands with différent intensifies (Fig.
1B, lanes 1,2). The distribution and the intensity of
the bands observed with the mutant and control cere-
bellar extracts (Fig. 1B, lanes 3,4) are different from
those seen with the cerebral préparations. Little or
no quantitative differences were observed between
the mutant and control préparations.

This data suggest therefore that in adulthood (1)
the expression of MAP2 but not that of tau is de-
creased in the cerebellum of the mutant; (2) there are
no changes in the concentration of these two markers
in the cerebrum.

Similar experiments were also performed with the
anti MAP2 antibody at other stages of development.
Fig. 2A shows for instance that the concentration of
MAP2 immunoreactivity decreases from day 20 to
day 75 postnatal in the cerebellar (Fig. 2, lanes 4–6)
but not in the cerebral extracts (lanes 1–3). Finally
when compared to the heterozygote (Fig. 2B, lane 2)
and the wild (lane 3) controls, the 20-day-old mutant
préparation (lane 1) seems to express lower levels of
MAP2 in the cerebellum, whereas the tau concentra-
tion seems to be identical in these 3 extracts (not
shown).

Densitometric analysis of the radioautographs al-
lowed a semi-quantitative estimation of MAP2. At
day 20 the values for three independent controls
were equal to 30.8–40.1 and 41.1 (arbitrary units).
Four assays with the mutants of the same âge gave
values ranging from 17–40% of the controls i.e. the

Fig. 1. Immunoblots of adult (44 days) nervous mutant (lanes
2,4) and heterozygote (lanes 1,3) extracts (5 txg protein) pre-
pared from the cerebrum (lanes 1,2) and the cerebellum (lanes
3,4). A: anti MAP2, B: anti-tau antibodies.

Fig. 2. Immunoblots with anti-MAP2 antibodies of cerebral (A,
lanes 1,2,3) and cerebellar (A, lanes 4,5,6 and B, lanes 1,2,3)
extracts (20 txg protein) prepared at different postnatal stages.
A: lanes 1,4: 20 days; lanes 2,5: 51 days; lanes 3,6: 75 days. B:
20-day-old mice: lane 1 nervous mutant; lane 2: heterozygote;
lane 3: wild.
decrease in MAP2 content averaged 65%. At later stages large variations were observed from experiment to experiment which were probably due to the fact that MAP2 was almost undetectable (more than 90% decrease).

Fig. 2A and B also show the presence of both MAP2a and b variants with probably and, as expected\textsuperscript{1,5} an increase in the proportion of MAP2b at adult stages compared to 20 day postnatal. Very small amounts of MAP2c were also still present at day 20 (Fig. 2B, lanes 2,3). However, because MAP2a and b are not sufficiently separated on the blot it has been impossible to estimate the quantitative changes in MAP2 variants. No immature tau variants\textsuperscript{11,15} were seen whatever the stage both in the mutant and the controls. According to these criteria, the nervous mutation does not seem to involve a retardation in the expression of the mature dendritic and axonal markers.

**Immunohistochemical labeling in the adult cerebellum of the nervous mouse; anti-MAP2 labeling**

At an adult stage anti-MAP2 antibodies stain different types of dendrites. In the central sections i.e. the vermis (Fig. 3) the dendritic tree of the Purkinje cells is clearly stained at all levels i.e. the dendritic trunk and the secondary and tertiary branches. However, according to the area of the vermis three different patterns of MAP2 positive Purkinje cells were observed: (1) a dense, intricated dendritic tree with an apparently normal cell density (Fig. 3A); (2) a decreased cell density with an apparently normally labeled dendritic tree of the surviving cells (Fig. 3B); (3) both a decrease in cell density and abnormalities of the dendritic shape and arborization (Fig. 3C), i.e. enlarged and irregular dendritic caliber, asymmetrical pattern of the secondary dendrites, reduced number of tertiary and terminal branching. In the molecular layer fine stained varicose fibers extending up to the surface are probably Golgi cell (Fig. 3) dendrites since some of them clearly originate from the granular layer. In this latter layer a dense network of labeled dendrites probably belongs in majority to the granule cells.

A different picture is seen in the lateral sections of the cerebellum i.e. in the hemispheres (Fig. 4). No labeling of the Purkinje cells is observed in most areas (Fig. 4A). Only rare Purkinje cells often

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Fig. 3. Sections of the cerebellar vermis in the sagittal plane of a 44-day-old nervous mutant mouse. Anti-MAP2 labeling showing 3 different patterns: (A) and (B) show a normal dendritic tree but differ in Purkinje cell densities; (C) shows abnormalities of the Purkinje cell dendrites. Arrows in (C) point on the differences in shapes and arborization of the secondary dendrites of a same Purkinje cell. P, Purkinje cell body; G, Golgi cell dendrites; GR, granule cell layer. Bar = 20 \( \mu \text{m} \).
grouped in small clusters with a dystrophic dendritic tree were scattered in the cortex of the hemispheres (Fig. 4B). The absence of the Purkinje cells also allows a better visualization of dendrites (Fig. 4) originating from the granular layer and extending up through the molecular layer (probably Golgi cell dendrites). The granular layer also contains a dense labeled network probably the granule cell dendrites. Thus, with the exception of the Purkinje cells, all the other types of dendrites seem to be equally well stained in the lateral sections compared to the control sections.

Anti-tau and antineurofilament labeling

Anti-tau antibodies stain (Fig. 5) in the molecular layer a dense array of fibers which are probably the parallel fibers of the granule cells. In the regions where the Purkinje cells are still present the parallel fibers appear as heavily stained bundles which are separated by the unstained dendrites of the Purkinje cells (Fig. 5A). In contrast in the lateral sections of the cerebellum the staining is compact with no obvious trace of the unstained Purkinje cell dendrites (Fig. 5B). Round unstained surfaces are present which correspond probably to the cell body of the basket cells in the lower molecular layer and, near the surface, of the stellate cells.

The layer where the Purkinje cells were located before their death is unstained by the anti-tau antibody. However, the shape of the cell body of the dead Purkinje cell can still be seen in this layer. This might correspond to the persistence of the perisomatic fibers of the basket cells which have been shown to survive after Purkinje cell death. Staining with an anti-neurofilament antibody which has been reported to label preferentially basket cell axons in the cerebellar cortex confirmed the survival of these fibers in their initial localization (Fig. 6). Moreover, the density and the staining of the fibers seem to be greater in the mutant than in the control. Since the antibody used stains phosphorylated epitopes of the neurofilaments it might be that such a difference re-

Fig. 4. Sections of the cerebellar hemispheres in the sagittal plane of a 44-day-old nervous mutant mouse. Anti-MAP1 labeling showing two areas: (A) without surviving Purkinje cells and (B) with a small cluster of surviving dystrophic Purkinje cells. Golgi cell dendrites originating from cell bodies localized in the granule layer are well seen in the molecular layer arrow heads. Bar = 20 μm.
Fig. 5. Sagittal sections of the cerebellum of a 44-day-old nervous mutant mouse. Anti-tau labeling: A, vermis: bundles of heavily stained parallel fibers run in between the unlabeled Purkinje cell dendrites; B, hemispheres: no unlabeled Purkinje cell dendrites are seen in between the heavily stained parallel fibers bundles. Round unlabeled surfaces in the molecular layer probably correspond to the cell bodies of basket and stellate cells (arrows). Arrowheads point on the unlabeled Purkinje cell dendrites. PA, parallel fibers. Bar = 20 μm.

Fig. 6. Sagittal sections of the cerebellar hemispheres in a 44-day-old nervous mutant (A) and a heterozygote control mouse (B). Anti-neurofilament labeling shows not only the persistence but an increased staining intensity of basket cell axons in the Purkinje cell layer in the nervous mouse. BA, basket cell axons. Bar = 20 μm.
Fig. 7. Sagittal sections of the cerebellar vermis of a 20-day-old heterozygote mouse (A,B) and nervous mutant (C,D) of the same strain. Anti-MAP₂ labeling (A,C) shows a similar well-developed Purkinje cell dendritic tree and varicose dendrites belonging to the Golgi cells (arrows). Anti-tau labeling (B,D) shows the punctate labeling of parallel fibers sections in between Purkinje cell dendrites which are unlabeled (arrow heads). Bar = 20 μm.
Fig. 8. Sagittal sections of the cerebellar hemispheres of a 20-day-old heterozygote mouse (A,B) and nervous mutant (C,D) of the same strain. Anti-MAP2 labeling (A,C) shows shorter, dilated, vertically oriented dendrites of the Purkinje cells with reduced branching and heavily stained patches (arrows) in the nervous (C) as compared to the heterozygote control (A). Anti-tau labeling (B,D) shows a similar distribution of the immunoreactivity among parallel fibers in the nervous (D) and the heterozygote control (B). Bar = 20 μm.
reflects under lying modifications in the structure and/or activity of the Basket cell axons. Finally anti-tau antibodies stain in the granule layer a fine network of fibers which might be the different types of axons present in this layer (climbing fibers, mossy fibers and glomeruli, axons of the Golgi cells, etc).

**Immunohistochemical labeling in the young nervous cerebellum**

Anti-MAP2 antibodies stain at day 20 postnatal the Purkinje cell dendrites both in the central (Fig. 7) and the lateral (Fig. 8) sections of the cerebellum. In the central sections the results are identical when one compares the labeling obtained both with the mutant (Fig. 7C) and the heterozygote control (Fig. 7A). In contrast the situation is different when one compares the lateral sections of the mutant (Fig. 8C) and of the control (Fig. 8A). In the mutant several abnormalities are clearly seen: shorter and dilated dendrites aligned vertically with severely reduced branching compared to the controls are present in more than approximately 1/3 of the Purkinje cells. Furthermore the intensity of the staining along the dendrites is uneven and large, heavily stained patches are observed.

Anti tau antibodies clearly stain at this stage and whatever the section, the parallel fibers (Figs. 7 and 8). However in the lateral sections (where several abnormalities of the dendrites of the Purkinje cells are observed) the unstained traces of the dendritic trees are less apparent than in the central sections.

Thus, at day 20 postnatal, immunostaining of the dendrites and the axons with anti-MAP2 and anti-tau respectively reveals several abnormalities in the lateral sections of the cerebellum which were not previously described. In contrast the sections of the vermis of 'nervous' are indistinguishable at this stage from those of the unaffected normal mice of the same age.

**DISCUSSION**

Labelling the lateral hemispheres of the cerebellum of the nervous mutant mice with an anti MAP2 antibody revealed that, at day 20 postnatal, a large proportion of the Purkinje cells present in the lateral hemispheres is already profoundly disorganized: anti MAP2 stained in many cells a large flat dendritic tree trunk with a paucity of labeled branches and processes; moreover, in this region, the label was unevenly distributed along the primary dendrite, large, heavily stained dots or patches being present along the neurites. This suggests that, in the lateral hemispheres a large proportion of the Purkinje cells are probably already near dying at day 20 postnatal although it has been reported that at this stage, the Purkinje cells are present in normal numbers. The decrease in MAP2 content, which was estimated by protein immunoblot to be approximately equal to 60%, might actually reflect either impaired activity of the altered cells or even the beginning of a necrotic process. The alterations in the shape and branching of the Purkinje cell dendritic tree are also more profound than suspected from previous light- or electron-microscopic examinations; for instance Landis reported that elaboration of the dendritic tree halts midway whereas anti MAP2 staining revealed much more profound alterations.

In contrast, at this stage, all the Purkinje cells present in the vermis are indistinguishable from those of unaffected normal mice.

Labelling with anti-MAP2 antibodies also confirms that almost all the Purkinje cells have disappeared in the lateral sections at adult stages; only small clusters of stained surviving cells are present and most of them have a marked altered shape of the dendritic tree which is similar to that seen at earlier stages. In contrast, in the vermis the situation appears to be different in several respects both from that seen in this region at day 20 postnatal and in the lateral sections at adult stages; three types of Purkinje cells were stained by the anti MAP2 antibody: (1) clusters of cells with a normal density and an intricate and extensive pattern of dendritic arborization; (2) isolated surviving cells showing either an apparently normal or (3) an altered dendritic tree. In this latter case the alterations seemed to be different from those seen in the lateral sections, the shape of the dendritic tree being maintained as well as a certain degree of arborization; it is not clear, however, whether this type of alteration is really different from that observed in the lateral sections or if it represents an earlier step of the same process of dendritic degeneration which would be slower in the vermis than in the lateral hemispheres.

MAP2 is both a marker of the dendrites and a promotor of microtubule polymerization.
In addition, several lines of evidence suggest that microtubules are important to define the shape and the stability of the dendrites and the axons (see ref. 27 for a review). The change in MAP\textsubscript{2} staining of the dendrites of the altered Purkinje cells of the nervous mutant mice might mean that either the expression or the transport of this protein is abnormal or that MAP\textsubscript{2} is dissociated from the microtubules and accumulates into the heavily stained areas. A decrease in expression, is suggested by the immunochemical evaluation of MAP\textsubscript{2}. The finding that MAP\textsubscript{2} is already decreased by 60% at day 20 postnatal i.e. when almost all the Purkinje cells are still present, would favour the idea that such a decrease is responsible for dendritic regression and finally cell death. Conversely, and more probably, the changes in MAP2 concentration might be a consequence rather than a cause of Purkinje cell degeneration which is already very important at day 20 as shown by the immunohistochemical data. The same type of question may be addressed to the observations made by Landis\textsuperscript{16} who detected early changes in the mitochondrial shape of the Purkinje cells of the nervous mutant: such a mitochondrial alteration might be a consequence and not a primary effect of the mutation.

It is not clear also whether the loss of MAP\textsubscript{2} can be entirely attributed to the Purkinje cell dendrite alterations. At an adult stage, in the large regions of the lateral hemispheres where almost all the Purkinje cells have disappeared, the anti MAP\textsubscript{2} antibody stains both thin varicose fibers, probably the dendrites of the Golgi, basket and stellate cells in the molecular layer and a dense network in the granular layer, probably the dendrites of the granule cells: the overall intensity of MAP\textsubscript{2} staining seems to be much lower in the molecular layer of the mutant than in that of the unaffected normal mice. Thus, although the relative contribution of each cell type to the total MAP\textsubscript{2} pool cannot be estimated precisely by the immunohistochemical method, the disappearance of almost all the Purkinje cells in the hemispheres might explain most if not all MAP\textsubscript{2} loss.

The presence in the lateral sections of the adult mutant mouse of surviving clusters of altered Purkinje cells and in the vermis of at least 3 categories of normal or altered cells differently stained by the anti-MAP\textsubscript{2} antibody might be related to the existence of subsets of cells differing in sensitivity to the effects of the nervous mutation. Several lines of evidence demonstrated two distinct subpopulations of Purkinje cells in the normal cerebellum\textsuperscript{6,13,17,24}. Recently Wassef et al.\textsuperscript{33} have shown a differential sensitivity of subsets of Purkinje cells to the nervous mutation which is topographically determined. For instance these authors observed that in the anterior vermis of control mice 3 thin sagittal bands are labeled by a monoclonal antibody (Q113) and that the nervous mutation affects specifically the survival of these cells. However, in other regions the cells labeled by the same antibody are not affected by the mutation suggesting a complex chemical heterogeneity of the Purkinje cells of the vermis; such an heterogeneity, which is also revealed by staining with anti-MAP\textsubscript{2} antibodies, might reflect the existence of Purkinje cell subpopulations differing in both their sensitivity to the mutation and in the stability of their dendrites.

The results of the immunoblots and of the immunohistochemical studies performed with the anti-tau and the anti-neurofilament antibodies also shed some light on the problem of the axonal survival in the cerebellum of the nervous mutant. The early ultrastructural studies of Landis\textsuperscript{16} showed that 'as nervous Purkinje cells are inadequately elaborating their dendritic components they receive all the major classes of synaptic contacts'. Sotelo and Triller\textsuperscript{20} also showed that, in spite of the important Purkinje cell loss, presynaptic elements belonging to the parallel and basket fibers persist long time after deprivation of their postsynaptic targets. Their conclusion was that the presynaptic fibers are stabilized, because they have been functioning, even if their postsynaptic partners have disappeared later on.

Our results are consistent with these conclusions: both the immunoblots and the immunohistochemical data show that tau immunoreactivity does not change whatever the stage and the region of the cerebellum with the exception that the parallel fibers appeared to be more densely packed in the areas devoid of Purkinje cells. However, labelling with a monoclonal anti-neurofilament antibody\textsuperscript{35}, which specifically stains the axons of the basket cells, revealed significant differences between the mutant and the unaffected mice cerebellum. In the zones devoid of Purkinje cells the axons of the basket cells are densely packed and stained whereas in the controls a well-defined nest delineates the Purkinje cell body. Thus,
not only a large majority of basket fibers, if not all of them, seems to survive in areas completely devoid of Purkinje cells but their density and staining seem to be increased in the mutant.

In conclusion the data reported in this work suggest that: (1) a marked decrease in expression of the microtubule-associated protein, MAP₂, and alterations in dendritic morphology are early and correlated events of Purkinje cell degeneration. It is not clear whether the altered morphology of dendrites is a primary effect or, more probably, a result of the mutation; (2) as judged by the expression of tau, a marker of different axonal fibers, the majority of the afferents seems to be established during development and stabilized in adulthood. In contrast, the morphology and the density of the basket cell fibers is altered in the regions devoid of Purkinje cells.

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