Cerebellar Defect and Impaired Motor Coordination in Mice Lacking Vimentin

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ABSTRACT Vimentin belongs to the family of intermediate filament (IF) proteins. During the nervous system development in mammals, it is transiently expressed in precursor cells of neuronal and glial lineages, and then it is progressively replaced by other types of IF proteins. Surprisingly, mice knock-out for vimentin develop and reproduce without any apparent defects (Colucci-Guyon et al. Cell 79:679–694, 1994). In adult rodents, Bergmann glia (BG) of the cerebellum continue to express vimentin together with glial fibrillary acidic protein (GFAP). A careful analysis of cerebellar morphology and ultrastructure in mutants showed poorly developed and highly abnormal BG, whereas the migration of granular neurons proceeded normally. Moreover, many Purkinje cells (PC) appeared stunted with a loss of spiny branchlets, and some of them were necrotic. Finally, impaired motor coordination was evidenced by behavioral tests. These observations demonstrate a role for vimentin in contributing to the normal development and morphology of BG and reveal a hitherto unreported functional relationship between BG and PC. GLIA 25:33–43, 1999.

INTRODUCTION

Vimentin, a cytoskeletal protein belonging to the family of intermediate filament (IF) protein, is expressed in the developing embryo in different types of precursor cells. During the development of the nervous system, it is transiently expressed in virtually all the precursors cells of both neuronal and glial lineages; when these cells differentiate, vimentin disappears and is replaced by two other types of IF proteins, the neurofilament protein in neurons and the glial fibrillary acidic protein (GFAP) in astrocytes (Lazarides, 1980). Thus, in the astroglial cell lineage, vimentin is the only IF protein expressed in radial glia and immature astrocytes in the embryonic nervous system. After birth, there is a dramatic increase in GFAP expression, which becomes the major IF of mature astrocytes in adulthood (Oblinger et al., 1993, for review). However, Bergmann glia (BG), a subset of astrocytes in cerebellar cortex, continue to express vimentin together with GFAP in adults, suggesting a functional role for vimentin in these cells (Shaw et al., 1981). Interestingly enough, in recently generated vimentin-null mutant mice (Colucci-Guyon et al., 1994), BG fail to assemble GFAP into filaments, which results in the total absence of IF network in these cells (Galou et al., 1996). The present study was undertaken to investigate whether the absence of vimentin impairs BG development and physiology and whether cerebellar organisation and function can consequently be affected. Indeed, recent studies (Liedtke et al., 1996; McCall et al., 1996; Shibuki et al., 1996) have evidenced structural and functional disturbances in GFAP null mutant mice. An abnormal organization of the white matter and an alteration in the blood–brain barrier were evidenced in aged animals, which led to late-onset CNS dysmyelin-
However, it should be noted that histological analysis, including Golgi staining, electron microscopy, and immunochemical staining, did not evidence any morphological abnormalities in BG of mice devoid of GFAP, except for the lack of GFAP network, and that these mutant mice did not present any motor impairment (Shibuki et al., 1996). In contrast, we show here that vimentin null mutant mice exhibit morphological defects of BG and PC cells, and present a severe equilibrium deficit and a disturbance in the coordination of actions. This strongly suggests that vimentin plays a crucial role in the structural support of BG, and that its absence in these cells in turn induces defects in cerebellar neuronal physiology.

**MATERIALS AND METHODS**

**Materials**

**Animals**

Vimentin-null mutant mice were generated as described previously (Colucci-Guyon et al., 1994). All experiments described here were performed on Vim1 knock-out mice. For morphological studies, mice bearing the Vim1 allele either on mixed genetic background or on the inbred 129/sv background were used. Behavioral tests were performed on mice with a mixed genetic background except for the rotating rod test, which was performed on two groups of animals: those with a mixed background and 129/sv inbred animals.

**Chemical products**

Glutaraldehyde, paraformaldehyde, silver nitrate, uranyl acetate, and toluidin blue were from Merck (Dornstadt, Germany); potassium dichromate was from Rectapur Prolabo, (Paris, France); osmium tetroxide was from Euromedex (Souffleuwersheim, France); and Araldite was from Fluka (Buchs, Switzerland).

**Light Microscopy**

Adult animals (3 months old) were perfused intracardially with 1% paraformaldehyde and 1% glutaraldehyde in 0.12 M phosphate buffer with 0.02 mM CaCl₂. After postfixation for 24 h in the same fixative, 50-µm vibratome sections of the cerebellum were processed for Nissl staining. For rapid Golgi, the multiple impregnation technique was used (Palay and Chan-Palay, 1974), and thick sections (200 µm) were then performed.

**Electron Microscopy**

For electron microscopy, animals were perfused intracardially with 1.5% glutaraldehyde and 1% paraformaldehyde in 0.12 M phosphate buffer. The cerebellum were removed, dissected, and postfixed in 1% osmium tetroxide in the same buffer. After inclusion in Araldite, semithin sections (1 µm) were stained with toluidin blue. Ultrathin sections (100 nm) were contrasted with uranyl acetate and examined with a Zeiss-900 electron microscope.

**Behavioral Tests**

Adult female mice were used at 9–15 weeks of age with an average weight of 21 g for mutant and 20 g for wild type.

**Open-field behavior**

The open-field was conducted as described (Maurice et al., 1995), in a 75-cm-diameter arena with a 25-cm-high wall, divided into 13 partitions. Each mouse was placed at the center, and its movements recorded on a videotape for 10 min.

**Rotarod test**

Animals were placed on a rotating rod (diameter 2.5 cm, length 15 cm, rotation speed 20 rpm), and the falling latency was measured up to 10 min.

**Spontaneous alternation behavior in the Y-maze**

Each arm is 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, converging with 120° angles (Maurice et al., 1995, 1996). Each mouse freely explored the maze during an 8-min session. The series of arm entries was recorded using an Apple IIe computer, and the number of arm entries and the percentage of correct alternations, defined as entries into all three arms on consecutive occasions, were calculated.

**Black/white exploration model**

Activity in the black/white exploration box was measured in a two-compartment open plexiglass box, 45 × 27 cm high, one, 15 × 27 cm, being black and the other being white and illuminated (60 W) (Hendrie et al., 1993). A small passage, 7.5 × 7.5 cm, was open between these two compartments. Each mouse was placed in the center of the white section, and its exploratory activity was recorded on a videotape for 5 min. The total time spent in the white section and the number of crossings through the passage were calculated.

**Place learning in the water-maze**

The procedure was conducted as detailed (Maurice et al., 1995, 1996). The water-maze consisted of a black
plexiglass rectangular pool, 30 x 60 x 36 cm high, filled with water at a height of 25 cm. Milk powder was used to render the water opaque, and water temperature was maintained at 26 ± 2°C by means of a bath heater. A transparent plexiglass platform, 5 x 8.5 cm, was fixed in one corner of the pool, 1 cm below the water surface. Each mouse was placed at the middle of the side of the pool, opposite the platform location, and the latency to find the platform was recorded. The mouse remained on the platform for 20 s. If it did not find the platform within 60 s, it was manually placed on it for 20 s. Each animal was allowed five trials on day 1 and three trials on day 2. Intertrial time interval was about 15 min. The starting position and platform locations did not change throughout all training sessions. On day 4, 48 h after the last training session and the last drug injections, the animals were tested for retention. The platform was removed. Each mouse was again placed at the starting position and observed for 60 s. The latency to reach the platform position and the time spent on it were recorded. Results were statistically analyzed using Student's t-test and Mann-Whitney's test.

RESULTS

Generation of Vimentin-Deficient Mice

Targeted mutagenesis of the vimentin gene by homologous recombination resulted in the generation of mice totally devoid of vimentin. Surprisingly, these vimentin-null mice appeared to develop and reproduce without an obvious phenotype (Colucci-Guyon et al., 1994). Furthermore, analysis of the IF content in these mice did not reveal any compensatory expression of another IF.

Morphological Abnormalities in Bergmann Glia and Purkinje Cells

Macroscopical examination of the cerebellum in adult animals showed no differences in size or gross anatomy between wild-type and vimentin-null mice (Fig. 1A).

In 10-day-old mutant mice, electron microscopy reveals a striking disturbance of the ultrastructure of BG in cerebellum. Cell bodies, located in the vicinity of Purkinje cells (PC), appear poorly differentiated with a lack of the ultrastructural nuclear and cytoplasmic characteristics found in wild-type animals. Apical processes appear stunted with a discontinuity of the glia limitans separating the external granular layer from pia mater (Fig. 2A), and a striking absence of the characteristic thin profiles isolating parallel fibers from PC dendrites and from other elements of molecular layer (Fig. 2C,D); similarly, vascular end-feet are poorly developed. However, the migration of external granular neurons seems to proceed normally (see Fujita, 1967, for review). We found bipolar neuroblasts migrating across the molecular layer (Fig. 2B), which, however, are for the most part not in contact with glial guides, because these guides are not present in the tissue (Fig. 2A,C,D).

In adult animals, most, but not all BG appeared hypertrophic with a massive thickening of the processes and a lack of the characteristic delicate appendages observed with the Golgi method on sagittal sections (Fig. 3B,C). This disposition was not homogenous throughout the cerebellum, and a large spectrum of abnormalities was observed. Nevertheless, the general geometry of the cells was maintained with processes abutting onto the pia. Electron microscopic examination revealed that BG fibers, which normally constitute a narrow cytoplasmic investment of PC dendrites (Fig. 4A), formed now large electron-lucent lakes, in which dendritic thorns and parallel fiber boutons appeared completely embedded. (Fig. 4B). Structural abnormalities were not limited to BG. Indeed, some PC were lacking (Fig. 1D) and the geometry of many other PC was markedly disturbed, as was seen on Golgi-impregnated sections. The dendritic tree of many cells appeared stunted with scarce dendritic thorns or in many instances smooth branchlets (Fig. 3A). In an effort to quantify these abnormalities, Nissl-stained vibratome sections were examined at high magnification. In some instances (Fig. 1C,D) the density of PC appeared reduced in the mutant mice; however, the high variability from one lobule to another, and from one animal to another, precluded any systematic quantitation.

With the electron microscope, affected PC showed retracted spiny branchlets with a paucity of dendritic thorns, and a corresponding loss of parallel fiber synapses (Fig. 4B). Around PC perikarya, synapses of basket axons were reduced in number, and most of the perikaryal surface was occupied by dystrophic BG processes (Fig. 5B). We also found scattered necrotic PC, which appeared on semithin sections as dark, pyknotic perikarya with a short dendritic stump (Fig. 5A). Careful survey with the electron microscope disclosed PC exhibiting early signs of degeneration, i.e., with a densification of the hyaloplasm, dilatation of rough endoplasmic reticulum (RER) and Golgi cisternae, and rarefaction of basket axon synapses (Fig. 5B).

In such instances, a few necrotic granule neurons were also found in the underlying granular layer, probably resulting from a degeneration “en cascade” (Fig. 5A). Other neuronal populations (basket-stellate) of the molecular layer evidenced various signs of degeneration in those areas where PC were most affected (data not shown).

In the cerebellar white matter, we did not detect any qualitative or quantitative abnormality other than a discrete rarefaction of astrocyte processes, which otherwise contained morphologically normal glial filaments.

In the oldest animals (14 months old), glial as well as neuronal abnormalities were qualitatively similar to those of young adults (Fig. 6). Because of the heterogeneous character of the lesions, it was very difficult to decide whether they were quantitatively more important than in young adults.
Impaired Motor Coordination

Vimentin-null mice do not demonstrate, at any age, gross motor deficits, such as tremor, ataxia, falling during locomotion, or even handicaps in climbing or rearing. However, in view of the various defects observed in the PC of mutant animals (and because PC are the only output element of the cerebellum), we decided to better explore the cerebellar function, using appropriate behavioral tests such as the general motility in an open-field test, the ability to remain on a rotating rod apparatus, the activity in the black/white exploration model, the spontaneous alternation in a non-aversive situation, and finally, place learning in a water-maze.

Spontaneous activity of vimentin-null mice recorded using an open-field test evidenced no spontaneous motor abnormality, in terms of latency to start exploring, numbers of rearings or groomings (Table 1A). However, compared to wild-type mice, vimentin-null mutant mice showed a significantly increased locomotion and a duration of immobility that was also slightly
Fig. 2. Development of BG and normal migration of external granule neurons in 10-day-old wild-type and vimentin-null mice. 

A: The glia limitans of a vimentin-null mouse is discontinuous, interrupted by the direct apposition of external granule cells onto the basement lamina (arrows). 

B: On a semithin section (1 µm) stained with toluidin blue, the molecular layer in a vimentin-null mouse contains many migrating bipolar neuroblasts (arrowheads). 

C: The molecular layer of a wild-type mouse exhibits the usual appearance of a neuropile composed of parallel fibers and dendritic profiles, some of which form synapses (arrowhead), between which astrocytic profiles occupy all the vacant volume (arrows). PCD, PC dendrite. 

D: The molecular layer of a vimentin-null mouse exhibits a neuropile where synapses are scarce, and where astrocytic profiles are almost absent. PCD, PC dendrite. Scale bars: A: 2 µm; B: 30 µm; C: 0.5 µm; D: 0.5 µm.
but non-significantly increased, resulting in an increased animal speed (Table 1A). Vimentin-null mice thus appeared hyperactive with respect to the wild-type mice. The number of rearings appeared non-significantly decreased, and the number of groomings was unchanged, confirming the lack of motor impairment. These observations point to disturbances in the coordination of actions rather than in the coordination of movements. Interestingly, the percentage of locomotion performed in the center circle of the arena was significantly decreased in mutant mice (Table 1A), suggesting an increased anxiety state in these animals. To further evaluate the motor coordination ability, we used the rotating rod task, which consisted in placing the animal on a rotating rod and measuring the falling latency during a 10-min test. This test revealed a dramatic difference between wild-type and mutant mice. When mice were put on the apparatus and the rod began to turn, wild-type mice stayed on the rod during all the observation time (Table 1B). In contrast, vimentin-null mice fell off very rapidly (Table 1B), showing a severe equilibrium deficit. Finally, convergent measures were obtained with a test specifically designed to assess the anxiety level of the animals: the black/white explora-

Fig. 3. Geometry of BG and PC illustrated with Golgi impregnation in adult wild-type and vimentin-null mice. A: In a vimentin-null animal, PC located in the center of the picture (arrows) exhibit a paucity of spiny branchlets and a disorganized dendrites geometry, at variance with those located to the right of the picture (light arrow), which appear normally organized. B: In a wild-type animal, a BG exhibits the typical chandelier architecture, with the usual fine appendages. C: In a vimentin-null animal, BG appear hypertrophic, with a massive thickening of processes. Scale bar: 100 µm.
Fig. 4. Ultrastructural examination of BG and PC dendrites in wild-type and vimentin-null adult mice. A: In a wild-type animal, the neuropile of the molecular layer exhibits the usual thin BG processes isolating neural profiles in the vicinity of a PC dendrite (PCD). B: In the molecular layer of a vimentin-null mouse, a PC dendrite (PCD) is surrounded by dilated astroglial profiles devoid of IF where dendritic thorns and parallel fiber boutons are embedded (arrows). Scale bars: A: 0.5 µm; B: 0.5 µm.
demonstrate that vimentin-null mice did not show the normal tendency to spontaneously alternate during the Y-maze exploration, which may be due either to a learning impairment, because spatial working memory is primordial for correct alternation, or to perturbations in the response strategies in the maze. Mnesic capacities were examined using place learning in the water-maze (Fig. 7). During acquisition sessions, both groups showed correct learning abilities, as the percentages of animals to criterion increased (Fig. 7A) and the latencies to find the platform decreased over trials (Fig. 7B). However, if the two groups performed similarly on day 2, marked differences appeared on day 1. Vimentin-null mice showed the lowest percentage of animals correctly avoiding the platform (Fig. 7A) and increased latencies to find it (Fig. 7B). These differences were not due to motor deficits, as swimming speeds were similar in both groups (22.5 ± 1.4 cm/s for wild-type mice vs. 20.1 ± 1.8 cm/s for mutant mice, P > .05). They are directly related to the disturbances already detected, hyperactivity and poor coordination of actions, which impeded the correct performance in the water-maze on the 1st training day. However, mnesic capacities were unaffected in mutant mice, as revealed during the 2nd training day. Indeed, vimentin-null mice appeared to have learned the platform location better than wild-type mice, because during the retention test performed 48 h after the last training, latency to swim on the platform location was significantly shortened (Fig. 7C), and the time spent on the platform location was significantly increased (Fig. 7D). These observations thus confirm that the reduced tendency to spontaneously alternate in the Y-maze is not related to impaired working memory, but rather to disturbances in the strategies, which retard the animals in mastering the task but enhance their learning in more difficult spatial tasks, such as place learning in the water-maze.

Taken together, these behavioral observations have evidenced not only a severe equilibrium deficit in vimentin-null mutant mice but also a marked hyperactivity and a general disturbance in the coordination of actions rather than at the execution of a single movement, resulting in an impaired motor coordination, an anxious-like behavior, and disturbances in spontaneous alternation. However, mnesic capacities appeared unaffected, if not improved.

DISCUSSION

In this study, we have used our recently generated vimentin-null mutant mice to investigate the involvement of vimentin in the development and function of cerebellum.

We have shown that, in the absence of vimentin, the architecture of BG failed to develop normally. Such results strongly suggest that vimentin plays a key role in the structural support of BG during development and in the adult. BG normally coexpress GFAP and vimentin in adult animals; however, in vimentin mu-

Fig. 5. PC in vimentin-null mice. A: On a semithin section (1 µm) stained with toluidin blue, some necrotic PC (arrow) can be seen distributed among other ones, which did not differ from those found in wild-type mice. Necrotic granule neurons can be seen in underlying granular layer (arrowheads). B: In the pycnotic perikaryon of a Purkinje neuron, the nucleus is retracted, the rough ER, and the Golgi are dilated, and the hyaloplasm appears densified. Synapses of basket axons are scanty on its surface (arrow), and the enlarged light profiles of surrounding BG are devoid of glial filaments. BG, Bergmann glia. Scale bars: A: 20 µm; B: 5 µm.
tant mice, we have recently shown that BG are completely devoid of any IF network. Thus, the absence of vimentin hinders GFAP assembly into a network (Gallou et al., 1996). In this context, it is interesting to note that mice lacking GFAP, which seem to express vimentin normally (Shibuki et al., 1996), do not exhibit the structural and functional defects that we have observed in mice lacking vimentin. This strongly suggests that vimentin has a specific role that cannot be taken over by GFAP. Conversely, the latter appears to play a

**TABLE 1.** Behaviors exhibited by mutant and wild-type mice in (A) the open-field test, (B) the rotarod test, (C) the black/white exploration model, and (D) spontaneous alternation performances in the Y-maze test.

<table>
<thead>
<tr>
<th>Behavioral parameter</th>
<th>Wild-type</th>
<th>Mutant</th>
</tr>
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<tbody>
<tr>
<td>(A) Open-field</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to depart(s)</td>
<td>11 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Locomotion (partitions)</td>
<td>111 ± 6</td>
<td>141 ± 10*</td>
</tr>
<tr>
<td>Immobility (s)</td>
<td>165 ± 17</td>
<td>202 ± 20</td>
</tr>
<tr>
<td>Speed (m/min)</td>
<td>2.81 ± 0.09</td>
<td>3.88 ± 0.15***</td>
</tr>
<tr>
<td>Rearings</td>
<td>35 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Groomings</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Locomotion in center (%)</td>
<td>33.0 ± 2.1</td>
<td>25.0 ± 1.3**</td>
</tr>
<tr>
<td>(B) Rotarod</td>
<td></td>
<td></td>
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<tr>
<td>Falling latency(s)</td>
<td>600 [600–600]</td>
<td>44 [26–600]†</td>
</tr>
<tr>
<td>(C) Black/white exploration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity in white section (%)</td>
<td>32.1 ± 3.5</td>
<td>25.1 ± 2.2*</td>
</tr>
<tr>
<td>Crossings between sections</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Time in white/crossing(s)</td>
<td>19.2 ± 1.4</td>
<td>12.9 ± 0.7***</td>
</tr>
<tr>
<td>(D) Spontaneous alternation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent alternation</td>
<td>64.9 ± 1.1</td>
<td>51.9 ± 2.6†</td>
</tr>
<tr>
<td>Number of arm entries</td>
<td>27 ± 1</td>
<td>21 ± 1†</td>
</tr>
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*Results are expressed as mean ± SEM, except in (B): median latency and interquartile range. *P < .05, **P < .01, ***P < .001, Student’s t-test; †P < .01, Mann-Whitney’s test; n = 11 for wild-type mice; n = 15 for mutant mice.

Fig. 6. Ultrastructural alterations in BG and PC of 14-month-old vimentin-null mice. A: In the molecular layer, dilated BG processes (arrows) devoid of IF are dispersed throughout the neuropile. B: Some PC dendritic thorns appear embedded in a large lucent BG profile (arrows), while another one forms a synapse with a parallel fiber bouton (arrowhead). Scale bars: A: 2 µm; B: 1 µm.

Fig. 7. Place learning in the water-maze test. Acquisition sessions (upper panel): A: Percentage of animals showing correct avoidance (which found the platform within 60 s and did not return to swimming). B: Median latency to reach the platform. Retention session platform (lower panel); C: Latency to reach the platform location. D: Time spent on platform. *P < .05, **P < .01, Mann-Whitney’s test), with n = 11 for wild-type and n = 15 for vimentin-null mice.
specific role in the maintenance of myelination and white matter architecture (Liedtke et al., 1996).

The present study contributes to the knowledge of interrelationship between BG and neurons in the developing and adult mouse cerebellum. Classical work on the weaver mutant led to the hypothesis that intact BG processes are mandatory for the inward migration of granule neurons during cerebellum development (Rakic and Sidman, 1973; Rakic et al., 1994). This view was later supported by Rezai and Yoon (1972), Hirano and Dembitzer (1973), and tentatively substantiated by the in vitro studies of Messer and Smith (1977). The biochemical nature of the interaction was later investigated by Lindner et al. (1986) and by the group of Hatten (Gasser and Hatten, 1990; Hatten and Mason, 1990; Fishell and Hatten, 1991). Specifically, the neural antigen astrotactin was found in vitro to constitute a neural receptor system for granule neuron migration along glial fibers. However, the hypothesis of necessary interaction between BG and granular cells has been challenged (Sotelo and Changeux, 1974; Das, 1974; Privat et al., 1979; Sotelo and Rio, 1980; Zagon et al., 1985). In the weaver mutant, the target of mutation was found to be the granule neuron itself rather than the BG, the latter being only secondarily affected as reactive glia.

In vimentin-null mutant mice, despite the marked disruption of BG development and the disturbance of the regular glial scaffolding normally present in young animals (Dupouey et al., 1985), granule cell migration does not seem to be impaired, thus substantiating the view that the apposition of granule neurons on intact BG processes is not essential for neuronal migration and suggesting also that the geometry of BG does not play a crucial role in directing that migration. Therefore, it could be hypothesized, as expressed earlier by Sotelo and Changeux (1974) and Privat et al. (1979), that this regular glial scaffolding offers a preferential axis for migration, whereas when this geometry is disturbed at the time of granule cell migration, pioneer migrating neurons can set up alternative axes, which are then used by followers.

The present study has also evidenced a specific pathology which develops in adult PC. Because vimentin is not expressed in adult PC, it is very unlikely that this pathology is a direct effect of the lack of vimentin, but it might primarily be attributable to the disruption of BG development and/or function. Stunting of PC dendrites and accumulation of membranous material have been found in many pathological conditions, such as malnutrition (Griffin et al., 1977), phenytoin therapy (Ghatak et al., 1976), and in pcd, nervous, and brindled mutant mice (Landis, 1973; Landis and Mullen, 1978; Yamano and Suzuki, 1985). However, in all these instances, it was assumed that PC were the target of the mutation or of the disease. In contrast, in the present case, it could be hypothesized that BG devoid of vimentin are unable to support the homeostasis of PC. Indeed, we cannot preclude which of the many crucial glial-neuronal interactions is disturbed in vimentin-null mice. In any event, the finding of a major functional deficit in young adult vimentin-null mice (impaired motor coordination), where many PC appear unaffected, favors the hypothesis of a permanent systemic abnormality of cerebellar neural function. The latter may be associated with an ionic disturbance, due to astrocyte dysfunction. However, many other causal relationships are also likely, given the intricate ionic and metabolic relationship between neurons and astrocytes. In this context, it is interesting to note that a similar functional deficit (impaired motor coordination) was observed in cerebellar X-ray-irradiated rats (Pellegriino and Altman, 1979), in which the structural abnormalities are very different from those found in vimentin-null mice. In addition, it is worth noting that the absence of a GAFP network evidenced in vimentin-deficient mice (Galou et al., 1996) is not, per se, responsible for the present phenotype, as mice lacking GFAP were devoid of major gross neurologic, behavioral, or structural CNS abnormality (Gomi et al., 1995; Pekny et al., 1995; McCall et al., 1996; ShibukI et al., 1996) with the exception of the late onset of white matter disturbance (Liedtke et al., 1996).

Recently, impaired motor coordination has been described in several gene knock-out mice, in which the inactivated gene was related either to a glutamate receptor or to a transduction system possibly involving calcium homeostasis. PKCy mutant mice exhibit an impaired motor coordination, but are fully capable of motor learning (Chen et al., 1995). The only structural abnormalities found in these mutants was the persistence of climbing fiber multiple innervation (Kano et al., 1995). Similarly, mice devoid of the NR2A and NR2C components of the NMDA receptor showed no impaired movement in the motor coordination, but fell off a rotating rod (Kadotani et al., 1996). Finally, mice lacking the GluR2 subunit of the glutamate receptor (Kashiwabuchi et al., 1995) were found to be devoid of structural abnormalities, except for the presence of non-synaptic spines surrounded by large sheets of BG. These mice exhibited moderate locomotor defects and a major deficit on the rotarod test. All these mutations focus upon PC function either through the parallel fiber input, or climbing fiber, or both. Comparison with vimentin mutant mice points to the role of BG in supporting the homeostasis of PC function, possibly through ionic control.

Finally, in the context of this study, very recent results should be recalled. A massive loss of PC has been described in aged mice lacking the prion protein PrP. PrP is expressed in both astrocytes and PC and exhibits a strong affinity for GFAP (DeArmond et al., 1987; Oesch et al., 1990; Sakaguchi et al., 1996). Although the loss of PC in vimentin mutant mice is moderate and takes place earlier in life, there remains the intriguing possibility that the effect of BG on PC which we have revealed involves PrP in some way. Thus, it would be interesting to generate mice bearing both PrP and vimentin null mutations and to monitor the loss of PC in these mice.
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