Molecular Neurobiology

ISSN0893-7648/03/28(1): 65–106/$25.00

The Wobbler Mouse

A Neurodegeneration Jigsaw Puzzle

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Abstract

Various mutations in humans and animals lead to the selective and progressive degeneration of motoneurons, resulting in muscular weakness, subsequent paralysis, and death *(1–3)*. Amyotrophic lateral sclerosis (ALS) is the most common adult human motoneuron disease, but the vast majority of sporadic and familial cases of ALS are still of unknown origin *(4)*. Murine models of motoneuron diseases, derived from spontaneous mutations in the colonies, have been known for half a century. Prior to the first identifications of the mutated proteins in human ALS, they have largely been used to explore the disease etiology. The chromosomal localization of these mutations does not favor a genetic similarity between these murine models and the few human forms of the disease for which the mutation or the chromosomal localization is known. Yet the fact that most human ALS cases are of unknown etiology and the recent discovery of molecules with no known role in motoneuron survival *(5–7)*, indicate that these murine mutants may still contribute to the understanding of motoneuronal degenerative processes. This can be exemplified by the work performed on the wobbler mouse, one of the oldest and most extensively studied models, which is reviewed here.

Index Entries: motoneuron; amyotrophic lateral sclerosis; neurodegeneration; astrocyte; microglia; gliosis.

Introduction

Falconer originally described wobbler *(8)* as a mutation that appeared spontaneously in the C57BL/Fa strain and was associated with a wobbly gait characteristic of the mice. The appearance of the first clinical signs of the mutation in juvenile mice, linked to the observation of motoneuronal degeneration in the spinal cord and the brainstem, has first led to consider the wobbler mouse as a model of spinal muscular atrophy (SMA). The identification of the mutated Survival Motor Neuron

Received 12/23/02; Accepted 3/21/03
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gene in SMA patients *(9)* and the discovery of degeneration markers in cortical neurons of the wobbler brain *(10)* have now prompted researchers to reconsider the wobbler mouse as a model that is closer to ALS than to SMA. Until now, the identity of the mutation has been elusive. In contrast, very diversified explorations of the morphological and metabolic alterations that take place in the wobbler mouse have been carried out, facilitated by the progressive nature the wobbler neurodegenerative process. As in humans, motoneuronal death in the wobbler is not a rapid, sudden event, but extends over time. The wobbler model has thus allowed investigators to explore the course of neurodegeneration at the clinical, cellular, and molecular levels. This revealed, in particular, that motoneuron degeneration is accompanied by complex modifications of the cellular environment. The main etiopathological hypotheses that have been proposed over the years as a source of neurodegeneration—excitotoxicity, defective free radical detoxification pathways, and accumulation of toxic protein aggregates—have been approached in the wobbler model, and the results have pointed to defects common to other types of motoneuron degeneration as well as other neurodegenerative illnesses.

After a short presentation of the clinical syndrome and the current state of knowledge about the mutation, this article will review cellular and molecular data on motoneuronal degeneration according to the chronology of disease progression. This process can be subdivided into three successive phases: presymptomatic, evolutionary, and stabilization. The diverse etiopathological hypotheses are discussed when appropriate.

The Wobbler Mutation

Chromosomal Mapping of the Wobbler Locus

The first genetic data provided by Falconer *(8),* based on the statistical analysis of the wobbler strain progeny, showed that the mutated gene responsible for the disease was autosomal, recessive, and apparently unique. The wobbler karyotype did not reveal any visible sign of chromosomal deletion or translocation *(11).*

The wobbler gene is localized on chromosome 11 *(12–15)* (Fig. 1). The region of interest, identified by recombination, presently covers 4 Mb, corresponding to a distance of 1.2 ± 0.9 CM *(14),* and presents a conserved synteny on human chromosome 2p13–14 *(16).* All genes localized in the locus are believed to be identified *(16),* but all candidate genes for the wobbler mutation have been excluded. The levels of the transcripts of otx1, Hcc8, Homoloc13, Ugp2, Kiaa0903, PeliI, Homoloc2 are the same in wobbler mice and in wild-type controls *(16,23).* Sequence analysis of the cDNA and part of the 3′ and 5′ untranslated repeats of Hcc8, Ugp2, Kiaa0903, and Homoloc13 did not reveal a mutation *(16).* The activity of cytoplasmic malate dehydrogenase, encoded by Mor2, is similar in wobbler and wild-type mice *(23).* In addition, a knockout mouse for Otx1, a homeobox gene that encodes a protein required for the development of cortical neurons, presents an epileptic phenotype that is unrelated to the wobbler one *(21).* These results raise the possibility that the wobbler mutation involves a noncoding sequence that could be localized outside the wobbler region, as defined by recombination *(16).*

Genetic Diagnosis and Genetic Modifiers of the Wobbler Gene

The genetic mapping of the wobbler mutation has led to the development of hybrid mice that allow a genetic diagnosis of the disease *(24,25).* These hybrids were obtained by breeding C57BL/6 mice carrying the wobbler mutation with mice of the NZB strain that have a gln-ps1 pseudo-gene longer than the one in the C57BL/6 strain. The polymorphism of this pseudo-gene, which is localized at 1 CM of the wobbler mutation, allows mutant mice to be identified *(24,25).* (Fig. 1). More recently, an intra-strain polymorphism in one of the Cct4

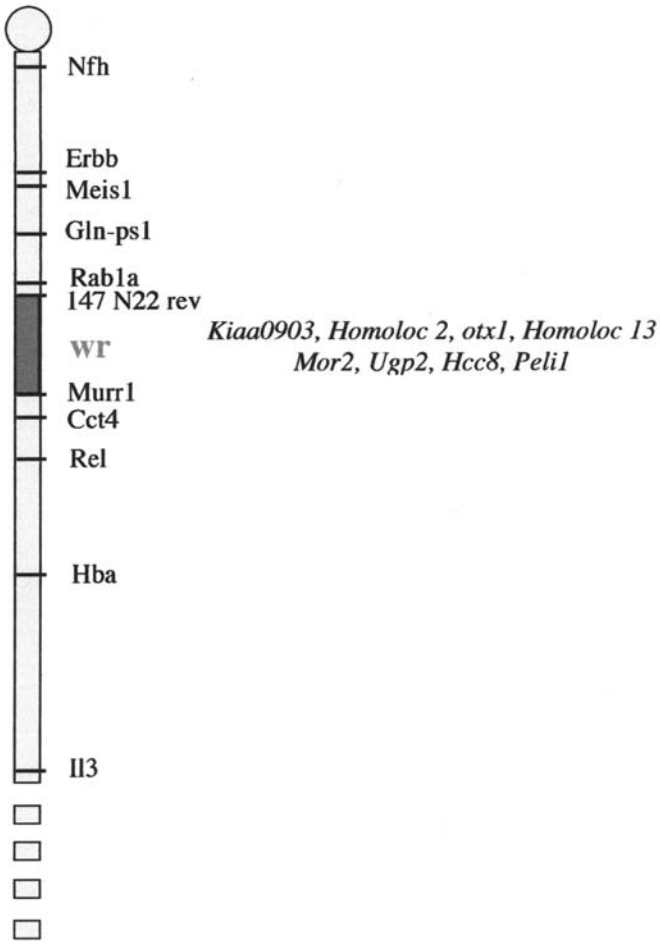

Fig. 1. Schematic representation of the wobbler locus (dark gray box) on the murine chromosome 11. Adapted from Fuchs et al. (2002) *(16).* The genes positioned in the region of interest are noted in italic. 147 N22 rev and Murr1 (for "the gene that locates in the mouse U2af1-rs1 region") *(17)* are the closest markers currently known. Gln-ps1 and Cct4 are the polymorphic genes that allow the identification of the wobbler allele by genotyping. The genes localized in the wobbler locus correspond to Ugp2, which in humans encodes a muscle isoform of UDP-glucose pyrophosphorylase *(18),* peli1 (Pelle adaptator protein Pellino), believed to be a protein adaptor involved in signal-transduction pathways *(19,20),* Otx1, a homeobox gene involved in the control of brain development *(21),* Hcc8, identified as a hepatocellular carcinoma antigen *(22),* mor2, which encodes cytoplasmic malate dehydrogenase, and Homoloc13, Kiaa0903, and Homoloc2 of unknown functions.

alleles has been discovered, which permits a genetic diagnosis of the mutation in the original wobbler strain *(26)* (Fig. 1).

The development of hybrids has revealed the existence of genetic modifiers of the wobbler phenotype. The breed of wobbler mice from the C57BL/6J strain with *Mus Spretus* mice can lead to a heterogeneous phenotype that corresponds to variations in the weight loss and severity of the neurological symptoms *(12).* In addition, the breed of wobbler C57BL/6J mice with mice from the *Mus Castaneus* strain exacerbates the wobbler neurological symptoms. In this case, segregation analysis showed a localization of the modifier gene locus on chromosome 14 *(27).* The hybrids resulting from the breed of the C57BL/6J that carry the wobbler mutation and the NZB strain showed either the same symptoms as the original wobbler strain *(24)* or slightly exacerbated ones *(25).* In accordance with the existence of genetic modifiers of the wobbler gene, differences in the time-course of the disease have been reported between the available wobbler strains, and other variations exist, including those at the molecular level. It is thus important to keep in mind that strains of wobbler mice are not identical from one laboratory to another, because of the use of different hybrids or the breeding of local lines by each laboratory, even when using the original strain.

Evidence for a Cell-Autonomous Action of the Wobbler Mutation

The association of the wobbler neurodegenerative syndrome with male sterility *(28,29)* has raised the possibility that the wobbler mutation corresponds to a humoral factor that is able to affect different organs. To explore this hypothesis, Augustin et al. *(30)* obtained two chimeras by aggregation of wild-type and wobbler embryos. Although clinically normal, these chimeras presented scattered degenerating motoneurons in the spinal cord and defective spermatozoa in the testis *(30).* These results indicate that the mutation acts at the level of the affected cells themselves.

Our unpublished observation of organotypic cultures of 7-d-old wobbler and wild-type spinal cords also support a cell-autonomous effect of the mutation. Such cultures can be maintained over a period of several weeks, allowing the degenerative process to take place, and can preserve the complexity of the tissue. Nine-to-twelve slices were obtained from each spinal cord and cultured following the method of Stoppini and colleagues *(31).* After 6 wk in culture, the slices were fixed and the motoneurons were identified by their immunoreactivity for the motoneuron marker choline acetyltransferase (ChAT). Analysis of the ChAT-immunoreactive neurons localized in the ventral horn revealed higher cell-surface areas in the wobbler slices than in the controls (Fig. 2). This result is consistent with the in vivo occurrence of degenerating motoneurons that present a marked swelling *(32–34).* As previously observed in rat spinal cord slices *(35),* the number of ChAT-immunoreactive motoneurons per slice was highly variable (2–52 in controls, 1–30 in wobblers). To reveal a reduction of motoneuron numbers in wobbler slices, the cumulative distribution of the number of motoneurons per slice in controls and wobblers was compared. This analysis showed a statistically significant 30% reduction in motoneuron numbers per slice of wobbler vs control spinal cords (Fig. 2), confirming that motoneuron degeneration induced by the wobbler mutation may be maintained in isolated cervical spinal cord slices. Associated with the observations on chimerical mice, these findings indicate that the wobbler mutation does not affect the humoral environment of the spinal cord cells.

Wobbler: A Progressive Neurodegenerative Disease Associated With a Defect in the Reproductive System

In the mice that are homozygous for the mutation (wr/wr), the first motor defects appear at the juvenile stages (e.g., at 3–4 wk of age) and stabilize in older animals (e.g., 3–5 mo of age). Although the wobbler mice are first known for their neurological syndrome, the males also suffer from infertility *(29).* The mice heterozygous for the mutation present no sign of the wobbler disease during their entire life span.

Wobbler Males Are Sterile

Wobbler males are unable to reproduce, despite the normal appearance of their genital apparatus and normal levels of testosterone *(28,29).* However, there is a 20–30% reduction in the number of spermatids in the testis *(29),* and only a small number of motile spermatozoa in the sperm of wobbler mice *(28).* This defect appears to be linked to morphological abnormalities in the spermatozoa tail. The spermatozoa tail comprises an axoneme composed of doublets of contractile microtubules. In wobbler male mice, a large percentage of spermatozoa lack the normal amount of microtubule doublets *(28).* As the microtubules confer their contractile property to the spermatozoa tail, the development of abnormal axonemes, which is aggravated during the maturation of spermatozoa, may explain the weak motility of the wobbler spermatozoa *(28).* The other main defect of wobbler spermatozoa is the lack of a real acrosome that normally appears during the maturation of the spermatozoa *(29).* In wobbler mice, the acrosome does not form a cap around the nucleus, but rather remains as vesicles in the cytoplasm *(29).* As a result, the spermatozoa head displays a rounded instead of a crescent shape. Until a recent study on the expression of the heat-shock protein of 40 kDa, Msj-1 (Mouse sperm DnaJ first homolog) in the testis of wobbler mice *(36),* no molecular defect had been associated with these reproductive abnormalities. Initial studies indicating that Msj-1 expression increases during the course of the spermatogenesis, associated with the localization of the protein in a peri-acrosomal position in the mouse spermatozoa *(36–38),* were the

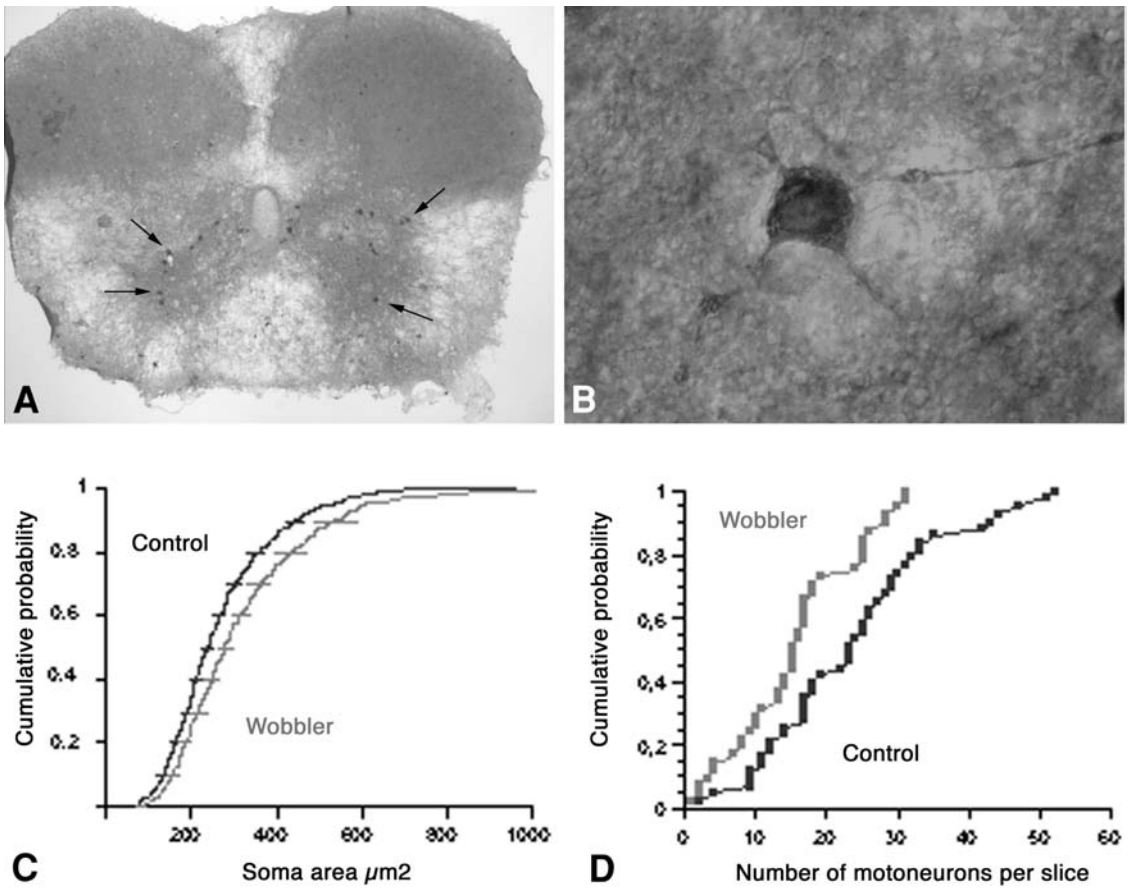

Fig. 2. Organotypic cultures of wobbler cervical spinal cord slices. **(A)** and **(B):** ChAT-immunoreactive labeling. **A** General view of a cervical spinal cord slice obtained from a 7-d-old control pup after 6 wk of culture. Note the ChAT-immunoreactive cells localized in the ventral horn of the spinal cord (arrows). **B** High-power view of a ChAT-immunoreactive neuron of the ventral horn displaying the large multipolar cell body characteristic of the motoneurons. Bar = 500 µm in **A,** and 20 µm in **B (C).** Analysis of the cumulative distribution of the soma size of ChAT-immunoreactive motoneurons in slices of wobbler (gray line) and control (black line) cervical spinal cord slices after 6 wk in culture. The slices were obtained from 7-d-old wobbler and wild-type pups. Note the shift to the right of the wobbler distribution, marking the presence of motoneurons displaying higher soma-cell sizes in the mutant than in the control (Kolmogorov-Smirnov test, $p = 0.01$, four animals per group, two independent experiments). **D** Analysis of the cumulative distribution of the number of ChAT-immunoreactive motoneurons per slice in the wobbler (gray line) and the control (black line) groups. Note the shift to the left of the wobbler distribution, indicating that the number of motoneurons detected in wobbler slices is reduced as compared to the control slices (Kolmogorov-Smirnov test, $p = 0.05$, four animals per group, two independent experiments). Methods: ChAT immunohistochemistry was performed using a polyclonal antibody (AB144P, Chemicon, Euromedex, France, 1:800 in PBS Triton 1%). Detection of the immunohistochemical signal was achieved using the avidin-biotin complex immunoperoxidase technique (1: 300, Vector) and diaminobenzindine as a chromogene. The analyses of the number, the soma area, and the maximal diameter of ChAT-immunoreactive neurons were performed using a Leica DMIRB microscope (Wetzlar, Germany) and an image analysis software (Starwise Morphostar, IMSTAR, France) on all ventral horn ChAT-immunoreactive neurons throughout the whole thickness of each slice.

origin of this work. The finding that expression levels of Msj-1 are downregulated in wobbler testis as compared to controls *(36)* provides a first clue to the identification of the molecular abnormalities underlying the inappropriate maturation of the spermatozoa in the wobbler testis, although the participation of Msj-1 to the establishment of the acrosome has not yet been defined.

In wobbler females, no pregnancy in mutant mice has been reported to date, but to our knowledge, no study has been undertaken to explain this infertility. The wobbler mutation may affect the female gametogenesis, but the muscular weakness of the mutants and a consequential change in sexual behavior could easily impair the mating of these mice.

The Clinical Evolution of the Neurological Syndrome is Progressive

More than 30 years ago, Duchen and colleagues *(33)* provided a heavily detailed description of the clinical symptoms, which is still in use. These clinical symptoms—which primarily result from the progressive loss of cervical spinal motoneurons, and to a lesser extent of brainstem motoneurons—can be divided into three phases. The presymptomatic phase comprises the first 3 wk of postnatal life, during which the mice that carry the mutation do not present clinical signs of the disease. This is followed by the evolutionary phase, when the symptoms develop—a period that lasts until the second to third month of life. Then there is a stabilization of the clinical symptoms.

Despite its denomination, the presymptomatic phase may correspond to a subtle alteration of motor functions. The NFR/wr line displays an anomaly in the righting reflex at three to seven postnatal days of age *(39),* and an impaired performance during grid walking at 2 wk of age *(40).* In the other hybrids or the original wobbler strain, no clinical sign of the disease has been reported in the mice that are homozygous for the wobbler mutation prior to 3 wk of age.

From the third to the fourth week, wobbler mice are 40–50% smaller than their control littermates, and remain smaller thereafter *(11,40,41)* (Fig. 3A) This reduction in size results partially from nutritional deprivation, probably linked to muscle atrophy in the face and forelimbs, since drop feeding of the mice compensates for some of the weight loss, although it does not modify other aspects of the disease *(40).* Assisted feeding does not reduce the loss in the weight of the forelimb muscles and of the spinal cord observed in wobbler mice, which remain 50% and 30% inferior to the normal weight ranges, respectively *(40).* At this early stage of the disease, the mutant mice present an unsteady gait with a discrete head tremor. Thereafter, instability and wobbling of the gait develop progressively. The muscular strength is diminished in the forelimbs at 3 wk of age *(25,42).* Muscular tension is affected at 4 wk, and decreases instead of increasing with age as in control mice *(43).* The muscular weakness in the forelimbs is visible at the fourth to fifth wk of age. The capability of the fore paws to cling is particularly affected (Fig. 3C). The weakness of the muscles of the forelimbs, the head, and the neck worsens over this period, and the hind limbs remain marginally affected until the 12th week of life. The wobbler mice become progressively unable to extend their forelimbs at their wrists, leading to difficulty in climbing and walking. Muscle atrophy in the face results in a muzzle with a pointed appearance (Fig. 3D). When the mouse is suspended by the tail, the hind limbs are flexed instead of extended (Fig. 3B). At the end of the evolutionary period, in 3-mo-old mice, the electromyographic recordings of the forelimbs muscles display characteristics of muscular denervation. In contrast, the hind limb muscles are electromyographically normal *(11).* The evolution of the motor syndrome is progressive in most animals, but some of them exhibit a stepwise evolution *(33).* Such heterogeneity within a particular colony may be aggravated by the crossing of the original strain with other mouse strains, resulting in modified time-courses in the development of the neurological symptoms *(12,27).* These inter-individual variations extend to the life span: some mice survive up to 1 yr, and others present a more severe disease and

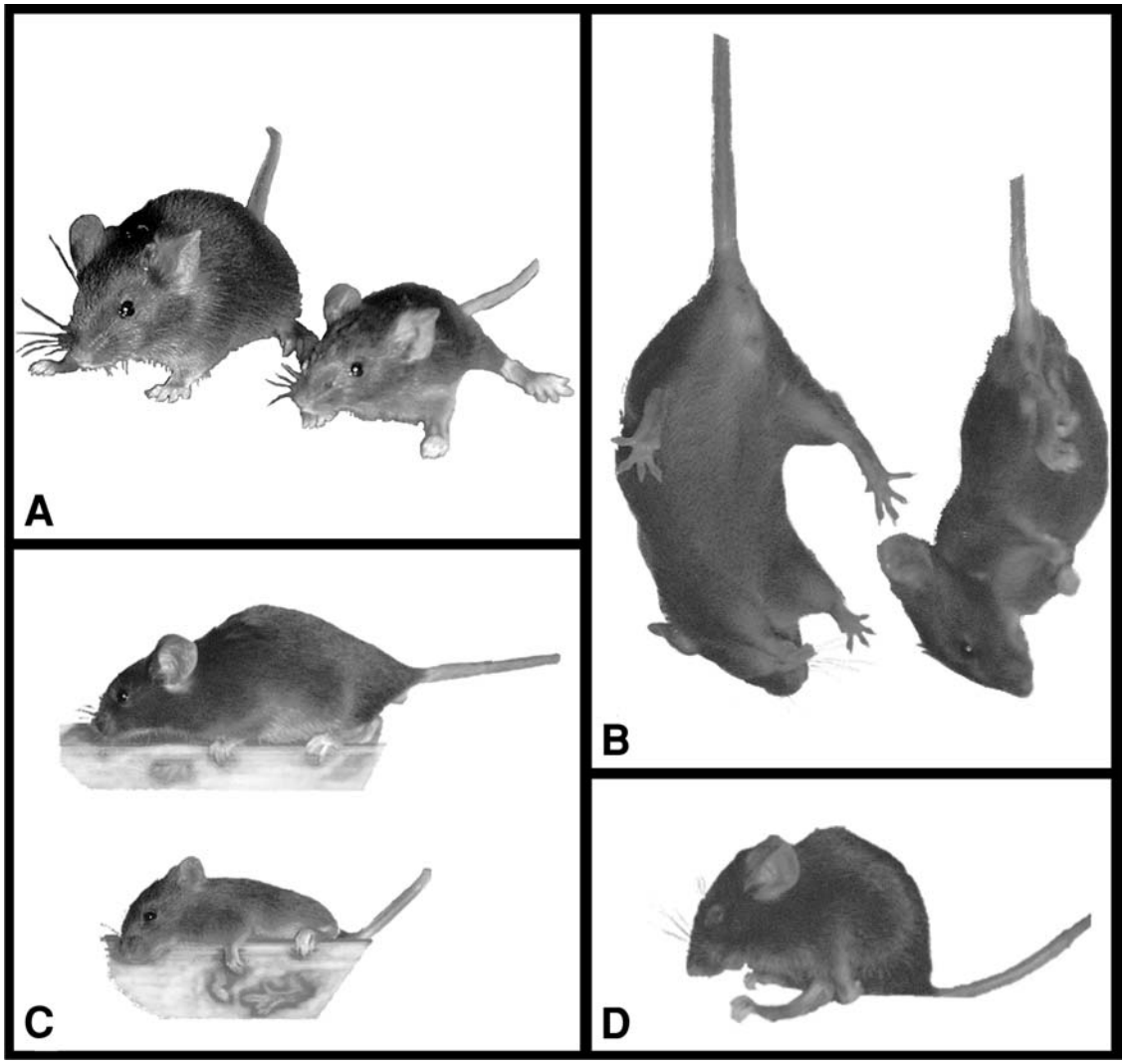

Fig. 3. The wobbler mouse. **(A)** Two litter mates during the evolutionary phase of the disease—on the left, a control mouse and on the right, a wobbler mouse. Note the difference in size. **(B)** Same mice as in **A,** suspended by the tail. Note that the wobbler mouse (on the right) folds up its hind limbs, whereas the control mouse (on the left) spreads them. **(C):** Same mice as in **A** and **B,** subjected to the test of walk on the edge of a cage. The control mouse (top) clings perfectly on the edge of the cage, whereas the wobbler mouse (bottom) is not able to hang on with its forelimbs. **(D)** An aged wobbler mouse, during the stabilization phase of the disease, presenting the folding up of the wrists of the forelimbs and the "pointed" muzzle that is characteristic of the disease.

die between the third and the fourth month *(33)*, or even the third and the fourth week, as in the NEW strain *(24)*. The longest lifespan reported has been 16 mo *(11)*.

Various histological and molecular alterations of the spinal cord cells cause these clinical symptoms. During the presymptomatic phase, cellular morphological variations are subtle, but variation in molecular expressions is already established. During the evolutionary phase, this worsens, and modifications of the neuronal and glial environment of the affected

motoneurons occur. The phase of clinical stabilization corresponds to an arrest of the degeneration process.

Subtle Morphological Changes and Marked Molecular Alterations Characterize the Presymptomatic Phase

Most histological and molecular studies of the presymptomatic phase have focused on the cervical spinal cord of the mutant, which is the central nervous system (CNS) region most affected by the degenerative process. However, it is important to remember that approx 50% of the cervical spinal cord motoneurons survive the wobbler degenerative process, but that reciprocally, the wobbler mutation also affects motoneurons in the brainstem and other neurons in the cerebral cortex.

Morphological Alteration of the Motoneuron Soma Precedes Axonal Anomalies

Cytoplasmic vacuolization is the histological landmark of the wobbler degenerative process in spinal motoneurons *(33,44)*, but this spectacular change in motoneuron structure is rare in the spinal cord prior to the evolutionary phase of the disease. During the presymptomatic stage, morphological alteration of neurons is more subtle. Affected motoneurons are primarily characterized by an enlarged soma and a weak labeling of Nissl bodies *(33,45)*. Stereological measurements have confirmed the enlargement of motoneuron perikaryon in the cervical spinal cord of 18- to 22-d-old wobbler mice *(32)*. At this age, cells with diverse anomalies have also been reported in the brainstem, the ventral reticular magnocellular nucleus, and the motor nuclei of the cranial nerve V and VII. In contrast, abnormal neurons were rarely found in the thoracic and lumbar spinal cord, the red nucleus, the substantia nigra, the anterior and posterior colliculi, and the deep nuclei of the cerebellum *(33)*. No anomaly was reported at this stage in the cerebral cortex *(33)*. Rare cytoplasmic vacuolization of the motoneurons has been reported in the cervical spinal cord at 2 wk of age, but none at either 1 d or 1 wk of age *(26,46,47)*. Sporadic degenerating neurons characterized by faded Nissl staining or chromatolysis have also been described in thalamic nuclei that belong to the extrapyramidal system and in the brainstem at 13 d of age *(26)*. Despite these morphological abnormalities, the numbers of motoneurons in 3-wk-old wobbler and control mice are identical in the median nerve nucleus, a region of the cervical spinal cord that is later severely affected by the disease *(34)*. Together, these data indicate that motoneuron death does not take place during the presymptomatic phase. At 2 wk of age, the cervical ventral-root nerves appear to be similar in wobbler and control mice *(46)*. In 19- to 22-d-old mice, some intramuscular nerve trunks contain fragmented axons, and many pre-terminal axons present a fine sprouting and innervate more than one muscle fiber *(33)*. The first degenerating myelinated fibers appear only during the third week of age *(46)*. Microtubule segregation, intra-axonal invagination of the Schwann cells, dilation of the endoplasmic reticulum, and the presence of large and dense secondary lysosomes characterize the ultrastructure of affected axons *(46)*. In accordance with this lack of marked axonal alteration, only a few muscle fibers exhibit an abnormal centrally located nucleus, and no muscular atrophy is observed *(33)*.

The sequence of histological events during the presymptomatic phase indicates that the wobbler disease primarily affects the perikaryon of the motoneurons, with the axonal alteration appearing secondary to the cell-body degeneration. The fact that motor functions are already impaired at the end of this period, despite the modest changes in cellular morphology and the lack of significant motoneuron loss, indicates that the biology of the motoneurons is already deeply affected.

Abnormal Accumulation of Neurofilaments Precedes Motoneuron Degeneration

Excessive accumulation of proteins is one of the hallmarks of neurodegenerative processes. They often correspond to neurofilaments, which are intermediate filaments that form part of the neuronal cytoskeleton. Their abnormal cytoplasmic accumulation frequently occurs in motoneurons in patients with sporadic *(48–51)* or familial ALS linked to a mutated SOD1 gene *(52).* Such an accumulation is also found in transgenic mice that overexpress human mutated SOD1 *(53–56)* and in the murine mutant *motor neuron degeneration (57).* Mutations in neurofilament subunits have been identified in approx 1% of the ALS patients examined *(58).* It is unknown whether these mutations are sufficient by themselves to account for the neurodegeneration. Pernas-Alonso and colleagues *(59)* have studied the rates of expression of mRNA for the three neurofilament subunits, heavy (NFH), medium (NFM), and light (NFL), in the spinal cord of all members of several litters of parents who were heterozygous for the wobbler mutation. The evaluated NFM mRNA levels can be segregated into three distinct groups that are compatible with Mendelian statistics. The supposed wr/wr mice displayed 4 × higher NFM mRNA levels than the control wild-type mice. The group most represented, which would correspond to mice heterozygous for the mutation, had intermediate expression levels. The enhanced NFM mRNA levels were observed between embryonic d 15 (E 15) and postnatal d 21, the oldest time-point examined. Increased NFL and NFH levels were only detected in the supposed wobbler group and at later ages (E 18 for NFL, and P5 for NFH). The same team recently confirmed these results in wobbler mice genotyped using the amplification of the Cct4 gene, and their results point to a gene-dosage effect of the wobbler mutation on NFM gene expression *(60).* These transcriptional changes however, are not visible at the protein level by immunohistochemistry prior to the 10th postnatal day *(60).* No alteration in neurofilament expression was detected in any other CNS area at this stage. These results are consistent with the hypothesis of an involvement of neurofilaments accumulation in motoneuron degeneration, although the genetic manipulations of NF expression in mice have resulted in paradoxical results. NFL deletion delays the onset of the disease and slows its progression *(61)* in SOD1-mutant mice, which exhibit many inclusions that contain only the NFL subunit *(62).* Surprisingly, the overexpression of NFL, as well as that of NFH, also extends the life span of these mice *(63),* whereas in contrast, overexpression of either NFL or NFH in transgenic mice that do not carry the SOD1 mutation results in motoneuron degeneration *(64,65).* NFM effects have not been explored. Cleveland and Rothstein *(4)* suggest that these paradoxical observations may result from different subcellular redistribution of the neurofilaments, in the perikaryon or in the axon, upon each of these genetic manipulations. Currently, defects in neurofilaments in motoneuronal degeneration are viewed as contributing/aggravating factors, or as common final targets, rather than as primary causal factors *(4,66).* Likewise, NFM does not correspond to the wobbler gene because it is localized on a different chromosome. But the dependence of NFM levels on the number of wobbler alleles points to NFM as a primary target of the mutation. In any case, motoneuronal neurofilament accumulation in murine models of neuronopathy, as well as in sporadic and familial cases of ALS, reveals a surprising commonality in the mechanisms that take place during motoneuronal degeneration. This reaffirms the high sensitivity of neurofilaments metabolism to a broad range of genetic insults in motoneurons.

Altered Expression of a Chaperone Protein During the Presymptomatic Phase

The neurofilaments are not the only proteins that accumulate in the neuronal soma during the course of neurodegenerative diseases. The presence of protein inclusions, often conjugated

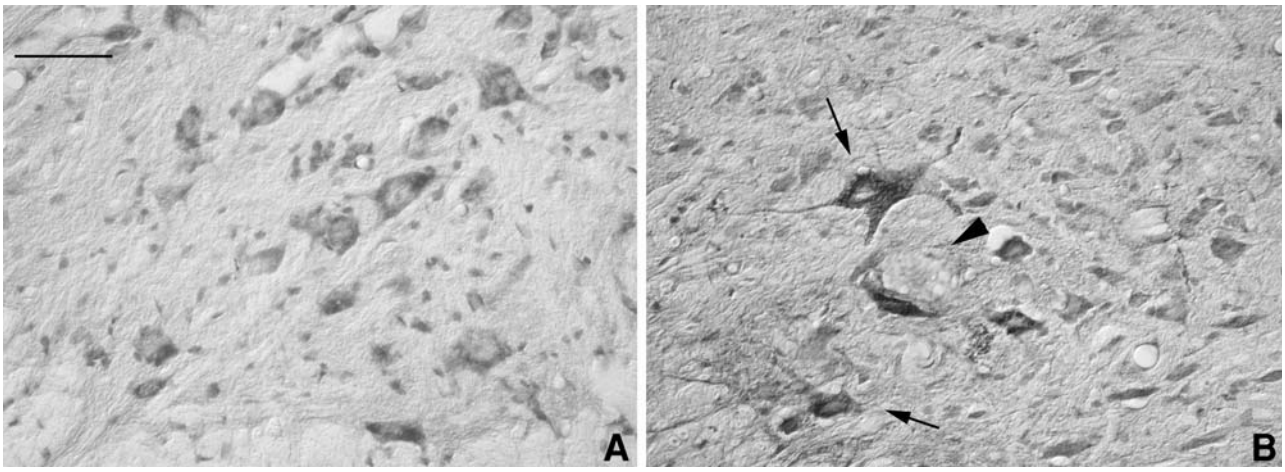

Fig. 4. Immunohistochemical localization of ubiquitin in the ventral horn of the cervical spinal cord of 6-wk-old wobbler (wr/wr, **B**) and wild-type (+/+, **A**) mice, associated with a cresyl violet counterstaining. Nomarski micrographs: Arrows point to ubiquitin-immunoreactive neurons and the arrowhead to a degenerating neuron totally filled with vacuoles. Mice were deeply anesthetized before they were perfused transcardially with warm heparinized saline followed by a phosphate-buffered saline solution (PBS 0.1 M; pH 7.4) containing 4% paraformaldehyde. The brain and the cervical portion of the spinal cord were immediately removed and post-fixed for 4 h at 4°C in the same fixative. Tissues were frozen after 24 h incubation in a 30% sucrose cryoprotective medium. Cryostat sections (20-µm thickness) were cut in the frontal plane. Immunohistochemistry was performed on free-floating sections. Ubiquitin immunohistochemistry was performed using a rabbit polyclonal antibody (1: 600, DAKO, France). Detection of the immunohistochemical signal was achieved using the avidin-biotin complex immunoperoxidase technique (1: 300, Vector) and diaminobenzindine as a chromogene. Images were acquired on a DXM 1200 digital camera using the Lucia software (Nikon, France). Scale bar = 50 µm.

with ubiquitin, is a common phenomenon in neuropathologies such as ALS, Alzheimer's disease, Parkinson's disease, and several hereditary neurological syndromes caused by expansions of poly-glutamine tract *(67–71).* These observations have led to the proposal that altered chaperone activity may contribute to neurodegeneration *(72–74).* The chaperone proteins ensure the prevention of protein misfolding and the correct maintenance of protein conformation. They also promote ubiquitination and subsequent degradation of abnormal proteins by the proteasome complex *(75–80).* A protective role of these chaperone molecules in the nervous system is supported by the fact that experimental upregulation of heat shock protein 70 (HSP70) and/or of members of the DNAJ/HSP40 co-chaperone family diminishes the intracellular inclusions of altered proteins, and protects cells in cellular models of the polyglutamine diseases, and of ALS linked to SOD1 mutation *(81–88).* HSP70 and DNAJ/HSP40 proteins act in a cooperative and adenosine 5'-triphosphate (ATP)-dependent manner to prevent the accumulation of improperly folded proteins *(75–80,89,90).* In wobbler mice, the accumulation of abnormal proteins other than neurofilaments has been reported in the cytoplasm of the spinal motoneurons *(44,46),* and ubiquitin-immunoreactive deposits have been observed at the end of the presymptomatic phase and during the evolutionary phase of the disease *(91)* (abstract) (MPJ, unpublished results, Fig. 4). As mentioned previously, the

testes of wobbler mutants exhibit a decreased expression of the DNAJ/HSP40 chaperone protein Msj-1 *(36).* Msj-1 mRNA levels are also decreased in the cervical spinal cord of wobbler mice at 2 wk of age, prior to the loss of motoneurons, and at 5–6 wk of age, when motoneuron degeneration is at its height *(92).* In control mice, the Msj1-immunoreactive signal is found in spinal motoneurons, yet no signal is observed in age-matched wobbler mice *(92),* suggesting that Msj-1 downregulation occurs independently of the degenerative status of the motoneuron. Together with the observed downregulation of Msj-1 in sperm cells *(36),* these observations suggest that Msj-1 plays a central role in defects linked to the wobbler mutation. No mutation has been found in the coding region of msj-1 *(92),* and the Msj-1 gene appears to be localized on chromosome 1 (as determined by the alignment of the msj-1 sequence and the mouse genome with the web server ensembl.org). Like the neurofilaments, Msj-1 thus appears as a target protein for the wobbler mutation. The existence of an altered chaperone activity in human neurodegenerative diseases to date remains a matter of hypothesis, largely based on the finding of intracellular protein aggregates in most of these diseases *(72–74).* In the spinal cord, a decrease in overall chaperone activity has been detected in SOD1-mutant mice, but no specific defect in the expression of a chaperone protein was identified *(83).* In the *paralysé* mutant mouse *(93),* which undergoes a rapid degeneration of its spinal lumbar motoneurons, the small Hsp25 levels diminish, although it is unknown whether this decrease occurs prior to the loss of motoneurons or simply results from it *(93).* In wobbler mice, the impact of Msj-1 downregulation on neuronal degeneration is a matter of speculation. It remains to demonstrate that it is accompanied with a decreased chaperone activity in the affected neurons, and that this is harmful to the cell. Msj-1 expression in wobbler nevertheless provides the first example of an association between transcriptional repression of a chaperone protein and a neurodegenerative process.

Changes in Growth Factors and Growth-Factor-Receptor Expression: A Motoneuron Attempt at Coping With the Disease Process?

The studies described in the preceding section clearly show that the presymptomatic phase of the wobbler disease corresponds to a period of "motoneuronal suffering," which occurs well before the death of these cells. The existence of such an intermediate period may be seen as a result of the time lag necessary to the full development of the toxicity of the mutation, or/and as the result of synthesis of novel molecules capable of delaying the neurodegenerative process. To address this question, cellular patch-sampling and reverse transcriptase-polymerase chain reaction (RT-PCR) were used to obtain DNA pools complementary to the cytoplasm mRNA content of sound motoneurons in control mice and of morphologically affected ones in wobbler mice at 21–24 d of age *(94).* This study, based upon the analysis of the RNA messengers in cells characterized using morphological criteria, was set to reveal a variation of expression levels related to the affection of motoneurons that may have been undetectable when the whole tissue was analyzed. Exploration of transcriptional alterations of genes that encode growth factors believed to be involved in motoneuronal development revealed an upregulation of the neurotrophin brain-derived neurotrophic factor (BDNF) in the affected motoneurons. In contrast, expression of neurotrophin-3 (NT-3) as well as that of trkB and trkC, the high-affinity BDNF and NT-3 receptors, was found in both control and wobbler motoneurons. The low-affinity receptor $p75^{NTR}$, common to all neurotrophins, was upregulated in the wobbler motoneurons. Immunohistochemical detection of corresponding proteins corroborated these observations *(94,95).* Expression of a member of the epidermal growth factor (EGF) family, the neuregulin transcript sensory motor neuron-derived factor, was detected in both control and degenerating motoneurons, while trans-

forming growth factor-α (TGF-α), the functional homolog of EGF, was present only in the affected motoneurons *(94,96).* These results indicate that motoneurons affected by the wobbler mutation can initiate novel syntheses, leading to the production of molecules endowed with glio- or neurotrophic properties *(97–102).* Interestingly, similar changes have been observed in axotomized adult motoneurons *(103–105),* which survive to the cut of their axons *(106).* These similarities indicate that these changes in growth factor and growth-factor receptors are unlikely to participate in the degeneration process. Instead, they suggest that at least part of the response of motoneurons to an injury is stereotyped and unrelated to the identity of the inflicted insult.

Other Molecular Alterations

Alterations in the synthesis of a range of other molecules has been reported, either within the motoneurons or in their environment during the presymptomatic stage (Tables 1 and 2). The observation in the ventral part of the cervical spinal cord, at the end of the presymptomatic period, of an important number of immunoreactive fibers for the thyreotropin-releasing hormone (TRH) *(131),* and of increased number of TRH receptors in the dorsal horn of the spinal cord *(128),* is worth noting. Enhanced densities of nerve fibers that are immunoreactive for serotonin, met-enkephalin, leu-enkephalin and cholecystokinin (CCK) are also visible at this stage *(40,132).* Unfortunately, the origin of these fibers—e.g., from within the spinal cord or from upper CNS regions—is unknown. These changes in ventral-horn neuropeptide levels have been proposed to provide enhanced deleterious excitatory inputs to the degenerating motoneurons, but the hypothetical participation of such an excitatory component to the disease has not yet been documented *(131).*

In addition to these various molecular alterations, an intriguing modification of the DNA structure has been reported in the wobbler spinal motoneurons and glial cells between the 18th and 21st postnatal day *(47).* At these ages, a transitory dUTP nick-end labeling (TUNEL) of the DNA occurs in motoneurons and glial cells, which is not observed prior to or after this time frame *(47).* TUNEL labeling of motoneurons has also been reported in 21 d-old animals in an independent study *(115).* TUNEL labeling of the nuclei occurs when the cell DNA is fragmented, and this technique is widely used to identify cells that are dying by apoptosis. In wobbler mice, no chromatin condensation, no apoptotic body, no DNA laddering, and no motoneuron death at the issue of these 3 d are associated with this positive TUNEL staining. In this case, TUNEL staining reveals DNA breaks unrelated to apoptosis. Blondet and colleagues have suggested that these breaks may result from DNA oxidative damage *(47).* However, no indices for altered free radical detoxifying pathways have been provided in the presymptomatic wobbler mouse. Alternatively, a defect in DNA repair mechanisms may be envisaged, as proposed in the wasted mouse, which presents a complex immune and motoneuron syndrome *(133)* caused by a deletion in the gene Eef1a2, which encodes an isoform of the translation elongation factor 1α *(134).* Such a possibility is supported by the recent demonstration that DNA-repair enzymes are required for normal development of the CNS *(135).* In any case, the transitory aspect of this phenomenon remains puzzling.

The studies performed on the wobbler mouse during the presymptomatic phase show that the soma of the motoneurons is the primary affected site. Changes in the motoneuron metabolism precede and accompany the morphological alteration. At least one of these changes, the intra-cytoplasmic accumulation of neurofilaments, has been found in most types of human and animal spinal motor neuropathy. The extent to which these early modifications in motoneuron metabolism take part in the degeneration process has not yet been determined.

Table 1
Molecular Alterations in the Wobbler Motoneurons and Their Glial Environment During the Presymptomatic and Evolutionary Phases of the Disease

Molecule	Variation	Tissue	Phase	Method	References
BDNF	+	MTN	P	RNA: RT-PCR Protein: ICC	*(94)*
	+	MTN, SC	E	Protein: ICC	*(95)*
TGFα	+	dMTN	P E*	RNA: RT-PCR Protein: ICC	*(94,96)*
SMDF	0	dMTN	P	RNA: RT-PCR	*(94)*
NT-3	0	dMTN	P	RNA: RT-PCR Protein: ICC	*(94)*
TNF-α, IL-1β	+	SC, brainstem, cerebellum	E	RNA: RT-PCR	*(107)*
IL-2, IL-6, IL-10, IL-12, IL-18, IFN-γ	0	SC, brainstem	E	RNA: RT-PCR	*(107)*
Trk B	+	dMTN	P	RNA: RT-PCR	*(94)*
Trk C	0	SC dMTN	pn5 P P	RNA: RT-PCR	*(94,108)*
C-Jun	+	dMTN	P	RNA: RT-PCR	*(94)*
P75^{NTR}	+	dMTN	P	RNA: RT-PCR Protein: ICC	*(94)*
Neurofilament (NF)					
NF light	0 +	SC	ω1 ω2 pn5	RNA: RT-PCR Protein: western (21j)	*(59)*
	+	dMTN	P	RNA: RT-PCR	*(94)*
NF medium	+		ω1 ω2 pn5 P	RNA: RT-PCR Protein: western (21j)	*(59)*
NF heavy	0 +		ω1 ω2 pn5 P		
GAP-43	+	MTN, dhSC	E	RNA: ISH Protein: ICC	*(109–111)*
Adhesion molecule F3	0	SC	pn5 P	RNA: RT-PCR	*(108)*
peripherin	0 –	SC	pn5P P	RNA: RT-PCR	*(108)*
ubiquitin	+	Cortical neurons	E	ICC	*(10)*
NOS (NAPDH-diaphorase)	+ 0	dMTN	E*	Activity: histochemistry	*(110,112)*
PAR-1	+	SC	E	RNA: RT-PCR	*(113)*
TRPM-2	+	SC, MTN	E	RNA: ISH, northern	*(119)*
ADAM-8	+ 0	SC (MTN, astro, μglia), brainstem cortex	E	RNA: RT-PCR Protein: ICC, western	*(107)*

The variations as compared to control litter mates are symbolized with + (increase), – (decrease), 0 (no change). MTN, motoneurons; dMTN, motoneurons presenting histological signs of degeneration; astro, astrocytes; μglia, microglial cells; SC, spinal cord; dhSC, dorsal horn of the spinal cord; P, presymptomatic period of the disease; ω1, embryonic d 15; ω2, embryonic d 18; pn5, postnatal d 5 and 10; E, evolutionary phase of the disease; S, stabilization phase of the disease; E*, scattered motoneurons expressing the indicated molecules have been reported during the stabilization phase of the disease; ICC, immunocytochemistry; ISH, *in situ* hybridization; SMDF, sensory and motor neuron-derived factor; TRPM-2, testosterone-repressed prostatic message 2. TGF-α and TNF-α are putative candidates for a role of motoneuronal inducers of astrogliosis (*see* pp. 62–66). Activation of the cell-surface receptor of the activated protease (PAR-1) by the serine protease thrombin may underlie an eventual reciprocal action of the reactive astrocytes upon the motoneurons. Activation of PAR-1 by thrombin has recently been shown to result in apoptosis of various neuronal populations and of avian motoneurons in particular *(114).* In wobbler spinal cords, PAR-1 mRNA levels are slightly increased at birth, and multiplied by 5 in 4-wk-old mutants. Its cellular sources correspond to spinal motoneurons *(113,115)* and in vitro wobbler astrocytes release enhanced levels of thrombin-like activities *(115).* A deleterious effect of the thrombin/PAR-1 couple has not yet been demonstrated in mouse motoneurons. *See* text for further explanations.

Table 2
Molecular Alterations in the Wobbler Spinal Cord Involved in General Metabolic Pathways and in Neurotransmitter Synthesis

Molecule	Variation	Tissue	Phase	Method	References
Protein synthesis	–	MTN	E	*Incorporation	*116*
RNA synthesis	–	MTN	E	*Incorporation	*117*
cGMP	–	SC, cerebellum, cortex	E-S	Enzymatic analysis	*118*
cAMP	0	SC, cerebellum, cortex			
ATP	0	SC, cerebellum, cortex			
Amino acids:			E	Chromatography	*119*
Glu, Asp, Gly, Thr, glutathione	0	Brain			
Ser, Ala	–	Brain			
Gln	+	Brain			
Ser, Ala, Thr, glutathione	0	SC			
Glu, Asp, Gly	–	SC			
Glucose, glycogen	+	Cerebellum	E-S	Enzymatic analysis	*118*
	0	SC, cortex			
lysosome enzymes	+	SC white matter	E-S	Histochemistry	*120*
Lactate dehydrogenase	0	SC	E-S	Histochemistry	*120,121*
Glucose-6-phosphate dehydrogenase	+	SC	E-S	Histochemistry	*120*
Glycerol-3-phosphate dehydrogenase	–	vhSC	E-S	Histochemistry	*120*
	+	SC white matter			
acidic protease	+	SC	E	Activity, OD	*122*
Ornithine decarboxylase	+	SC	E	Activity	*123*
glucocorticoids II receptors	0	SC	E	Binding	*123*
Thyrotropin releasing hormone (TRH)	+	SC	P	RIA	*121,124–126*
	0+	SC, pons	E		
	+	SC, pons	S		
	0	vhSC	S		
	+	Brainstem	E-S		
	+	Hypothalamus	E-S		
	–	Midbrain	S		

Substance P	0	vhSC	P	RIA	*121,124–127*
	0	SC, pons	E-S		
	+	SC	S		
	+	Hypothalamus	P		
	+	Brainstem, midbrain, hypothalamus	E-S		
	+	Cerebellum	S		
Enkephalins	–	Cerebellum	P-E-S	RIA	*124–127*
Leu-enkephalin	+	vhSC	P		
	+	Hypothalamus	E		
Met-enkephalin	0	vhSC	P		
Leu- and Met-enkephalin	+	SC, brainstem, midbrain E-S			
Somatostatin	+	Brainstem	E-S	RIA	*121,126*
	0	SC, pons			
Neurotensin	0	SC, pons	E-S	RIA	*121*
GABA	0	SC, cerebellum, cortex, brain	E-S	Enzymatic analysis chromatography	*118,119*
TRH receptors	+	dhSC	P	Binding	*128*
	–		E-S		
5-HT receptors	0	dhSC	E-S	Binding	*128*
NMDA, kainate, AMPA receptors	+	dhSC	S	Binding	*129*
Glu metabotropic receptors	0	SC			
ChAT	+	vhSC	P	RIA	*59,125,130*
	–	vhSC	S	Activity, RIA	
	0	SC	E		
	0	SC	P§	RNA, RT-PCR	
Acetyl Choline Esterase	–	SC	P	RNA, RT-PCR	*108,125*
	–	vhSC	S	Activity	
Pyroglutamyl-aminopeptidase, proline-endopeptidase (degrades TRH)	0	SC	E	Activity, OD	*121*
	+		S		

The variations as compared to control littermates are symbolized with + (increase), – (decrease), 0 (no change). MTN, motoneurons; SC, spinal cord; vhSC, ventral horn of the spinal cord; dhSC, dorsal horn of the spinal cord; OD, optical density; RIA, radioimmunoassay; *, incorporation of [^{3}H] leucine to evaluate protein synthesis, and [^{3}H] uridine to evaluate the synthesis of RNA; NMDA, N-methyl-D-aspartate; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid. *See* text for further explanations.

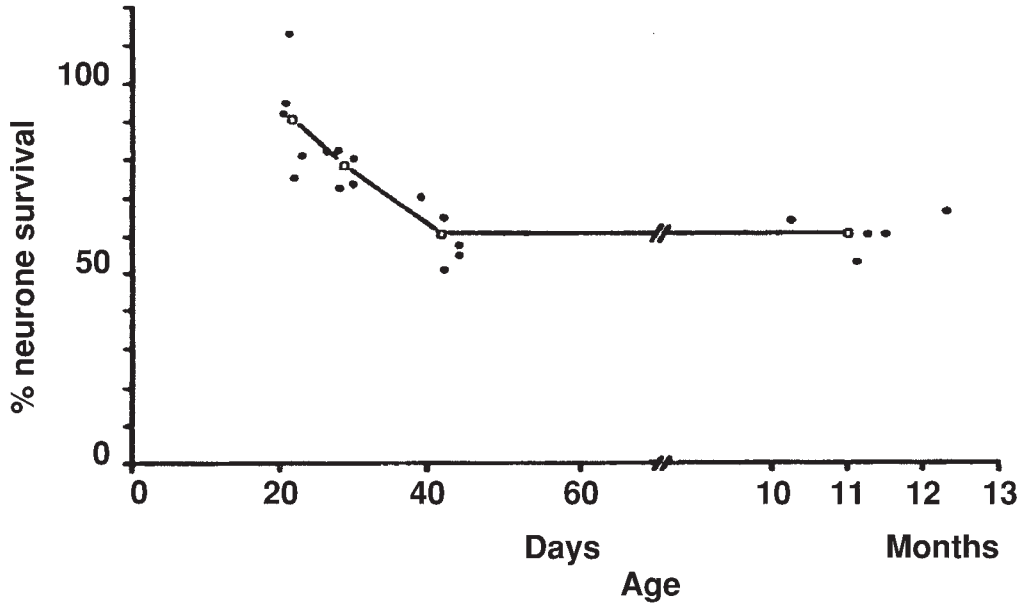

Fig. 5. Time-course of motoneuron loss in the median nucleus of the cervical spinal cord. Note that the cellular loss begins at the third week of life, progresses through the sixth week of postnatal life, and then stabilizes. The presymptomatic period of the disease extends until the third week. The evolutionary phase of the disease lies between the third week and the third month. Although the motoneurons loss is not marked any more during the third month, the presence in a significant number of vacuolated motoneurons led to the inclusion of this period in the evolutionary phase of the disease. Adapted from Pollin and colleagues (1990) *(34).*

During the Evolutionary Phase of the Disease, Motoneurons Present Major Morphological Alterations But Are Still Metabolically Active

Cytoplasm Vacuolization of the Spinal Motoneurons Is the Histological Landmark of Wobbler Disease

The progressive loss of motoneurons begins during the fourth week of life with the appearance of clinical signs, and characterizes the evolutionary phase of the disease. Through retrograde labeling of the median nerve motoneurons, Pollin and colleagues *(34)* showed that approx 50% of the motoneurons of this area had disappeared in 6-wk-old mice (Fig. 5). The neuronal loss is then stabilized, but morphologically abnormal motoneurons are still visible beyond. The characteristics of the affected motoneurons of wobbler are atypical. Vacuoles appear in the cell body and become increasingly large and numerous, until they completely fill the soma *(33,44,136)* (Fig. 6). These vacuoles, which are present in the affected and swollen motoneurons, originate from the dilation of the endoplasmic reticulum *(33,44)* (Fig. 7). Their composition is unknown. They are not electron-dense, and thus appear empty *(33).* Such figures of degeneration have also been described in the murine models of motoneuron disease, *muscle deficient (140)* and *wasted (141).* Other ultrastructural anomalies, like the presence of tubular structures and an increase in the number of lysosomes, occur in affected wobbler motoneurons *(44,136).* Motoneuron death takes place mainly in the cervical part of the spinal cord and the brainstem *(33,34,142).* Within the cervical spinal cord, the number of degenerating motoneurons varies according to the segment *(34)*—but at all

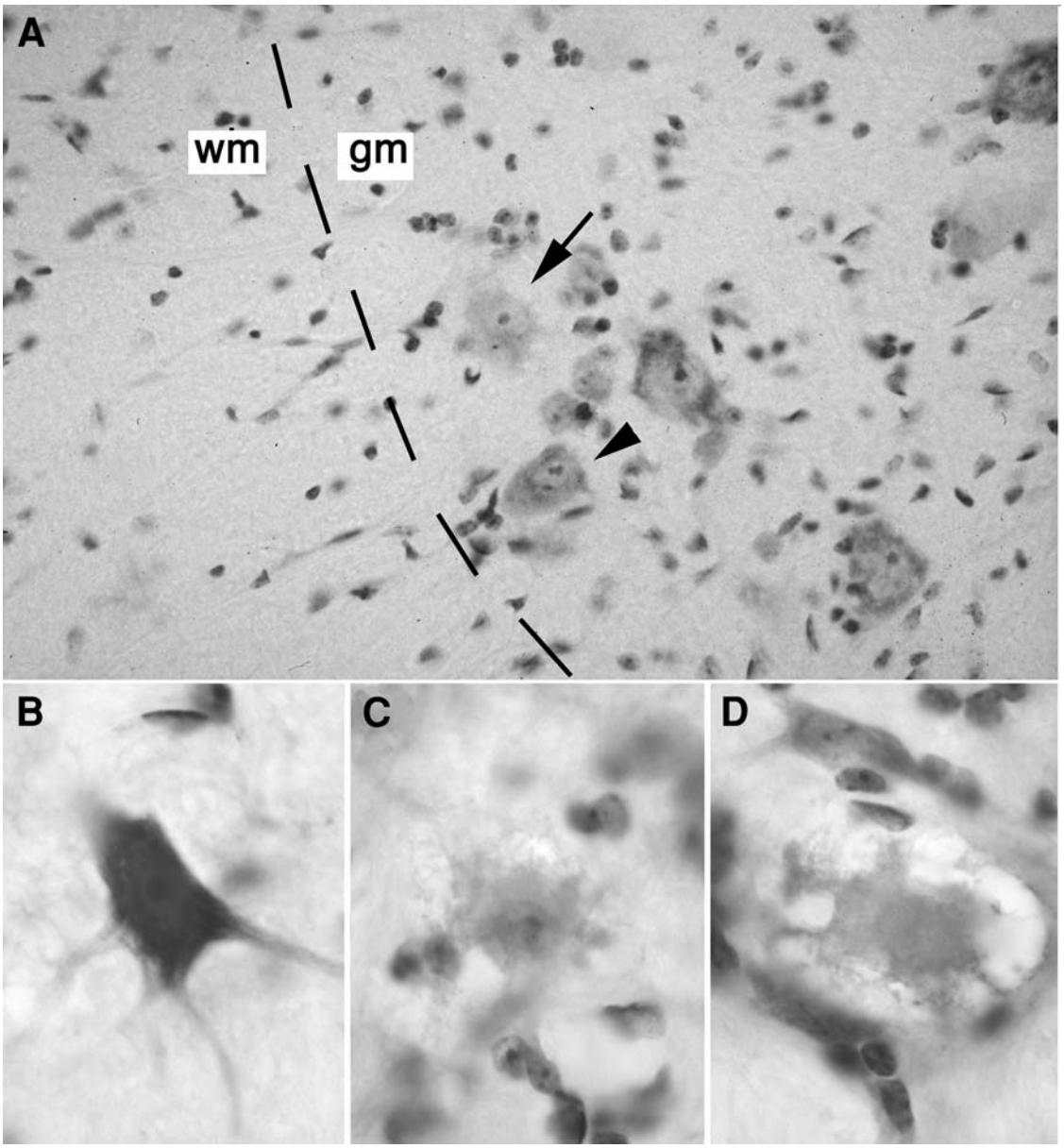

Fig. 6. Degenerating motoneurons in the wobbler cervical spinal cord as revealed by cresyl violet staining, a marker of the Nissl bodies. **(A)** Six-week-old wobbler cervical spinal cord. The arrow points to a vacuolated motoneuron, and the arrowhead to a motoneuron with a normal appearance. gm: gray matter, wm: white matter. **(B)** A motoneuron in the cervical spinal cord of a 6-wk-old control mouse. **(C)**, **(D)** Vacuolated motoneurons in the ventral horn of 6-wk-old wobbler mice. Note the weak intensity of the Nissl staining, the swelling of the cell body, and the presence of vacuoles.

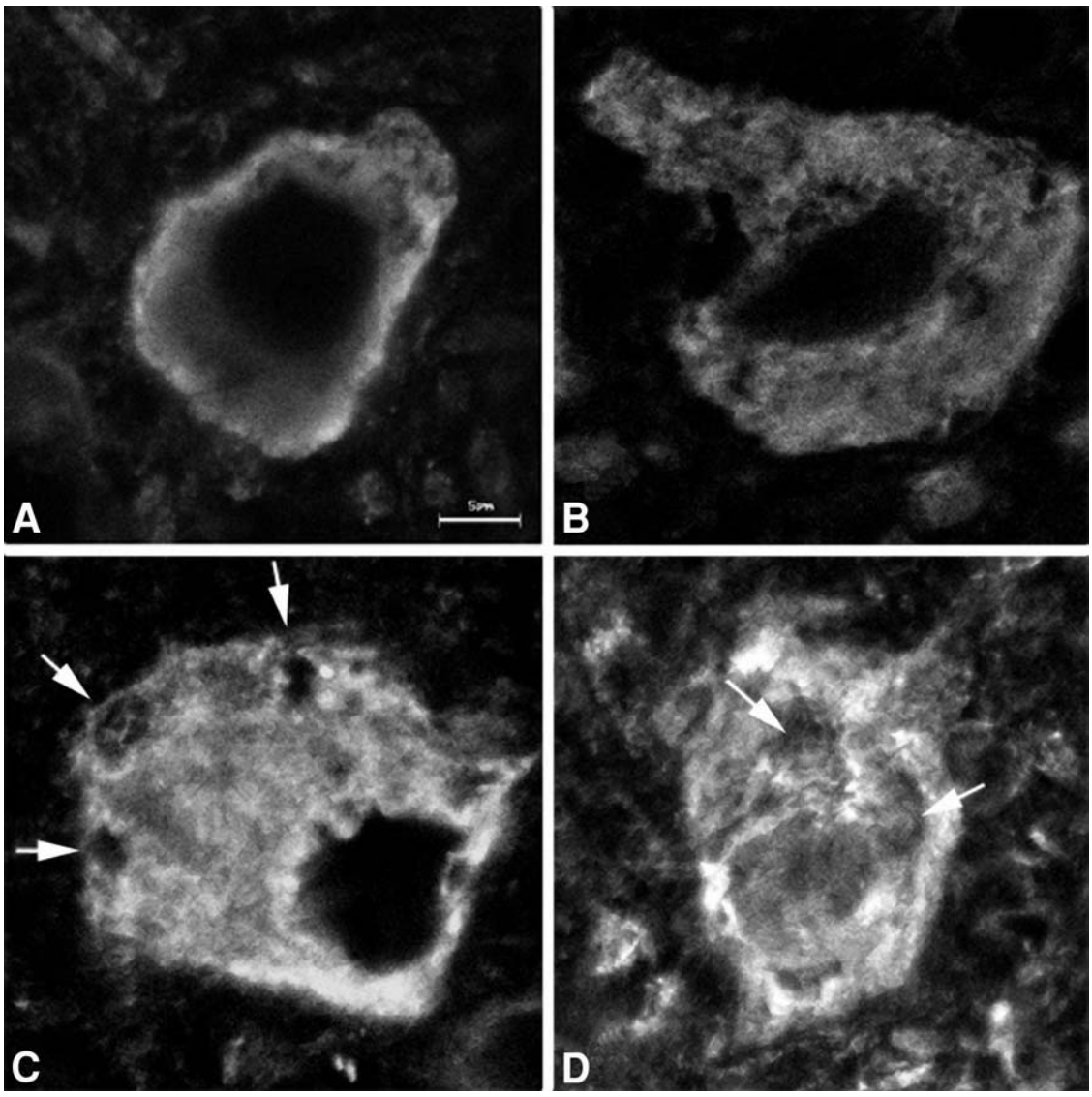

Fig. 7. Confocal visualization of the immunofluorescent labeling of Bip, an Hsp70 protein specific to the endoplasmic reticulum *(137–139),* in motoneurons of the cervical spinal cord of a 6-wk-old wild-type **(A,B)** and wobbler **(C,D)** mouse. Note the disorganization of the endoplasmic reticulum in the wobbler motoneurons (arrows) as compared to the controls. Immunohistochemical procedures were as described in Boillée et al. 2002 *(92).* Scale bar = 5 µm in **A,** 4.7 µm in **B,** 5.9 µm in **C,** and 5.3 µm in **D.**

times, motoneurons displaying the entire panel of morphological possibilities, from totally sound to degenerated, co-exist in the same segment *(33,96).* In the motor nuclei of the brainstem, the extent of motoneuron loss is much less important than in the cervical spinal cord. For instance, in the facial motor nucleus, only 15% of motoneurons have disappeared in 5-wk-old mutants *(41).* During the evolutionary phase, figures of degeneration other than vacuoles are observed in the brain. In the abducens nucleus, degenerating motoneurons display a

loss of labeling of the Nissl bodies but are rarely vacuolated, despite a 31% loss at 4–5 wk *(143).* Other anomalies, such as an eccentric location of the nucleus, have also been observed at an advanced stage of degeneration *(33).*

Alteration of the soma is accompanied by morphological and functional axonal changes. The morphological alteration described at the early stage is amplified. The thin, beaded axons sprout, and the number of motor nerve terminals is reduced *(33,143).* The number of large-diameter fibers is the most significantly reduced, and this decrease increases with age *(41,46,142,144,145).* Signs of axonal degeneration are also associated with a reduced diameter of the myelin sheath and even a loss of myelin, leading to the presence of non-myelinated fibers of large diameter *(136,144,145).* The inter-axonal space is increased and filled with collagen *(144).* These morphological anomalies are accompanied with an alteration of axonal transport systems, which may be related to the accumulation of abnormal neurofilaments observed during the presymptomatic period *(66).* The quantity of proteins transported by slow axonal transport *(136,146),* fast anterograde *(147),* and fast retrograde transports *(148)* is decreased. The axons maintain their regenerative capacities in wobbler mice in response to a crush or a cut, although the process is slower than in healthy mice *(149).* Some vacuolated neurons are able to maintain their axons at the periphery and to ensure their growth *(150).* Axonal collateral sprouting is often observed, and could compensate—partially and only during a limited period of time—for the functional consequences of the neuronal loss *(33,130).*

During the evolutionary stage of the disease, neurons other than motoneurons are also affected. It is difficult to determine whether these anomalies are the result of motoneuron death, or whether these neurons are also directly affected by the mutation. At the beginning of this period, interneurons of the spinal cord layers V–VIII present a higher density of dendritic spines than in age-matched controls, whereas the average size of the cellular bodies is normal *(151)* and the levels of γ-aminobutyric acid (GABA) appear unchanged *(118).* Rare occurrences of vacuolated cells are observed in the cervical ganglia *(33,144,152).* Although the wobbler disease was classified for a long time in the group of the SMA because no neuronal anomaly was detected in the cortex, this has been contradicted in a recent study using proton magnetic resonance spectroscopy *(10).* Moreover, the cell bodies and the neurites of neurons of the neocortex present a strong immunoreactivity for ubiquitin. An accumulation of the phosphorylated heavy chain of the Neurofilament (NF) is also observed in neuronal-cell bodies in the cerebral cortex *(10),* as has been observed in human ALS *(153,154).* No vacuolization has been reported in the cortical neurons that exhibit these anomalies *(10).* A 29% decrease in the cGMP levels has been reported in the cortex of 1.5–14.5-mo-old wobbler mice *(118),* reinforcing the case of neurodegeneration in this brain area. Although the actual loss of neurons has not yet been demonstrated and evaluated in the cortex, these results show that neuronal target cells of the wobbler mutation are not restricted to motoneurons, but extend to upper brain areas. This finding compares more to ALS than to SMA.

A Non-Canonical Type of Neuronal Death in Wobbler Mice

The different histological figures of motoneuron degeneration observed in the wobbler mice strongly differ from the typical figures of apoptotic degeneration. Apoptosis is characterized by a condensation of the chromatin and a fragmentation of the nucleus, which precede the alteration of the cytoplasmic membrane *(155).* In wobbler mice, chromatin condensation has not been observed *(41).* Apoptosis is also characterized by an internucleosomal degradation of DNA *(155).* Such degradation has not been observed during the evolutionary phase of the disease with the TUNEL technique *(109).* The early and transitory TUNEL-positive motoneuron- and glial-cell labeling observed at the end of the presymptomatic phase does not result in cell

apoptosis *(47).* Crossing wobbler mice with Bcl2-overexpressing transgenic mice has indirectly confirmed that neuronal death in wobbler mice does not mimic the apoptotic motoneuron death that takes place during embryonic development. Bcl2 is an inhibitor of apoptotic cell death *(156),* which prevents cytochrome c release from the mitochondria and the subsequent activation of caspases, the downstream effectors of apoptosis, and also presumably acts by preventing the activation of several caspases independently of mitochondrial damage *(157).* Bcl2 overexpression rescues motoneurons from physiological developmental death *(158),* yet it does not prevent motoneuron degeneration caused by the wobbler mutation *(41,159).* These observations do not imply that wobbler death corresponds to necrosis, which is a rapid event characterized by the disintegration of the entire cell that does not require the mobilization of endogenous suicide mechanisms and is accompanied by the recruitment of numerous phagocytic microglial cells *(160,161).* Specific intracellular pathways mobilized during wobbler death have to be found. Nevertheless, the histological features of wobbler death are closer to the cytoplasmic form *(162)*—also known as type II *(162,163)* or type 3B of cell death *(164)*—than to necrosis. Initially described in the ciliary ganglion *(163)* and avian lumbar motoneurons *(162)* during the course of development, these types of death share with the wobbler at least a striking dilation of the endoplasmic reticulum and the occurrence of free ribosomes *(44).* A recent study has revealed that programmed motoneuron cell death is maintained (although delayed) in mice embryos that lack the genes encoding caspase 3 or 9 *(155,165).* In these mice, chromatin condensation is lacking, and the degenerating motoneurons display a vacuolization of their cytoplasm. These results further support the existence of death pathways independent of the currently known caspases.

Together, these results show that apoptosis does not account for motoneuron death in wobbler mice. The identity of the components of the death pathways that are mobilized in those mice has not yet been identified.

Motoneurons Remain Metabolically Active During the Evolutionary Phase of the Disease

Affected motoneurons, including those with severe signs of degeneration, continue to synthesize a number of different proteins during the evolutionary phase, particularly some that they begin to express at the end of the presymptomatic period (Tables 1 and 2, Fig. 8). This is the case for various growth factors *(94–96),* and neurofilaments *(60).* TNF-α is also expressed in motoneurons during the evolutionary phase of the disease, as well as the metalloprotease ADAM8 *(107).* The functional significance of these syntheses is discussed in the following section devoted to gliosis. Morphologically abnormal motoneurons are not the only ones that demonstrate an altered metabolism. A total reduction in the syntheses of mRNA (in 2-mo-old mutants) and proteins (in 1–14-mo-old wobbler mice) has been reported in morphologically intact motoneurons, and in small neurons of the ventral horn of the spinal cord *(116,117).* However, it is unknown whether these modifications of synthesis of messengers and proteins involve motoneurons that are eventually fated to die. In addition to the sustained syntheses described here, an enhanced expression of growth-associated protein 43 (GAP-43) has been observed at both the mRNA and protein levels in the motoneurons of the cervical spinal cord *(109,111),* although the status of the GAP-43-expressing motoneurons—e.g., intact or degenerating—is unknown. GAP-43 is strongly expressed during development, and its expression is associated with axonal growth and regeneration *(167).* GAP-43 upregulation is thus likely to reflect at the molecular level the axonal collateral sprouting and the poly-innervation of wobbler muscle fibers *(33,168).*

Oxidative damage associated with defective free radical detoxification pathways has been linked to various neurodegenerative processes *(169,170),* and evidence of oxidative damage

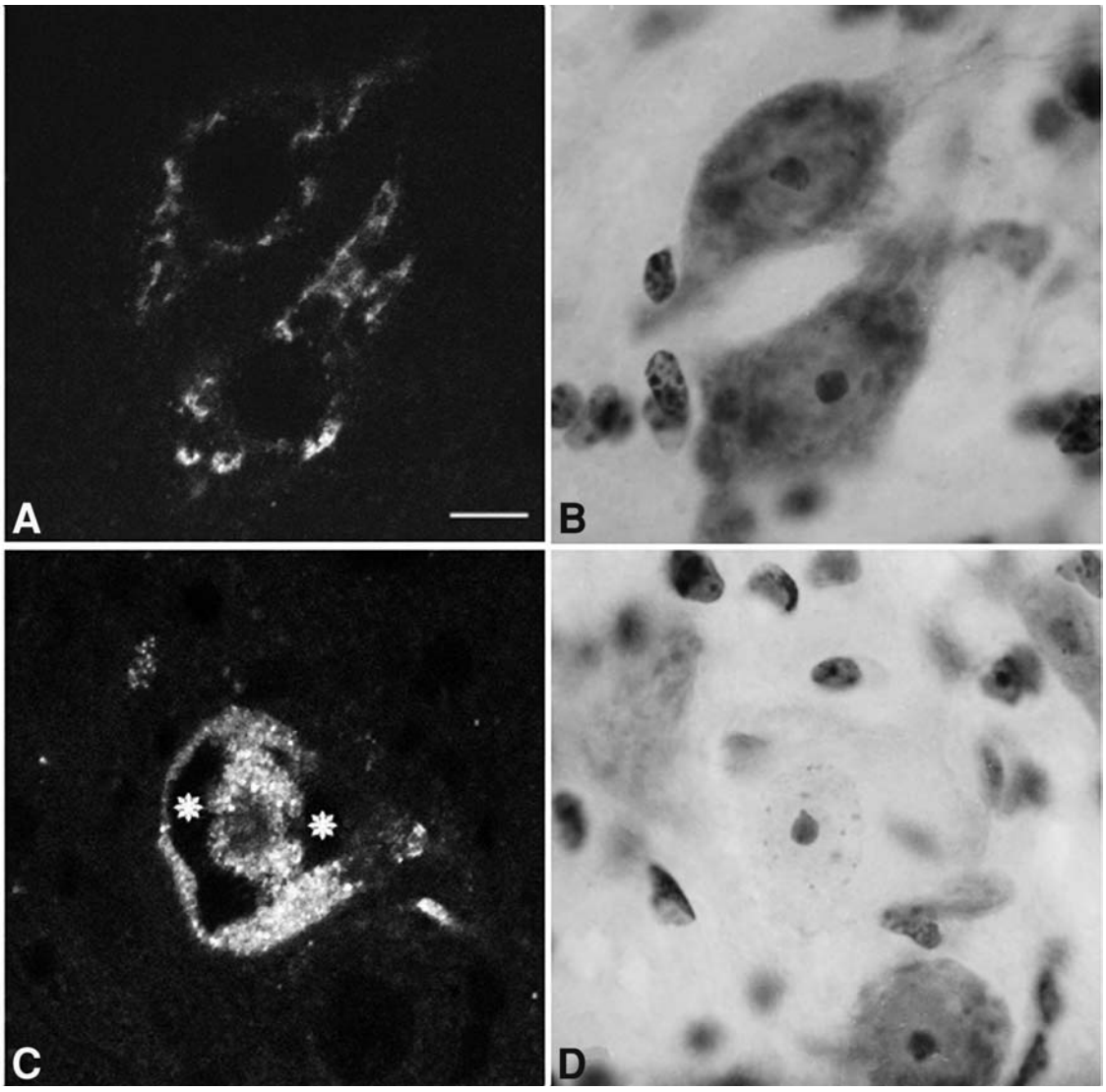

Fig. 8. CGRP-immunoreactive labeling and cresyl violet staining of 11-wk-old wobbler motoneurons displaying the typical vacuolization of their cytoplasm. **(A)** and **(B)** Control mice, **(C)** and **(D)** Wobbler mice. **(A)** and **(C)** CGRP-positive neurons visualized with a confocal microscope. Note the vacuoles in the wobbler motoneuron (stars). **(B)** and **(D)** Cresyl violet staining of the same motoneurons. Note the weakness of the Nissl staining in the degenerating motoneuron. Scale bar = 10 µm. Adapted from Boillée and colleagues (2001) *(166).*

has been reported in cases of sporadic and familial ALS *(171–173).* The Ca^{2+}-dependent enzyme neuronal nitric oxide synthase (NOS), which catalyzes the formation of NO *(174),* is not expressed in adult motoneurons under physiological conditions. On the opposite, it is detected at high levels in embryonic and adult motoneurons that undergo apoptosis following axotomy or ventral-root avulsion, and its inhibition rescues the injured motoneurons from death *(175–180).* Although motoneuron apoptosis does not take place *per se* in wobbler

mice, NOS induction has been reported, using NADPH-d (nicotinamide dinucleotide phosphate-diaphorase) histochemistry and n-NOS immunoreactivity *(95,110,112),* to be restricted to morphologically abnormal motoneurons. Moreover, NADPH-d positive motoneurons are rare at the beginning of the evolutionary period, their number augments sharply as the disease evolves, and they disappear in aged wobblers *(112).* Such a tight correlation between the time-course of degeneration and of NOS expression argues for the existence of an oxidative component in the death pathway. The relative beneficial effect of the administration of different antioxidants on the course of the wobbler disease supports this hypothesis. Intraperitoneal (ip) injections of 7-nitroindazole, a specific inhibitor of neuronal NOS slows down muscular atrophy, improves muscular strength, and reduces the loss of motoneurons in the cervical spinal cord *(181).* Similar results are obtained with an ip injection of Cu/Zn superoxide dismutase (SOD) modified to pass the blood–brain barrier (BBB) *(182).* An improvement of the wobbler pathology and a reduction in spinal motoneuron loss were also reported with oral administration of the antioxidant *N*-acetyl-L-cysteine *(183).* These effects were accompanied by a restoration to control values of the levels of glutathione peroxidase activity, a component of the free-radical scavenging pathways *(183).*

Gliosis Accompanies Motoneuron Degeneration in Wobbler Mice

Gliosis is characterized by profound changes in astrocytes and microglial cells, which evolve from quiescent to activated morphological and metabolic profiles *(184–186).* This process is systematically associated with neurodegenerative diseases and CNS trauma *(187,188),* and wobbler does not escape this rule (Fig. 9). Although no glial reactivity was detected during the first histological exploration of wobbler mice *(33),* the subsequent use of Glial fibrillary acidic protein (GFAP) immunohistochemistry made it possible to disclose astrogliosis in the cervical spinal cord and the brainstem of the mutants during the evolutionary phase of the disease *(26,96,189,190).* The characteristic hallmarks of reactive astrocytes—e.g., increased expression of GFAP coupled with a hypertrophied morphology including enlarged soma and thickened processes *(191–193)*—were observed in the cervical spinal cord and the brainstem of wobbler mice during the evolutionary phase of the disease *(26,96,189,190).* Likewise, the typical features of microglial activation—increased cell numbers and hypertrophied morphology *(188)*—have been found in the wobbler cervical spinal cord *(26,166).* No sign of gliosis has been observed in the brain areas that display occasional degenerating neurons *(26),* but the cortex has not yet been explored. In the original wobbler strain, reactive astrocytes have been detected in the ventral horn of the spinal cord as early as in 17-d-old wobblers *(26).* Until 1 mo of age, astrogliosis is confined to the ventral horn of the spinal cord; it then expands to the dorsal horn *(96,190).* The age of maximal astrogliosis coincides with the peak of motoneuron degeneration *(96,189).* Astrogliosis is transitory in hybrid wobbler mice, and no reactive astrocytes are visible in 3- to 5-mo-old mice *(96).* In the original wobbler strain the astrogliosis persists later *(189,190).* This difference may arise from the use of different wobbler strains, or from immunohistochemical protocols with different sensitivities. Activated microglial cells are detected in wobbler mice at 3 wk of age *(26,166).* At this time, microglial activation is discreetly localized around affected motoneurons, and there is no increase in the density of microglial cells *(166,189).* During the evolutionary stage of the disease, the number of microglial cells increases, until they are present in the entire ventral horn and in the ventrolateral white matter *(166).* A recruitment of lymphocytes is also noted at that time *(166),* although the BBB appears intact (Fig. 10). These lymphocytes are scattered in the ventral horn and are not in contact with the motoneurons, ruling out a potential attack of motoneurons by cytotoxic T-lymphocytes.

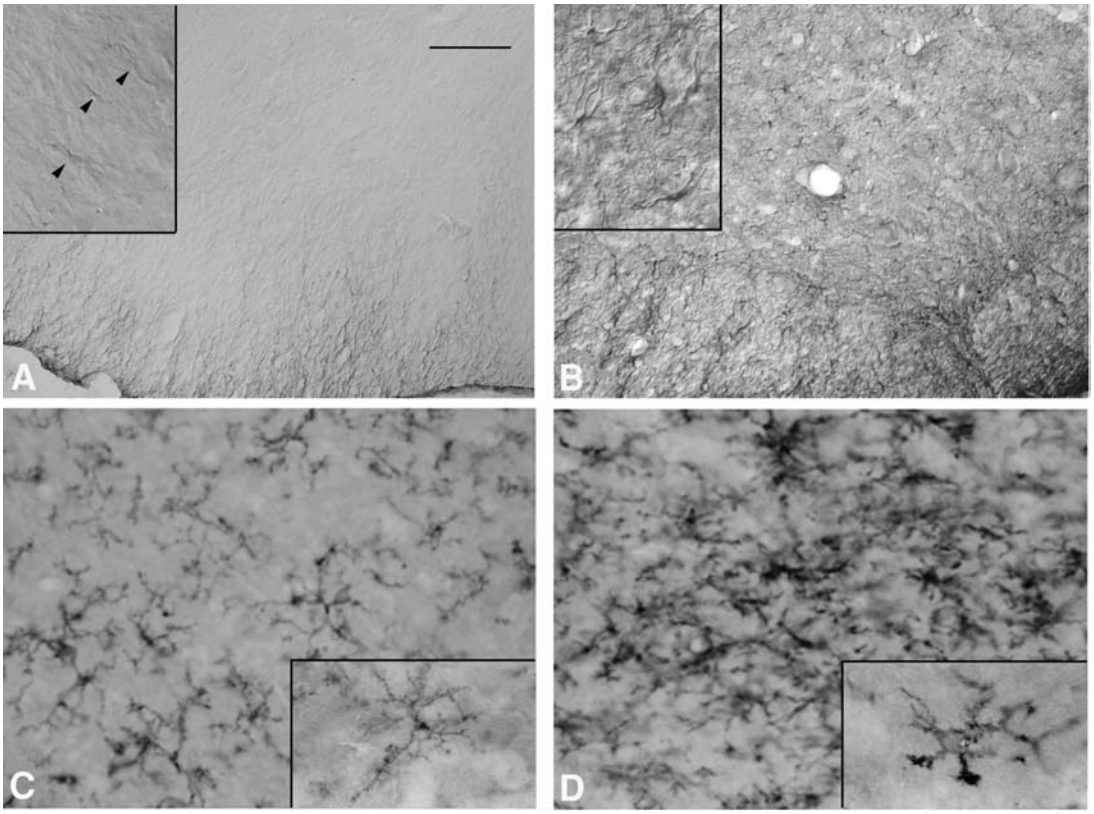

Fig. 9. Gliosis in the cervical spinal cord of wobbler mice during the evolutionary phase of the disease. **(A)** and **(B):** GFAP-immunoreactive labeling in the ventral horn of the cervical spinal cord of an 8-wk-old control **(A)** and wobbler **(B)** mouse. Nomarski micrographs: Note the numerous GFAP-immunoreactive astrocytes present in the gray matter of the wobbler spinal cord and their hypertrophic morphology (inset in **B**) as compared with the thin and weakly GFAP-labeled astrocyte processes in the gray matter of control spinal cord (inset in **A,** arrowhead). Immunohistochemical procedures were as described in Junier et al. (1994) *(96).* **(C)** and **(D)** Mac-I-immunoreactive microglial cells in the ventral horn of the cervical spinal cord of 6-wk-old control **(C)** and wobbler **(D)** mice. The morphological differences of the activated microglial cells—e.g., the enlarged cell body and the thicker and shorter processes as shown in insets of **C** and **D**—are visible as soon as at 3 wk of age in the wobbler cervical spinal cord. Adapted from Boillée and colleagues (2001) *(166).* Scale bar = 100 µm in **A** and **B,** 35 µm in insets of **A** and **B,** 28 µm in **C** and **D,** 32 µm in insets of **C** and **D.**

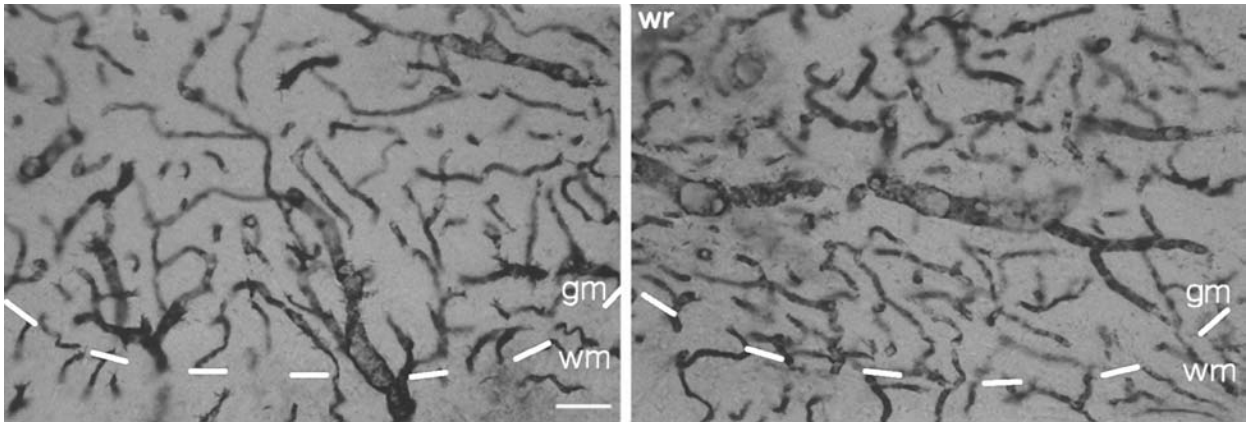

Fig. 10. Visualization of the blood brain barrier in the ventral part of the cervical spinal cord of HRP injected 7-wk-old control (left panel) and *wobbler* (right panel) mice. The procedures are described in Boillée et al. (2001) *(166),* and use 3,3′,5,5′ tetramethyl benzidine (TMB) as the substrate for peroxidase following the protocol of Mesulam (1978) *(194).* gm, gray matter; wm, white matter. Scale bar = 50 µm.

Toward the end of the evolutionary stage, reactive microglial cells and reactive astrocytes are present throughout the ventral and dorsal horns of the spinal cord *(166).* Despite their abundance, microglial cells scarcely display the typical rounded shape attributed to microglial cells engaged in phagocytosis *(166,188,189).*

Some of the molecular changes detected in the wobbler spinal cord have been related to the development of gliosis. Prior to the onset of astrogliosis, degenerating motoneurons express TGF-α, a chemokine endowed with glio-trophic properties *(100),* and the number of TGF-α expressing motoneurons is at its peak when activated astrocytes, which bear the TGF-α. receptor, invade the spinal cord *(96).* At that time, the reactive astrocytes also express TGF-α. These observations, coupled with the demonstration that TGF-α overexpression is sufficient to trigger astrogliosis in the CNS *(101),* have led us to propose that TGF-α is one of the neuronal inducers of astrogliosis in wobbler mice *(96).* Another putative candidate for this role is the cytokine TNFα. Although its main cellular sources correspond to astrocytes and microglial cells, it is also detected in degenerating motoneurons during the evolutionary phase of the disease *(107,195).* However, the comparison of the time-courses of synthesis of the cytokine and its receptors with that of the degeneration process has not been made. Gliosis is often accompanied by enhanced protease activities, believed to underlie extracellular matrix (ECM) remodeling consecutive to brain injury *(196).* In the wobbler spinal cord, the expression of two metalloproteinases, MT1-MMP and ADAM8, is enhanced. These proteins are expressed by reactive astrocytes and by both reactive astrocytes and microglial cells, respectively *(107,197).* The capability of TNF-α to enhance ADAM8 expression in cultures of astrocytes derived from wobbler mice that are sacrificed during the evolutionary phase of the disease *(107)* indicates that astroglia may indeed be a target of this cytokine. Astrocytes are at the core of the biological system that ensures the maintenance of glutamate to nontoxic extracellular levels in the CNS *(198).* Excessive excitatory inputs may be lethal to neurons *(199),* and an excitotoxicity caused by extracellular glutamate accumulations has been repeatedly evoked as one of the components of various degenerative processes including ALS *(4).* A comparison of cultured astrocytes derived from 1-mo-old wobbler and control spinal cords has revealed higher glutamate and glutamine levels in the extracellular medium of wobbler cultures, accompanied by decreased intracellular levels of glutamate, although this imbalance could be reversed by manipulation of glutamine concentration in the medium *(200).* This anomaly in astroglial glutamate metabolism in vitro has not been confirmed in vivo. The spinal cord glutamate contents are nearly normal, although slightly decreased *(119);* the protein levels of the glial glutamate transporters GLAST and GLT-1 are unchanged *(201),* and the glutamate-binding sites appear unaltered *(129).* Excitotoxicity therefore does not appear to be a major component of the wobbler degeneration process.

The fact that astrogliosis is not confined to the area of neurodegeneration, but rather extends to the dorsal part of the spinal cord, has led to researchers to contend that astrocytes could themselves be targets for a functional effect of the wobbler mutation *(189).* Arguments in favor of this hypothesis have been presented using cultures of astrocytes derived from 1-mo-old wobbler spinal cords. As compared to astrocytes obtained from age-matched healthy spinal cords, the wobbler astrocytes exhibit a reduced proliferation and an altered response to mitogens, and a glutamate metabolism imbalance *(202).* Some morphological changes are also observed—e.g., fewer contacts formed between the cells *(190).* In addition, these astrocytes differ from the control astrocytes because of their higher release of the IL-1β and TNF-α cytokines *(195)* and their decreased capacity to promote the survival of purified motoneurons in culture *(203).* However, it is not possible to conclude whether these differences are specific to wobbler astrocytes or are associated with a particular stage of astroglial activation. The rapid

expansion of astrogliosis to the dorsal horn could alternatively be linked to alterations in interneurons and in the extrapyramidal pathways, or to a spread of activating signals through the astrocyte network. Nevertheless, the observation of TUNEL-positive glial cells at the end of the presymptomatic phase *(47)* and during the evolutionary phase of the disease *(109)* supports the hypothesis of an endogenous defect in wobbler astrocytes. Along these lines, it is interesting to note that motoneuron degeneration linked to SOD mutation does not occur in mouse models, when the expression of the transgene is restricted to either neurons (although at low levels) or astrocytes *(204,205).* Although speculative, one possible interpretation of these results is a need for collaboration between glial cells and neurons for the full manifestation of degenerative processes.

Motoneuron degeneration is thus accompanied in the wobbler mouse by a reactivity of astrocytes and microglial cells. No astroglial or microglial reactivity has been reported to occur prior to the onset of histological signs of motoneuron affection, indicating that glial activation is consecutive to the neurodegeneration. Although no candidate for the role of neuronal inductor of microgliosis has been identified yet, the discrete location of the few activated microglial cells present at the beginning of the evolutionary phase in the proximity to morphologically affected motoneurons suggests that such a neuronal signal exists. Whether further interactions between microglial cells and astrocytes contribute to the subsequent reinforcement of the gliosis remains unknown. Astrogliosis and microgliosis may be independent phenomena—as shown, for instance, by transgenic mice that overexpress TGF-α or invalidated for the Neurofibromatosis type 1 gene, that exhibit a major activation of astrocytes in the absence of microgliosis *(101,206).* Likewise, the osteopetrotic mice, characterized by a defect in macrophage-colony-stimulating-factor expression, exhibit a downregulated microglial activation following axotomy, while astrogliosis is normal *(207).* The beneficial or detrimental nature of the potential effects of reactive glial cells upon neurons remains a matter of debate *(185,188,208–211).* The only study to address this issue in wobbler mice has yielded complex results, indicating the dual effects of reactive astrocytes on motoneurons, depending upon the presence or not of additional cell types *(203).*

The Phase of Clinical Stabilization is Characterized by the Arrest of Motoneuron Degeneration

The increase in motoneuron loss stops in wobbler mice after a few weeks. In the median nerve nucleus, the number of motoneurons is not modified after the sixth week *(34)* (Fig. 5). The number of the motoneurons that send their axons through the musculo-cutaneous nerve is identical at the age of 6–8 mo and 2–4 mo *(212).* Some vacuolated motoneurons may still be visible in aged wobbler mice in the cervical part of the spinal cord *(33,96),* but this is a very rare event at that stage. A precise quantification of the phenomenon *(117)* revealed that approx 9% of the motoneurons were vacuolated between 1 mo and 8 mo of age, whereas at 14 mo, the proportion was equal to 0. Large- and medium-sized motoneurons in "stabilized" wobbler mice (2–6 mo of age) present a modified neurite arborization, characterized by shorter and thinner dendrites and a reduction in the density of the dendritic spines and varicosities *(213).* In accordance with the lack of degenerating motoneurons, the molecules that are specifically expressed by the affected cells disappear *(96,112).* Spinal motor nerves present signs of degeneration in stabilized wobbler mice, and the number of motor nerve fibers is markedly reduced during the advanced stage of the disease *(33,46,144).* The volume of the spinal cord is reduced by a factor two in the wobbler mice compared to controls. This reduction comprises both the dorsal and ventral horns *(33,166,189,213),* and appears mainly as a result of cell loss, rather than as a side

effect of the impaired growth of the mutants. Indeed, 3-wk-old wobbler mice are often already smaller than their healthy litter mates *(40)*, yet the volume of their spinal cord is unchanged *(166)*. One intriguing fact is the escape from the degeneration process of a large part of the motoneurons within the cervical spinal cord population and the brainstem motor nuclei. This selective neurodegeneration may reflect a preferential use of the molecule(s) affected by the wobbler mutation by a subpopulation of motoneurons, or an enhanced vulnerability of part of the motoneurons to the mutation. It is not rare to find a molecular defect in a whole population of neurons while only part of them degenerate. This is well-exemplified by familial ALS-linked to SOD1 mutations and SMA, the mutated genes being expressed in both cases in a ubiquitous manner throughout the organism *(214,215)*. The sheer size of the motoneurons, and thus their high metabolic requirement, are often considered to be among the main factors underlying their sensitivity to a broad range of insults. However, this cannot account for the selectivity of most motoneuron degenerative diseases, which do not affect the whole population of spinal cord and brainstem motoneurons in a similar manner. The tremendous advances in the identification of the different transcription factors that control the genesis of motoneurons may help to address this issue. These studies have revealed that combinatorial expressions of these factors may distinguish subpopulations of motoneurons *(216,217)*. The use of such markers could help to identify the precise type(s) of motoneurons that are affected by degeneration in the wobbler mouse.

Muscular Alteration Becomes Significant After Motoneuron Degeneration is Established

Changes in muscle structure are not visible prior to the evolutionary phase of the disease *(33)*. During this period, progressive motoneuron degeneration is associated with muscular atrophy. This atrophy occurs in the same progressive manner as motoneurons degeneration. It affects the muscles of the head, the neck, the shoulders, and the forelimbs, in a symmetrical way, and less significantly, the posterior muscles. The muscles of the limbs in proximal position are more severely atrophied than those located in distal position. The muscles of the trunk and the diaphragm are spared *(33)*. The weight of the muscles is decreased, as well as the total number of muscle fibers *(218)*. Atrophied muscle fibers are visible from the 6th wk. The affected cells are first located at the periphery of the muscle fascicles, and gradually form clusters of abnormal fibers. These anomalies of muscle fibers are characterized by a reduction in their diameter, a widening of sarcolemmal nuclei with prominent nucleoli often located in central positions *(33)*, and a reduction in the length of the post-junctional membrane *(218)*. A study in the lateral rectus muscle in 4-wk-old to 2-mo-old wobbler mice showed that the three types of muscle fibers—distinguished according to their level of succinate dehydrogenase activity—were present in the same proportions as in the control mice *(218)*. However, a more recent study showed a conversion of the contractile and biochemical properties of the muscle fibers from a rapid to a slow type in the solear (muscle of the lower limb) and sternocleidomastoid (muscle of the shoulder) muscles of 5- to 7-wk-old mice *(219)* (Table 3). The muscles of aged wobbler mice display a mixture of normal and affected areas *(224)*. The size of the muscle fibers and of the end plates is decreased *(145)*. Anomalies of the neuromuscular synapses have been detected during both the evolutionary and stabilization phases of the disease. At the end of the evolutionary phase, 90% of the neuromuscular junctions are deprived of the neuronal-cell adhesion molecule (N-CAM) and have a reduced acetylcholine esterase activity. A reorganization of the neuromuscular contacts is also observed. An increase in the rate of acetylcholine receptors (AchR) is associated with the constitution of ectopic clusters of these recep-

Table 3
Molecular Changes Observed in the Muscles and the Intramuscular Nerves of the Wobbler Mouse

Molecule	Variation	Tissue	Phase	Method	References
ChAT	–	Biceps, gastrocnemius	E	Activity	*101*
Ach receptor	+	Biceps	E-S	Binding	*168,220*
Act α receptor	+	Triceps		RNA: Northern	
α-endorphin	+	IntraM soleus Nerve	E-S	ICC	*221,222*
β-endorphin	+	IntraM biceps Nerve	?		
α-MSH/ACTH	+	IntraM biceps Nerve	?		
α-melanotropin	+	IntraM soleus Nerve	E-S		
β endorphin receptor	+	Biceps	?	Binding	*221*
ACTH Receptor					
ClC-1 (chloride channel)	–	Triceps	E	RNA: Northern	*220*
parvalbumin	–	Triceps	E	RNA: Northern	*220*
Myogenin I	+	Triceps	E-S	RNA: Northern	*220*
Myosin:			E	Protein: Western	*219*
Heavy slow MHCI	+	Soleus, SCM			
Heavy fast MHCIIA	–	Soleus, SCM			
Light	0	Soleus			
	+	SCM			
Troponin C: slow	+	Soleus, SCM	E	Protein: Western	*219*
Troponin C: fast	0				
N-CAM, PSA-NCAM	+	Biceps	E-S	ICC	*168*
Urokinase plasminogen	+	Biceps, gastrocnemius	E	Protein: Western	*130*
LDH	0	Biceps	P	Activity: OD	*223*
	–		E-S		
Acidic protease	+	Forelimb muscles	E	Activity: OD	*122*

The variations as compared to control littermates are symbolized with + (increase), – (decrease), 0 (no change). SCM: sternocleidomastoid muscle. ICC: immunocytochemistry. Northern: RNA levels were evaluated using Northern blot assays. Western: protein levels were evaluated using Western blot assays. IntraM, intramuscular. *See* text for further explanations.

tors. The increasing levels of AchR, of the protein N-CAM and its polysialylated form (PSA-N-CAM), and the growth of terminal axonal branches observed in the wobbler, support the existence of a muscular denervation/reinnervation phenomenon in the mutant *(168).*

Therapeutic Trials in Wobbler Mice

Wobbler mice have been used to assay the efficacy of a number of drug treatments on the course of the motoneuron degeneration (Table 4). The majority of these molecules were given after the symptoms first appears, and their effects were evaluated for muscular strength. For some of them, the morphology of the muscle fibers and the number of motoneurons were also analyzed. Only some antioxidants and neurotrophic factors have exhibited some efficacy. The effects of the antioxidant molecules have been reported on pp. 62–64. The neurotrophic factors tested on the mutant—i.e., the neurotrophin BDNF and the cytokines CNTF, CDF/LIF, and IL-6, were chosen for their previously demonstrated ability to protect motoneurons from programmed developmental cell death. The isolated subcutaneous injection of each of these proteins led to only limited clinical improvement *(226–228,231,235),*

Table 4
Therapeutic Trials in the Wobbler Mouse

		Treatment		Test							
	Factor	Dose	Posology	Body weight	Forelimbs strength	Muscle mass or atrophy	Axons number	Axons caliber	Axons growth	Moto-neurons number	Refs
Anti-ox	PC-SOD[1,a]	10^4 ; 10^5U/Kg	Twice/d, ip		+	+				+	*182*
	N-acetyl-L-cystein[2,b]	1%	Water bottle		+	+		+		+	*183*
	OPC-14117[1,c]	10; 30 mg/Kg	Once/d, oral		+	+				+	*225*
	L-NAME[1,a]	50 mg/Kg	Once/d, ip		+	+				+	*181*
	7-nitroindazole[1,a]	5; 50 mg/Kg	Once/d, ip		+	+				+	*181*
NF	CNTF[1,a] (rat or human)	1 mg/Kg	3 ×/wk, sc	–(rat CNTF)	+	+	+		+	0	*226, 227*
	BDNF[1,a]	5 mg/Kg	3 ×/wk, sc		+	+	+			0	*228*
	CNTF + BDNF[1,a]	1 mg/Kg (CNTF) 5 mg/Kg (BDNF)	3 ×/wk, sc		+	+	+	+		+	*229* *229*
	CDF/LIF[1,a]	10 µg/Kg	3 ×/wk, sc		+	+	+			+	*230*
	IL-6 + sIL-6R[1,a]	1 mg/Kg (IL6)	Once/d, sc		+	+				+	*231*
	IL-6 or sIL-6R[1,a]	0,5 mg/Kg (IL-6R)	Once/d, sc		0	0				0	*231*
	IGF-I[1,b]	1 mg/Kg	Once/d, sc	+	+	+				0	*232*
	IGF-1 or GAGs[1,a]	Same as below	Once/d, sc	0	+	0/+			+		*233*
	IGF-1[1,b]	20 µg/Kg (IFG-1)	Once/d, sc	+	0				+	+	*234*
	GAGs[1,b]	1 mg/Kg (GAGs)	Once/d, sc	0	0					+	*234*
	IGF-I + GAGs[1,b]	Same as above	Once/d, sc	+	+	+				+	*234*
	bFGF[1,a]	1 mg/Kg	Once/wk, sc		+	+				+	*235*
	CT-1[1,a]	1 mg/Kg	Once/d, sc		+	+	+		+	0	*236*
Anti-ex	MK-801[3,c]	0,25; 0,5 mg/Kg	Once/d, sc		0						*237*
Others	T-588[1,a]	3; 10; 30 mg/Kg	Once/d, oral		+	+					*238*
	TRH[1,b]	50 mg/Kg	Once/d, ip		0						*239*
	JTP-2942[1,a]	2 mg/Kg	Once/d, ip		+	+				+	*240*
	Gangliosids[1,c]	50 mg/Kg	Once/d, sc		0						*241*

+, increase or improvement, 0, no effect, – decrease. Anti-ox: anti-oxidant molecules. NF: neurotrophic factors. Anti-ex: anti-excitatory molecules. L-NAME: Ng-nitro-L-argine-methyl ester is a NOS inhibitor and 7-nitroindazole is a neuronal NOS inhibitor. GAGs: glycosaminoglycans. CT-1: cardiotrophin-1. MK-801 is a NMDA receptor Antagonist. T-588: R(–)-1-(benzo [b] thiophen-5yl)-2-[2-(*N*,*N*-diethylamino) ethoxy] ethanol hydrochloride. JTP-2942 is a TRH analog. [1]treatment started at 3–4 wks of age, [2]treatment started at birth, [3]treatment started a 4 d of age. [a]3–4 wks long treatment, [b]6–9 wks long treatment, [c]≥ 3 month long treatment. ip: intraperitoneal injections. sc: subcutaneous injectons. *See* text for further explanations.

and motoneuron degeneration appeared to proceed unchanged. In contrast, the combined administration of BDNF and CNTF or of IL-6 and its soluble receptor sIL-6 R, has resulted in a clear behavioral improvement, associated with increased survival of the motoneurons *(229,231).* The subcutaneous co-administration of IGF-1 and glycosaminoglycans (GAGs) has resulted in an arrest of motoneuron degeneration during the 6 wk of treatment *(234).* Neurotrophic factors may thus have the capacity to stop the pathological death of motoneurons when they are suitably associated.

Despite these encouraging results in the wobbler mouse, the clinical trials of some of these molecules in ALS patients have not had significant success *(242–253).*

Conclusions

Since the initial identification of wobbler mutants, numerous studies have contributed to a better understanding of the origin of the disease, a precise knowledge of its developmental time-course, and the delineation of the nature of the pathological processes that may lead to neurodegeneration. Wobbler is now established as a neuronopathy, the degeneration of the perykaria preceding that of the axons. The finding that cortical neurons are also targets of the wobbler mutation allows researchers to view wobbler as a pathology resembling the human ALS, also characterized by a degeneration of both the upper and lower motoneurons.

The observation that motoneurons remain in altered states during various lengths of time before their final disappearance is, in our view, one of the most interesting outcomes of the exploration of the wobbler mouse. This finding implies that the clinical symptoms may appear while large numbers of motoneurons are still present and apparently alive, and that the mutation does not affect the entire population of neurons fated to die at the same time. This progressive nature of neurodegeneration contrasts sharply with the massive and temporally correlated disappearance of the motoneurons during developmental programmed death, or following axotomy in the newborn. Accordingly, the exploration of the death pathways mobilized in wobbler motoneurons has shown that they are not classical apoptosis pathways. As previously stated, the progressive nature of the neurodegenerative process may be viewed as the result of the time lag necessary for the full development of the toxicity of the mutation, and the overtaking of any compensatory metabolic attempts to restore the cellular homeostasis. The observation of diverse molecular anomalies prior to the appearance of the first histological signs of the disease suggests that the molecular defects require time to exert their full deleterious effect. On the other hand, the enhanced synthesis of growth factors endowed with neuro- and glio-trophic properties by motoneurons already engaged in the neurodegenerative process may indicate an attempt to counterbalance the toxic effects of the mutation. The fact that motoneurons submitted to a degenerative process do not remain "passive" but actively engage in novel syntheses during this intermediate period of motoneuronal suffering reinforces the importance of the need to explore the dynamic molecular evolutions that take place within the cell at that time. This period appears to be the most accessible time window for a therapeutic intervention, since the clinical symptoms are already discernable. Indeed, some of the attempts to counteract the wobbler neurodegeneration after the clinical onset of the disease have been successful.

Another important result of the studies performed on this mutant mouse is the disclosure of several similarities between the metabolic changes observed in wobbler motoneurons and those detected in other models of motoneuronal injuries, regardless of the traumatic or genetic nature of this injury. The fact that entirely different types of insults may trigger the same response in motoneurons, regardless of their anatomical localization, suggests that the adult motoneuron response to pathological situations is, at least in part, stereotyped. Some

of these similarities, best exemplified by the presence of ubiquitinylated protein aggregates, extend beyond the motoneuron pathologies, and are found in many other neurodegenerative diseases. These observations raise the possibility of designing treatments that would not depend on the prior knowledge of the identity of the mutation, a critical point for pathologies such as ALS, for which 90% of the cases are sporadic and of unknown origin.

The review of studies devoted to the wobbler mouse shows that there are numerous questions yet to be answered or without an answer. The wobbler mutation must be identified, but the lack of alteration in the sequence encompassing the wobbler locus defined by positional cloning emphasizes the difficulty of the task. The etiopathological mechanisms must also be defined. The available data do not favor the existence of an excitotoxicity resulting from increased levels of excitatory inputs to motoneurons. On the opposite, data have been provided in support of the other two classical etiopathological mechanisms believed to underlie neurodegeneration processes—e.g., oxidative damage linked to defective free radical detoxification pathways and accumulation of toxic protein aggregates caused by altered chaperone activity. The works published on these processes are nevertheless descriptive, and a functional demonstration of the involvement of these pathways has not yet been provided. As in human ALS, gliosis develops in the wobbler, and this phenomenon has clearly been shown to follow temporally motoneuronal degeneration. The synthesis of various proteins by wobbler motoneurons endowed with the capability to affect their glial environment raises the possibility that reactive glial cells may participate in the degenerative process. Here again, the functionality of such interactions has not been provided and, even more so, their positive or negative influences on the course of the degeneration. The difficulty to deliver pharmacological compounds in a controlled manner to the spinal cord without triggering further damage to the tissue is at the core of this lack of functional studies in wobbler mice. The breed of the mutant with strains bearing the adequate modified genes, for example, an overexpressed chaperone protein, may offer one solution. Another possibility is raised by preliminary results indicating that the wobbler neurodegeneration is maintained in vitro.

Despite all these unanswered questions, the various studies reviewed here reveal the value of exploring in a thorough manner a mouse model of motoneuron degeneration such as wobbler. The results gathered have already provided a dynamic view of the degenerative process that is inaccessible in humans, and revealed that motoneuron degeneration may share pathways during their course to death, regardless of the identity of the initial insult. Further exploration of the wobbler mouse is still likely to provide new insights into our understanding of motoneuron degenerative diseases, and hopefully, keys to their treatment.

Acknowledgments

We are deeply indebted to the members of our laboratory who participated in the studies on the wobbler mouse, and particularly to Gaël Grannec. Our research was supported by the Association Française Contre les Myopathies (AFM).

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