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Neuron volume in the ventral horn in Wobbler mouse motoneuron disease: a light microscope stereological study

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ABSTRACT

Previous pathological reports have indicated that swollen and vacuolated motoneuron cell bodies are the most predominant feature characterising Wobbler mouse motoneuron disease, but there has been little supportive evidence using area measurements. The present study focuses on the possible role of changes in neuronal nuclear and perikaryal volumes in the cervical spinal cord ventral horn, using new and traditional stereological probes which provide unbiased estimates of volume. Semithin sections from the ventral horn of Wobbler mice and age and sex-matched phenotypically normal littermates were examined at 2 ages (young and old). The young Wobbler group had significantly larger volume weighted mean perikaryal volumes compared with age-matched controls, reflecting the presence of large swollen cells characteristic of this group; this situation was reversed in the control group. Number-weighted perikaryal volume estimates in the old Wobbler group were smaller than in age-matched controls. The variation in perikaryal volume was greatest in the young Wobbler group in which the coefficient of variation was 127%. The mean number weighted and volume weighted mean nuclear volumes were significantly smaller in the old Wobbler group compared with age-matched controls and young Wobbler groups. The application of new stereological probes has enabled us to document more precisely these changes in neuronal structure in the Wobbler mutant mouse.

Key words: Spinal muscular atrophy; amyotrophic lateral sclerosis.

INTRODUCTION

The homozygous Wobbler mouse (*wr*) (Falconer, 1956) may represent a useful model for the investigation of human inherited motoneuron diseases (MNDs), including amyotrophic lateral sclerosis (ALS) and infantile spinal muscular atrophy (SMA) (Leestma, 1980; Murakami et al. 1981; Krieger et al. 1991). The clinicopathological features of ALS differ from Wobbler MND in the involvement of the corticospinal tract in the human disease (Bradley, 1984). Wobbler MND represents an example of spinobulbar muscular atrophy. Nevertheless, the pathogenesis of the mouse and human diseases may have some important common features. Furthermore, the limited availability of human tissues in the early stages of ALS, when therapeutic intervention may be of greatest value, emphasises the importance of using small animal models.

Some families of human familial ALS are known to be associated with mutations in the gene for copper/zinc superoxide dismutase (*SOD 1*) (Siddique et al. 1991). It has recently been shown that the majority of cases of infantile SMA are related to mutations in a gene known as SMN1 (survival motor neuron 1) (Lefebvre et al. 1995) and that severity may be determined by a neighbouring gene neuronal apoptosis inhibitory protein (NAIP) (Roy et al. 1995). Aetiology of ALS and of the motoneuron disease in the mouse mutant otherwise remains unknown. Several possible mechanisms have been proposed (Bradley & Krasin, 1982; Davidson & Hartmann, 1981; Murakami et al. 1981). In ALS, abnormal DNA transcription, due to a defect in the enzyme repair system in the motoneurons, has been suggested as a primary abnormality (Bradley & Krasin, 1982). Furthermore, decreases have been reported in the adenine and the adenine-uridylic acid (A/U) ratio of the RNA base molecules

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and the total RNA content taken from cervical spinal motoneurons (Davidson & Hartmann, 1981; Davidson et al. 1981). In the Wobbler mouse (C57B1/6N strain), decreased nuclear and nucleolar uptake of RNA precursors has been detected in normal-looking, as well as diseased, motoneurons in the cervical spinal cord (Murakami et al. 1981). This has led to the speculation that the decreased protein synthesis measured in the Wobbler MND is due to an early impaired synthesis of ribosomal and messenger RNA within the nucleus (Murakami et al. 1980). Collectively these reports indicate that the initial site for the pathogenesis of the Wobbler and human MND lies in the nucleus. Whilst nuclear volume reductions have been documented as an initial event in human motoneurons affected by ALS (Mann & Yates, 1974), there are no reports of significant nuclear size reductions in Wobbler MND (Murakami *et al*. 1980).

Light and electron microscope studies have revealed that the motoneurons in the Wobbler mouse cervical spinal cord and the brainstem are primarily affected (Andrews & Maxwell, 1967; Duchen & Strich, 1968; Andrews, 1975). Despite reports that the large, presumably α-motoneurons are the primary targets of Wobbler MND (Murakami, 1980; Ma & Vacca-Galloway, 1991*a*), there are indications that the medium α-motoneurons and the small γ-motoneurons, as well as the interneurons, are also affected (Murakami et al. 1980; Ma & Vacca-Galloway, 1991*b*, 1992). Histopathologically, perikaryal swelling and vacuolation have long been observed in the early stages of the Wobbler disease, followed by atrophy and the subsequent loss of motoneurons (Duchen & Strich, 1968; Andrews et al. 1974). However, these early reports lack quantitative data. In recent years, there have been very few quantitative studies using the Wobbler mutant. Applying various morphological approaches, including horseradish peroxidase tracer (HRP) and conventional histological sections, previous studies have focused on the extent of motoneuron loss (Papapetropoulos & Bradley, 1972; Baulac et al. 1983; LaVail et al. 1987; Cheng et al. 1990; Pollin et al. 1990). Electron microscopy and light microscopic Golgi studies have revealed defects within the motoneuron axons (Biscoe & Lewkowicz, 1982) and dendrites (Ma & Vacca-Galloway, 1991*a*, *b*). But there is a paucity of reports relating to the cytological changes that occur within the cell body. Based on qualitative observations, early cellular events in Wobbler MND include cytoplasmic swelling and vacuolation, whereas degenerative events predominate during the later stages (Duchen & Strich, 1968; Andrews et al. 1974). Quantitative evidence of cytoplasmic shrinkage was suggested by our previous Golgi study (Ma & Vacca-Galloway, 1991*a*). Another quantitative account of early cytoplasmic swelling (Ma & Vacca-Galloway, 1991*b*) suggested that only the large α-motoneurons are affected.

Using computerised morphometry, LaVail et al. (1987) reported no differences in the mean soma area measured from abducens neurons studied from young (aged 24–36 d) Wobbler (C57B1/6N strain) mice compared with the normal phenotype littermates. Using a similar computer-aided approach to study cervical spinal motoneurons, Ma & Vacca-Galloway (1991*a*) reported that the young Wobbler (NFR/*wr* strain) specimens (aged 21 d) taken at an early stage (Stage 1) of the MND possessed measurably enlarged somata within the category of large α-motoneurons. Applying volumetric measurements, Baulac et al. (1983) reported that significant cell swelling could be detected in the cervical spinal motoneurons studied from older (2–7 mo) Wobblers (C57B1/6N strain). In contrast, Ma & Vacca-Galloway (1991*b*) found no indications of cell size changes in older (aged 2–6 mo), although a distinct trend towards perikaryal shrinkage was noted among Wobbler specimens from a different strain (NFR/*wr*). Such diverse and contradictory findings may be due to differences in the measuring techniques employed, although strain and age differences cannot be discounted. Nevertheless, the varied data prevent clear conclusions being drawn about soma size during the course of the Wobbler MND.

All previous quantitative studies of soma size possess flaws in methodology; in particular, assumptions have been made on the shape of the cells and there is an inherent bias in the sampling strategies employed (see Baulac et al. 1983; LaVail et al. 1987; Ma & Vacca-Galloway, 1991*a*, *b*, 1992). The introduction of the new stereological probes has transformed the quantitation of neural structure, allowing assessment of size without assumptions about shape. These new methods are in general unbiased (Gundersen et al. 1988*a*; Mayhew, 1989). The primary aim of the present study was to determine whether or not nuclear and perikaryal size changes could be detected in the cervical spinal ventral horn of the Wobbler mouse taken at early (Stage 1, age 18–22 d) and late stages (Stage 4, age 3 mo) of the Wobbler MND. We have made 2 estimates of mean nuclear and perikaryal volume; the more usual number weighted mean volume estimate $\overline{V}_n$ and a volume weighted volume estimate $\overline{V}_v$. This latter estimate has been found to be of prognostic value in

cancer cell studies (Neilsen et al. 1986; Howard, 1986). Two populations of cells with the same mean volume but with differences in the variation in volume will be discriminated by the $\overline{V_v}$ but not $\overline{V_n}$ (Cruz-Orive & Weibel, 1990).

MATERIALS AND METHODS

Animals

Wobbler mice and their age and sex-matched normal phenotype littermates, male (n = 6) and female (n = 14) were used for the study. The animals were obtained from NIH stock (NFR/wr mouse strain, Bethesda, MD, USA) and raised in the Animal Laboratory Unit at the University of Hong Kong. The mice were first identified by behavioural tests. These tests were based on the assessment of front leg power, climbing and walking ability (Lange et al. 1983, as modified by Vacca-Galloway & Steinberg, 1986), as well as the presence or absence of the 'clasp knife reflex' response (Hanson, 1984). The Wobbler mice were then categorised into respective stages: early (Stage 1) and late (Stage 4) according to the extent of motor deficit (Vacca-Galloway & Steinberg, 1986). The mice identified at Stage 1 were aged 18–22 d and those at Stage 4 were 3 mo old. They are subsequently referred to as the 'young' and 'old' mice respectively. In both the young Wobbler and normal groups there were 3 females and 2 males and there were 4 females and 1 male in the older groups.

Tissue preparation and sampling

Prior to perfusion, the mice (n = 5/group) were anaesthetised with an intraperitoneal injection of 60% sodium pentobarbital (Nembutal) at a dosage of 60 mg/kg. They were then briefly perfused systemically through the left ventricle with 0.9% sodium chloride containing heparin (1 mg/ml), followed by a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 (Kidd et al. 1986). The cervical spinal cord (C3–C8) was removed and stored at 4 °C overnight in fresh fixative solution. The spinal cord was then cut transversely to its long axis into approximately 1 mm thick slices; these were trimmed under a binocular microscope to remove all but the ventral grey horn and the tissue slices were washed in several changes of 0.1 M phosphate-buffered saline (PBS), pH 7.4. Following postfixation in 2% osmium tetroxide for 2 h, the tissues were dehydrated in increasing grades of alcohol (75–100%) and haphazardly embedded in Epon 812 resin. This meant that the resultant sections were isotropic uniform and random in orientation (IUR). This randomisation of tissue organisation was essential for the application of the stereological probes used (Mayhew, 1989).

About 240 blocks were obtained, 12 blocks per animal. From these, 3 blocks per animal were selected by lottery. Sections (0.5 μm) for light microscopy were cut from the latter selected tissue blocks with glass knives using a Reichert OMU4 ultramicrotome. All sections were stained with toluidine blue.

Stereological methods

The study was confined to the neurons lying in the ventral grey horn region. For identification of neuronal profiles we used the morphological criteria described by Mayhew & Momoh (1974). No attempt was made to divide them into specific classes, either morphological (motoneurons, interneurons, Renshaw cells) or functional (large α or small γ). Following an initial randomisation, about 25 microscopic fields were selected in a systematic random fashion. Each field included of 3–5 cells. In a particular field, cells were selected regardless of their nuclear or nucleolar content. Using a drawing tube attachment and oil immersion, the neurons and their nuclei, when present, were traced at a final magnification of ×1667. These tracings were subsequently used to estimate the proportion of the neuron occupied by the nucleus and also nuclear and perikaryal volumes.

Nucleus: cell ratio (*Vv*). The volume proportion of the cell occupied by the nucleus was performed on the tracings using traditional point counting methods (Mayhew, 1991).

Volume estimates. Two stereological methods were employed to estimate nuclear volume by light microscopy.

1. *Volume-weighted mean volume* ($\overline{V_v}$ Nuc). This was determined by the point sampled intercept method (Gundersen et al. 1988*b*). A lattice of test points on lines was superimposed randomly onto the traced nuclear profiles in each particular field. When a point hit the nuclear profile, a line was drawn through the point from one nuclear margin to the other. These lines produced point-sampled intercepts whose length (lo) were measured, cubed and then the mean multiplied by $\pi/3$, and finally averaged over all intercepts to give an unbiased estimate of the volume-weighted mean nuclear volume ($\overline{V_v}$*Nuc*).

2. *Number-weighted mean nuclear volume* ($\overline{V_n}$ Nuc). The 'nucleator' principle (Gundersen, 1988) was used to estimate the number weighted nuclear volume. For this, neurons were sampled using physical disectors

(nucleoli were used as point identifiers (Mayhew, 1992). From 3 blocks of tissue from all animals in each group, a stack of tissue sections were cut from which pairs of sections (disectors) were chosen. An unbiased counting frame (Gundersen, 1977) was superimposed on one of the paired sections (the 'reference section'). At least 75 nuclei from each animal were selected for study if their nucleoli appeared in the reference section and not on the adjacent 'look up' section. The nuclear profiles of the selected nuclei were then traced and the position of the nucleolus noted. A 4-way nucleator was then applied to the profile and the distance measured from the centre of the nucleolus to the nuclear membrane (Gundersen, 1988; Tandrup, 1993). These lengths (lo) were measured, corrected for magnification, cubed and the mean multiplied by $\pi 4/3$ to give an unbiased estimate of individual nuclear volumes. The mean volume of selected nuclei gave an unbiased estimate of the number-weighted mean nuclear volume ($\overline{V_n}$ *Nuc*) for each animal in all groups.

The above 2 variables are related as follows: $\overline{V_v} = \overline{V_n}(1 + CV_n^2)$ (Gundersen & Jensen 1985; Cruz-Orive & Hunziker 1986) CV_n = coefficient of variation of the number weighted distribution.

Therefore, the distribution of nuclear volumes (CV_n %*Nuc*) can be estimated without having to measure every nucleus.

The $\overline{V_n}$ *Nuc* estimate for each animal was combined with the nucleus: cell ratio Vv to provide number weighted estimates of perikaryal (i.e. cell) volume ($\overline{V_n}$ *Peri*).

Volume weighted mean perikaryal volume ($\overline{V_v}$ Peri). The ($\overline{V_v}$ *Peri*) was obtained using the methods described by Mayhew (1989). The method applied was exactly the same as that used for nuclei (described above). For estimates of mean perikaryal volume, the cell body was truncated at the neurites (Mayhew, 1989).

Estimate of the perikaryal volume distribution (CV_n %Peri). An estimate of perikaryal volume, the distribution CV_n %*Peri* could then be calculated from the relationship $\overline{V_v} = \overline{V_n}(1 + CV_n^2)$ (Gundersen & Jensen, 1985; Cruz-Orive & Hunziker 1986).

Statistics

Values for each individual animal were used to derive group means, standard deviations (S.D.) for the young (Stage 1) and old (Stage 4) mice. Ratio estimates were logarithmically transformed prior to testing. A 2-way analysis of variance (ANOVA) was performed to assess the effect of age, disease and interaction. Comparisons between groups were undertaken by Tukey's test. A P value of $< 5\%$ was considered statistically significant. All statistical calculations were performed on an IBM PC using SPSS software.

RESULTS

Nucleus: cell ratio (Vv) (Tables 1, 2)

A 2-way ANOVA revealed no significant effect of age, disease or interaction on the proportion of the cell occupied by nucleus.

Table 2. *Vv Nucleus: cell*

	Control	Wobbler	*P*
Young	0.1538 ± 0.0196	0.1314 ± 0.0169	n.s.
Old	0.1439 ± 0.0145	0.1424 ± 0.0058	n.s.
P	n.s.	n.s.	

Values represent mean ± S.D. for 5 animals in each group.

Table 3. *Volume weighted mean nuclear volume* ($\overline{V_v}$ Nuc. μm^3)

	Control	Wobbler	*P*
Young	1618 ± 183	1831 ± 452	n.s.
Old	1777 ± 278	1106 ± 269	< 0.05
P	n.s.	< 0.05	

Values represent mean ± S.D. for 5 animals in each group.

Table 1. *Two-way analysis of variance: F values*

Feature	Age d.f. = 1, 16	*P*	Disease d.f. = 1, 16	*P*	Interaction d.f. = 1, 16	*P*
Vv Nucleus: cell	0.01	n.s.	3.11	n.s.	2.40	n.s.
$\overline{V_v}$ Nuc	4.13	n.s. (< 0.06)	2.70	n.s.	10.10	< 0.01
$\overline{V_n}$ Nuc	5.77	< 0.05	13.40	< 0.01	16.73	< 0.01
$\overline{V_n}$ Peri	4.10	n.s.	4.34	n.s. (< 0.06)	17.32	< 0.01
$\overline{V_v}$ Peri	21.57	< 0.01	0.02	n.s.	66.27	< 0.01

d.f., degrees of freedom numerator and denominator. Values represent data for 5 animals in each group.

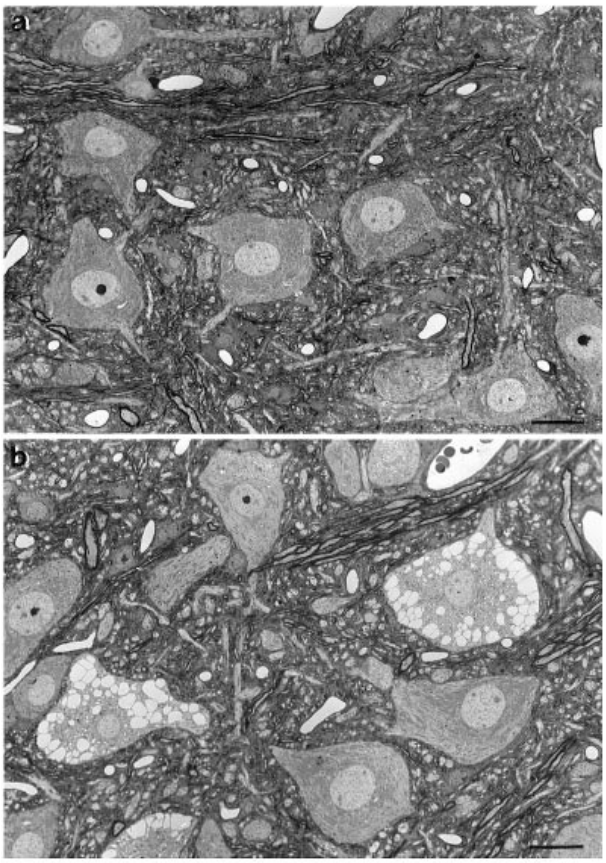

Fig. 1. Sections of ventral horn from young control mouse (*a*) and young Wobbler (*b*). Bars, 20 μm.

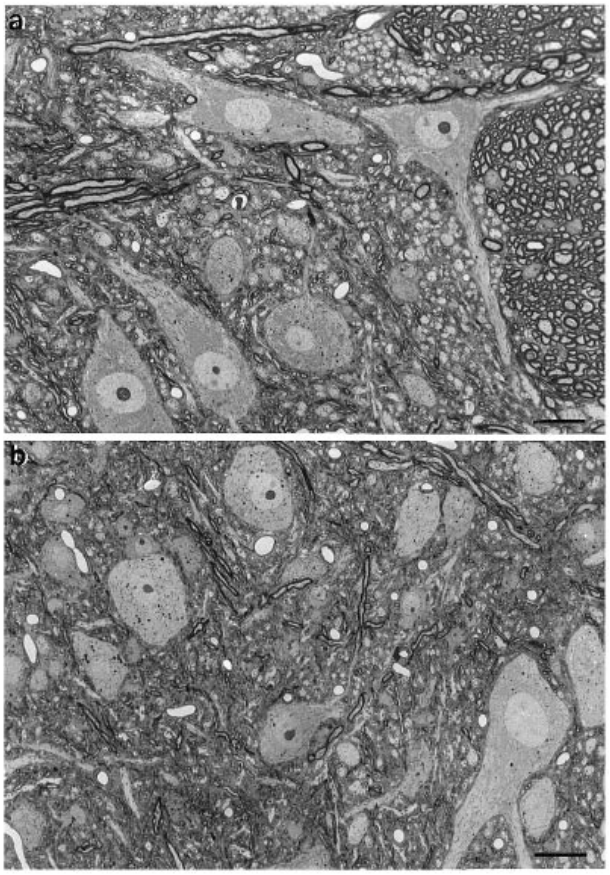

Fig. 2. Sections of ventral horn from old control mouse (*a*) and old Wobbler (*b*). Bars, 20 μm.

Table 4. *Number weighted mean nuclear volume*: ($\overline{V}_n$ Nuc. μm^3)

	Control	Wobbler	*P*
Young	1298 ± 90	1337 ± 1005	n.s.
Old	1450 ± 345	754 ± 151	< 0.05
P	n.s.	< 0.05	

Values represent mean ± S.D. for 5 animals in each group.

Table 5. *Nuclear volume: coefficient of variation for the number weighted distribution* (CV_n %)

	CV_n %
Young control	48 ± 13
Young Wobbler	54 ± 35
Old control	46 ± 26
Old Wobbler	65 ± 36

Values represent mean ± S.D. for 5 animals in each group.

Volume-weighted mean ($\overline{V}_v$ Nuc) (Tables 1, 3)

A 2-way ANOVA revealed a borderline effect of age ($P < 0.06$) and a significant interaction ($P < 0.01$). The mean nuclear volume in the Wobbler group was significantly smaller than young Wobbler (Fig. 1) and the age matched control.

Number-weighted mean nuclear volume: ($\overline{V}_n$ Nuc) (Tables 1, 4)

A 2-way ANOVA revealed that the number weighted volume showed a significant age ($P < 0.05$) disease ($P < 0.01$) and interaction ($P < 0.01$). The old Wobbler (Fig. 2) group was significantly smaller than age matched controls and young Wobbler groups. This may reflect the fact that there has been loss of many of the large motoneurons.

Nuclear volume distribution CV_n %Nuc (Table 5)

The mean CV_n %*Nuc* for control and Wobbler groups are shown in Table 5. The control group CV_n % *Nuc* for control was found to be 48 and 46 % for young and old groups the corresponding means for the Wobbler groups were 54 and 65 %. It should be noted

Table 6. *Volume weighted mean perikaryal volume*: ($\overline{V}_v$ Peri. μm^3)

	Control	Wobbler	*P*
Young	16271 ± 1457	26451 ± 2696	< 0.05
Old	20727 ± 4411	10159 ± 1901	< 0.05
P	n.s.	< 0.05	

Values represent mean ± S.D. for 5 animals in each group.

Table 7. *Number weighted mean perikaryal volume*: ($\overline{V}_n$ Peri. μm^3)

	Control	Wobbler	*P*
Young	8544 ± 1128	10175 ± 1294	n.s.
Old	10221 ± 2869	5314 ± 1077	< 0.05
P	n.s.	< 0.05	

Values represent mean ± S.D. for 5 animals in each group.

Table 8. *Perikaryal volume: Coefficient of variation for the number weighted distribution* (CV_n %)

	CV_n %*Peri*
Young control	95 ± 17
Young Wobbler	127 ± 15
Old control	104 ± 34
Old Wobbler	96 ± 14

Values represent mean ± S.D. for 5 animals in each group.

that the CV % (i.e. (S.D. × 100)/mean) between animals was 25 % and 26 % for young and old control groups and 39 % and 53 % for young and old Wobbler groups.

Volume-weighted mean perikaryal volume ($\overline{V}_v$ Peri) (Tables 1, 6)

A 2-way ANOVA revealed a significant effect of age ($P < 0.01$) and interaction ($P < 0.01$) but no effect of disease. The $\overline{V}_v$ Peri of the young Wobbler group was significantly larger than in the controls, possibly reflecting the presence of large swollen cells. In the old groups this situation was reversed, possibly indicating the loss of these cells. There was a significant age-related decrease in the Wobbler group, perhaps reflecting the loss of the large cells.

Number-weighted mean perikaryal volume: ($\overline{V}_n$ Peri) (Tables 1, 7)

A 2-way ANOVA revealed a borderline effect of disease ($P < 0.06$) and a significant interaction ($P < 0.01$). The old Wobbler was significantly smaller than the control. This may indicate some involvement of the remaining small neurons in the disease process.

Perikaryal population distribution CV_n %Peri (Table 8)

The mean CV_n %*Peri* increase from 95 % to 104 % in the control animals, perhaps reflecting shifts in the ratio between large multipolar and small fusiform cells. The young Wobbler had the largest CV_n %*Peri* (127 %), reflecting the presence of large swollen cells within the population. In the old group CV_n %*Peri* fell to 96 %.

The *CV* % (i.e. (S.D. × 100)/mean) between animals was 17 % and 33 % for young and old control groups and 11 % and 15 % for young and old Wobbler groups.

DISCUSSION

In the present study we have used new and traditional stereological probes to document the structural changes in the cervical ventral horn neurons of the Wobbler mouse and in the phenotypically normal littermates. Other investigators have measured motor or ventral horn neurons based on size, their presumed function (Campa & Engel, 1971; Cheng et al. 1990; Ma & Vacca-Galloway, 1991*b*), or selected neuronal profiles sectioned through the nucleolar plane (LaVail et al. 1987; Papapetropoulos & Bradley, 1972; Pollin et al. 1990). Previous studies of cell volume in the Wobbler (Baulac et al. 1983) and human MND (Davidson et al. 1981), besides being indirect, largely depended on shape assumptions. Various shapes of spinal motoneurons have been described in human MND (Nakano & Hirano, 1987) and Wobbler mouse spinal cord (Ma & Vacca-Galloway, 1991*b*). In contrast, the approach in the present study introduces no bias in neuron selection. Indeed, biasing towards nuclear, or soma dimensions is known to furnish gross overestimates for important parameters such as cell or nuclear volume (Mayhew & Momoh, 1973). One of the major difficulties encountered in the present study was the lack of clear distinction between the motoneurons and the remaining neurons in the ventral grey horn. Because of the limitations imposed upon the precise identification of the neuronal types, the present study includes all the neurons in the entire ventral horn. The same problem has also plagued previous investigators (Papapetropoulos & Bradley, 1972; Ma & Vacca-Galloway, 1991*a*, 1992). Uncertainty still prevails as to which types of neurons are affected and to what extent, by the degenerative process. It is rather spurious to assume that the only loss is in ventral horn motoneurons. If there is loss of moto-

neurons it is likely that other neurons synapsing on them might also degenerate. Therefore the unbiased approach adopted in the present study seems all the more appropriate.

Perikaryal changes

Earlier quantitative reports in the Wobbler suggest that the large presumed α-motoneurons are the primary targets (Cheng et al. 1990; Ma & Vacca-Galloway, 1991*a*, *b*). The present study seems to confirm this as the age-related significant decrease in $\overline{V_v}$ *Peri* in the Wobbler group suggests a loss of large cells.

A 2-way ANOVA on $\overline{V_v}$ *Peri* data revealed a significant effect of age and interaction. The young Wobbler mouse measurement was significantly larger than the age-matched controls, probably reflecting the presence of the large swollen cells characteristic of the Wobbler MND in the early stages. This situation was reversed in the old age group, perhaps reflecting the loss of the larger cells. In human MND, the swelling of the motoneurons has been described as an initial 'rounding up' process, (Mann & Yates, 1974). Quantitatively, our data seem to support the swelling phenomenon. The $\overline{V_v}$ *Peri* in the young (Stage 1) Wobbler specimens was found to be significantly larger than in the controls. Thus both in human ALS as well as in the Wobbler MND, cytoplasmic swelling is an early feature. It should be noted that the parameter $\overline{V_v}$ *Peri* was able to discriminate between young control and Wobbler groups whereas $\overline{V_n}$ *Peri* was not, again illustrating the usefulness of this parameter (Cruz-Orive & Weibel, 1990).

The $\overline{V_n}$ *Peri* from the mice in the old Wobbler group was significantly smaller than age matched controls. This may suggest an involvement of smaller cell types (γ-motoneurons, interneurons, Renshaw cells) in the disease process. Studies conducted in our laboratory have shown that the Wobbler MND is not restricted to the large presumed α-motoneurons, but also involves small presumed γ-motoneurons, the spiny interneurons and even peptidergic and serotonergic neurons (Ma & Vacca-Galloway, 1992; Yung et al. 1992; Zhang & Vacca-Galloway, 1992). The old (Stage 4) Wobbler specimens had smaller perikaryal volumes, both $\overline{V_n}$ *Peri* and $\overline{V_v}$ *Peri*, compared with age-matched controls. Small-sized neurons with atrophic cell processes have been observed during the late stages of ALS (Nakano & Hirano, 1987) and for the spiny interneurons of the old (aged 3 mo) Wobbler mouse spinal cord (Ma & Vacca-Galloway, 1991*b*). As in ALS, the large α-motoneurons in old Wobbler specimens also possess atrophic processes (Ma & Vacca-Galloway, 1991*a*). It has been documented that the spiny interneurons shrink during the late stage (Stage 4) of the Wobbler disease (Ma & Vacca-Galloway, 1992), although the trend towards shrinkage was present but not statistically demonstrated for the motoneurons (Ma & Vacca-Galloway, 1991*a*). Abnormal RNA metabolism and decreased protein synthesis are known to affect both the large and small ventral horn neurons, in which the small neurons include the γ-motoneurons and interneurons, including Renshaw cells (Murakami et al. 1980). Sakla (1969) reported that perikaryal volume in the albino mouse spinal cord increased from birth to 30 d with no further increase to 120 d. In the present study increases in perikaryal volume from young to old ages in control groups were noted for both volume-weighted (27%) and number-weighted (20%) mean perikaryal volumes, but these increases were not significant.

In the rat ventral horn motoneuron perikarya, Mayhew (1989) suggested a CV_n of 70% at 120 d and 45% at 20 d. In the present study a similar pattern was seen in the control animals with CV_n % 104% for old and 95% for young ones. This increase in variability of the perikaryal population may be explained in the shifts in the ratio between the large multipolar and the small fusiform cells (see Mayhew, 1989). In the Wobbler group CV_n% was 127% for the young Wobblers. This considerable heterogeneity may be accounted for by the presence of the large vacuolated cells at this stage. Shifts in the ratio of large multipolar and small fusiform cells have been hypothesised as part of the Wobbler disease (Ma & Vacca-Galloway, 1991*a*).

Nuclear changes

The results of the 2-way ANOVA of the $\overline{V_v}$ *Nuc* data showed a borderline effect of age and a significant interaction. Number-weighted mean nuclear volume showed a significant effect of age, disease and interaction. It should also be noted that the *CV*% for the number weighted data (Table 4) of the older groups was larger than that for the young groups; this interindividual variation may be a reflection of the increased size of the cord in the older groups, indicating some limitations in the sampling of the older group, perhaps requiring the examination of more blocks in the older groups. However, this age-related phenomenon is not seen in the volume weighted data or in the fractional volume data.

Having estimates of $\overline{V_v}$ *Nuc* and $\overline{V_n}$ *Nuc* enabled an

estimation of the distributions of nuclei: if the population of nuclei was homogeneous these 2 estimates would be equal and the CV_n% would be zero. The control groups show heterogeneity with CV_n% of 48% for the young and 46% for the old specimens. The CV_n% for Wobbler nuclei was found to be 54% in the young and 65% in the old specimens. Mayhew (1989) reported an age-related increase (68%) in nuclear $\overline{V_v}$ in rat cervical motoneurons between 20 and 120 d. In the present study both $\overline{V_v}$ and number-weighted nuclear volume estimates were found to increase (10% and 12%) in the controls between the young and old groups. However, these differences were not significant. $\overline{V_v}Nuc$ and $\overline{V_n}Nuc$ were significantly smaller in the old Wobbler groups when compared with age-matched control and young Wobbler groups. The reported CV_n for nuclei in the cervical ventral horn of the rat is 40% at 120 d (Mayhew, 1989). In the present study we found CV_n 48% for the young and 46% for the old controls. The CV_n for the young Wobbler group was 54% and 65% for the old. The larger CV_n in the young Wobbler probably reflects a greater heterogeneity in nuclear population.

A significant reduction of protein synthesis has been documented in 2-mo-old Wobblers (C57Bl/6J strain), thus implicating that one of the early expressions of the genetic defect may be an impairment of nuclear RNA synthesis in the neuron (Murakami et al. 1981). Yet previous nuclear area measurements showed no significant reductions (Murakami et al. 1980). In the present study, the absence of nuclear volume-weighted changes in the young Wobbler specimens compared with the age-matched normal phenotype controls may support those findings. Reduced nuclear and nucleolar uptake of RNA precursors has been demonstrated in the majority of neurons in the ventral horn of the Wobbler (C57Bl/6J strain) cervical spinal cord studies in 2-mo-old mice (Murakami et al. 1981). Alternatively, the nuclear volume measured herein in the old (aged 3 mo) Wobbler (NFR/*wr* strain) specimens suggests that nuclear events indeed occur late in the Wobbler MND. It might be presumed, however, that they may be preceded by molecular changes that are not detectable morphometrically. In human MND, affected motoneurons show a decrease in the nuclear volume prior to cytoplasmic RNA loss (Mann & Yates, 1974). Since the present study measured nuclei from all ventral horn neurons, such findings may have been obscured. It may be that the reduced volume only becomes evident very late in the disease following the loss of motoneurons.

The finding in the present study may also be related to the frequency of neurons exhibiting various stages of swelling and vacuolation, which is documented to be greatest in the early stage (Andrews et al. 1974; Pollin et al. 1990; Ma & Vacca-Galloway, 1991*a*). With increasing age, the variation seems less pronounced. Whether the latter feature relates to the stabilisation of the disease process or to the actual loss of large motoneurons (Andrews et al. 1974) needs to be explored.

The absence of differences in proportion of nucleus to perikaryon illustrates the danger of ratio estimates. Absolute data (volume estimates) enable more useful and meaningful comparisons to be made between groups than do density estimates (White & Gohari, 1982).

In summary, the present study using new stereological probes has documented changes in neuronal structure in the Wobbler mouse model which can be referred to for future studies. Our data indicate that perikaryal swelling characterises the early stage of the Wobbler MND, probably representing the involvement of the motoneuron pool. Later in the disease process there seems to be some involvement of other cell types.

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