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ORIGINAL ARTICLE

Loss of Motor Neurons innervating Cervical Muscles in Patients with Multiple System Atrophy and Dropped Head

(93 characters)

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ABSTRACT

We investigated whether loss of motor neurons innervating the neck muscles contributes to dropped head (DH) in multiple system atrophy (MSA). From 75 patients with autopsy-proven MSA, we retrieved 3 who had DH (MSA-DH), and examined the 4th cervical cord segments. Neurons of the medial and lateral nuclear groups (MNG and LNG) innervate the neck and shoulder muscles, respectively. We measured the area of individual neurons in the MNG and LNG, and created an area-frequency histogram. Neurons were classified as large or small based on their area, and their total numbers in the MNG and LNG were counted. In the MNG, the numbers of both total neurons and large neurons were significantly lower in MSA patients than in the controls (214.2 ± 91.4 vs. 521.3 ± 74.8, $P = 0.0030$, and 26.2 ± 9.1 vs. 88.0 ± 34.6, $P = 0.020$, respectively), and were significantly lower in MSA-DH than in MSA-nonDH (139.7 ± 7.6 vs. 288.7 ± 74.6, $P = 0.048$, and 18.0 ± 4.1 vs. 34.3 ± 4.1, $P = 0.016$, respectively). There were no differences in the LNG neuron counts between the MSA-DH and MSA-nonDH groups. Loss of cervical motor neurons may be responsible for DH in MSA.

INTRODUCTION

Dropped head (DH) is a condition characterized by severe neck flexion on standing or sitting, despite only minor thoracic or lumbar curvature (1). It markedly affects speaking and swallowing ability, and narrows the visual field. Parkinsonism-related disorders, including multiple system atrophy (MSA) and Parkinson's disease (PD), are major causes of DH. Because DH is observed more frequently in patients with MSA than in those with PD (2-3), and appears much earlier after disease onset in the former, it is regarded as a supportive feature for differential diagnosis of MSA (4).

With regard to the etiology of DH in patients with parkinsonism, neck dystonia involving the longus colli muscles and other deep cervical flexor muscles – a condition referred to as disproportionate antecollis – has been suggested (5). Other possible etiologies such as neck extensor myopathy, or imbalanced rigidity of the anterior and posterior neck muscles, have also been proposed (6). On the other hand, disorders associated with myogenic or neurogenic neck extensor muscle weakness, including myasthenia gravis and amyotrophic lateral sclerosis (ALS), can cause DH during their disease course. Therefore, it seems likely that DH can be a manifestation of cervical motor neuron loss (7).

In patients with MSA, although lower motor neuron signs are not a common feature, electromyography often demonstrates neuropathic changes (8). Consistent with this, histopathological examination of spinal cords obtained at autopsy have revealed loss of motor neurons in the ventral horn (9-11). Thus, similarly to patients with ALS, neck extensor muscle weakness caused by loss of lower motor neurons might be associated with DH in patients with MSA. However, it is still unclear whether depletion of these neurons is responsible for DH in this context.

In the present study, we sought to determine whether loss of cervical motor neurons innervating the neck muscles contributes to DH in patients with MSA. Using a morphometric approach, we evaluated the total number of these motor neurons in patients with MSA, and compared the data between those who had developed DH and those who had not.

PATIENTS AND METHODS

Subjects

We reviewed the medical records of 75 consecutive patients with pathologically confirmed MSA [43 males and 32 females; 43 patients with predominant cerebellar ataxia (MSA-C) and 32 patients with predominant parkinsonism (MSA-P) (6); aged 67.4 ± 6.5 (mean ± SD; range 50 - 84) years] who were referred to the Brain Research Institute, Niigata University, between 1970 and 2014. We retrieved three patients with MSA who suffered from DH (MSA-DH group). In these patients, the disease duration was 7.0 ± 0.9 years, and in accordance with the methods reported elsewhere (12), the severity of degeneration in the striatonigral (StrN) and olivopontocerebellar (OPC) systems was 2.3 ± 0.94 and 2.3 ± 0.47, respectively. DH was defined as a forward flexion of the neck evident while the patients were walking or sitting. We also retrieved three MSA patients without DH (MSA-nonDH group), in whom the disease duration (6.3 ± 0.9 years) and severity of StrN (2.0 ± 0.82) and OPC (2.0 ± 0.82) degeneration were similar to those in the MSA-DH group (Table 1). Age-matched individuals without any neurological disorders were included as a Control group (n = 3), and patients with pathologically confirmed ALS who developed DH (ALS-DH group, n = 3) and those

with PD who did not have DH (PD-nonDH group, n = 3) were also included as disease controls (Table 1). Written informed consent for autopsy including the use of tissue for research purposes was obtained from the next of kin. The present study was approved by the institutional review board of Niigata University School of Medicine, Niigata, Japan.

Clinical Features of Patients in the MSA-DH Group

The clinical phenotype of two of the MSA-DH patients was MSA-P, and that of the other patient was MSA-C (4). None of them had a family history of parkinsonism, cerebellar ataxia or DH.

MSA-DH 1: This patient, a 63-year-old male, developed gait disturbance. Neurological examination revealed bradykinesia and rigidity of the limbs. He was diagnosed as having PD. One year later, he showed DH accompanied by axial rigidity, sleep apnea, urinary disturbance, and impotence. The DH was initially improved after administration of a dopamine agonist, but it soon worsened thereafter. Around this time, severe forward flexion of the neck disturbed the patient's visual field, and diffuse muscle atrophy of the neck and limbs emerged. Asymmetric postural disturbance was not evident. By the age of 68 years he had become bedridden, and his head was fixed in the position touching on the bed. The patient died of pneumonia aged 68 years.

MSA-DH 2: This patient, a 53-year-old female, developed tremor and was admitted to a hospital for neurological evaluation. She was diagnosed as having PD, and levodopa was administered. Two years later, neurological examination revealed orthostatic hypotension and REM sleep behavior disorder for which levodopa was not effective. Brain magnetic resonance imaging (MRI) revealed putaminal atrophy and

linear hyperintensity signals along the lateral putaminal rim on T2-weighted images. At this time, the patient was diagnosed as having MSA-P. At the age of 57 years, she developed severe DH and forced laughter. Dystonic lateroflexion of the trunk and neck was unremarkable. Neurological examination revealed extensor plantar reflex. At the age of 62 years, she died of type 2 respiratory failure.

MSA-DH 3: This patient, a 64-year-old male, developed dysarthria and an ataxic gait. Genetic analysis was able to exclude spinocerebellar ataxia types 1, 2, 3 and 6, or dentatorubral-pallidoluysian atrophy. Brain MRI revealed cerebellar and pontine base atrophy. The patient was diagnosed as having MSA-C, and started on taltirelin. Two years later, he developed DH, as well as frozen gait, orthostatic hypotension, urinary disturbance, and severe obstructive sleep apnea syndrome requiring continuous positive airway pressure. Although mild rigidity of the lower limbs was detected, rigidity in the neck and trunk and postural asymmetry was unremarkable. Levodopa was prescribed for DH and frozen gait, but the symptoms were not improved. At the age of 68 years he became bedridden, and at the age of 71 years he succumbed to sudden death.

Tissue Processing and Immunohistochemistry

All the brains and spinal cords were fixed in formalin, and processed as described previously (13). All cases were assessed for neuronal and glial synuclein pathology using a rabbit polyclonal antibody against phosphorylated α-synuclein (1:1000; Wako, Osaka, Japan), and fulfilled the pathologic criteria for MSA (14). Sections 4 μm thick stained with hematoxylin and eosin were used for semi-quantitative analysis of neuronal cell loss in the striatonigral and olivopontocerebellar regions, using the method described previously (12). We also evaluated neuronal cell loss and gliosis in the motor

cortex, and degeneration of the spinal lateral columns.

The semispinalis cervicis, longissimus cervicis, and iliocostalis cervicis muscles are mainly responsible for neck extension. These muscles are innervated by the dorsal rami of C4-C8, C2-C8, and C4-C8, respectively (Susan Standring's *Gray's Anatomy* (15)) (16). Among these three muscles, the semispinalis cervicis is regarded as the most important posterior stabilizer (16). Therefore, for the quantitative analyses in the present study, we prepared transverse slices of C4 that is the median level from C2 to C8, and in which motor neurons innervate the semispinalis cervicis. The slices were embedded in paraffin, and serial sections 10 μm thick were cut. Ten sections, each separated by 100 μm, were subjected to Klüver-Barrera staining for identification of neurons.

Image Analysis and Quantification

The ventral horn of C4 was defined as the gray matter located on the ventral side of a vertical line through the central canal and perpendicular to the ventral sulcus. In accordance with James D. Fix's *Atlas of the Human Brain and Spinal Cord* (17), neurons in the ventral horn corresponding to Rexed's lamina IX were divided into medial and lateral nuclear groups (MNG and LNG) (Fig. 1A). Neurons of the MNG and LNG in the C4 segment innervate the neck and shoulder girdle muscles, respectively (Nancy Berryman Reese's *Muscle and Sensory Testing* (18)) (19).

Images of the bilateral ventral horns in ten sequential sections were taken through a x20 objective lens. Images were converted to gray scale images, adjusted for brightness and contrast, and then the area of each cell was measured by computer-assisted imaging algorithm using NIH Image J software (imagej.net) (Fig. 1B). Results were expressed in the form of an area-frequency histogram using the conventional square root choice

method. Based on the histogram obtained for the control patients, ventral horn cells with an area of ≥408.0 μm^2 and those with an area of 100.0 μm^2 − <408.0 μm^2 were regarded as large and small neurons, respectively (Fig. 1B, C). In accordance with this definition, the numbers of large and small neurons in the control group were 444.7 ± 56.6 [mean ± standard deviation (SD)] and 1111.0 ± 46.6, respectively. Cells with an area of <100.0 μm^2 were regarded as non-neuronal cells. The numbers of either total neurons or large neurons were counted automatically in the MNG and LNG separately. The numbers of neurons in a total of 10 sections were combined and computed for comparison.

Using the α-synuclein-immunostained sections, multiple images of layers 5 to 6 of the motor cortex, lateral columns and ventral horn of C4 were taken through a ×20 objective lens. The total area taken from a section in each case was 1.48 mm^2 in the motor cortex, 0.30 mm^2 in the lateral columns, and 0.89 mm^2 in the ventral horn. The total area of α-synuclein immunoreactivity ($\mu m^2 \times 10^3$) was measured using NIH Image J software.

Statistical Analysis

The total number of neurons, and the number of large neurons in the MNG, and those in the LNG, were compared between the controls and the MSA, ALS, and PD groups, and also in the MSA-DH and MSA-nonDH groups, using Student's t test for normally distributed data and Wilcoxon's rank sum test as a non-parametric test. Data were analyzed using SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA). Differences at P <0.05 were considered significant.

RESULTS

Histological Features and Neuronal Distribution

In patients with MSA, atrophy of the ventral horns was observed to various degrees, neuron loss in the MNG with gliosis being more prominent in the MSA-DH group than in the MSA-nonDH group. In ALS-DH group, marked atrophy of the ventral horns, and severe neuron loss in both the MNG and LNG with gliosis were evident. In contrast, in the PD-nonDH group, no such degenerative features were evident (Fig. 2A, *left columns*). In the control group, the total number of neurons was higher in the LNG than in the MNG, and moreover, large neurons were much more numerous in the LNG than in the MNG. The distribution patterns of large and small neurons in the PD-nonDH and control groups appeared similar, but were distinct in the MSA-DH, MSA-nonDH, and ALS-DH groups (Fig. 2A, *right column*).

Medial Nuclear Group

The numbers of total neurons and large neurons in the MNG of patients with MSA were significantly lower than those in controls (214.2 ±91.4 vs. 521.3 ± 74.8, P = 0.0030; and 26.2 ± 9.1 vs.88.0 ± 34.6, P = 0.020, respectively), and were significantly lower in the MSA-DH group than in the MSA-nonDH group (139.7 ± 7.6 vs. 288.7 ± 74.6, P = 0.048; and 18.0 ± 4.1 vs. 34.3 ± 4.1, P = 0.016, respectively). The corresponding counts in the ALS-DH group (240.3 ± 55.8 and 5.0 ± 1.4, respectively) were significantly lower (P = 0.015 and 0.046) than in the controls. On the other hand, the counts in the PD-nonDH group (574.3 ± 49.4 and 89.3 ± 10.1, respectively) were not significantly different (P = 0.45 and P = 0.96, respectively) from those of controls

(Fig. 2B, and Supplementary Table 1).

Lateral Nuclear Group

The number of total neurons in the LNG in patients with MSA was not significantly different from that in the controls (739.8 ± 170.5 vs. 1034.0 ± 154, $P = 0.090$); however, the number of large neurons in patients with MSA was significantly lower than that in the controls (204.5 ± 59.6 vs. 356.7 ± 69.1, $P = 0.020$). Surprisingly, the numbers of total neurons and large neurons in MSA-DH group were not significantly different from those in MSA-nonDH group (654.3 ± 208.1 vs. 825.3 ± 15.1, $P = 0.51$; 179.3 ± 65.6, 229.7 ± 39.2, $P = 0.42$, respectively). In the ALS-DH group, the corresponding numbers (379.0 ± 68.2 and 14.0 ± 6.7, respectively) were significantly lower than those in the controls ($P = 0.0053$; and $P = 0.019$, respectively). On the other hand, the counts in the PD-nonDH group (1060.0 ± 99.8 and 341.3 ± 135.6, respectively) were not significantly different from those in the controls ($P = 0.85$, and $P = 0.89$, respectively) (Fig. 2B, and Supplementary Table 1).

Severity of Degeneration in the Pyramidal Tracts in Relation to the α-Synuclein-Immunoreactive Area

The severity of neuronal loss and gliosis in the motor cortex and spinal lateral columns in both the MSA-DH and MSA-nonDH groups appeared to be similar. The α-synuclein-immunoreactive areas in the ventral horns, lateral columns, and motor cortex of both groups were not significantly different (1.53 ± 0.81 vs 3.52 ± 3.20, $P = 0.36$, 0.57 ± 0.10 vs 1.88 ± 1.04, $P = 0.050$, and 14.05 ± 8.65 vs 32.82 ± 28.10, $P = 0.33$,

respectively) (Table 2). The α-synuclein-immunoreactive areas appeared to substantially represent glial cytoplasmic inclusions (GCIs), as the latter were numerous whereas neuronal inclusions were sparse (e.g. less than 5 in the motor cortex of MSA-DH1, MSA-nonDH1, and MSA-nonDH3; and a total of 2 throughout the spinal ventral horn in all the cases examined). In addition, we encountered only 13 cytoplasmic inclusions in Schwann cells throughout the anterior nerve roots in all of the MSA patients examined

DISCUSSION

In the present study, we found for the first time that patients with MSA who develop DH during the course of their illness show selective vulnerability of motor neurons innervating the neck muscles. Interestingly, depletion of motor neurons innervating the shoulder girdle muscles at the same cervical cord level was not evident (Fig. 2).

To investigate the selective vulnerability of cervical cord neurons, we had performed a preliminary study of neuron numbers in the MNG and LNG of segment L4. We found a significant difference between the controls and patients with MSA, but no difference between the MSA-DH and MSA-nonDH groups (data not shown). The data appeared to be consistent with the notion that the depletion of cervical neurons observed in the MSA-DH group may contribute to the development of DH.

As shown in Fig. 2, even though the difference in the number of surviving motor neurons between the MSA-DH and MSA-nonDH groups appeared to be small, it may critically contribute to the clinical phenotype, as the statistical evaluation indicated significance. It is unclear whether the denervation resulting from cervical neuron loss is

solely responsible for DH, or whether other factors, including imbalanced denervation of the extensor and flexor muscles, may also account for to the development of DH in patients with MSA.

Consistent with the present findings, several previous histopathologic studies of the spinal cords of patients with MSA have demonstrated loss of motor neurons in the ventral horn (9-11) and small neurons in the intermediate zone (10). Interestingly, severe motor neuron loss was observed in a patient with MSA who manifested marked muscular wasting and fasciculation in the limbs (9), similar to the clinicopathologic features of patients with ALS. In the present study, DH appeared at an early stage in the clinical course in two patients (MSA-DH 1 and MSA-DH 3 in Table 1), one of whom (MSA-DH 1) exhibited remarkable axial and limb muscle weakness with atrophy. On the other hand, DH appeared at a late stage in the other patient (MSA-DH 2 in Table 1), who showed neither muscle weakness nor atrophy. On the other hand, in patients with ALS, both DH and muscle weakness may appear early in the clinical course (20-21).The different clinical pictures in patients with MSA and ALS may reflect differences in the severity and speed of motor neuron degeneration. Consistent with this, an electrophysiological study of limb muscles in patients with MSA demonstrated a chronic re-innervation pattern (8). In the PD-nonDH group, the spinal motor neurons were well preserved, as has been reported previously (22).

The pathomechanisms underlying the depletion of spinal motor neurons in MSA have remained unclear. Here we evaluated the severity of degeneration in the pyramidal tracts of the cervical cord, and the amount of phosphorylated α-synuclein-immunolabeled glial cytoplasmic inclusions in the ventral horn. However, it appears unlikely that these features might be associated with the depletion of the spinal motor neurons in MSA

(data not shown). Consistent with previous studies (23), we rarely encountered phosphorylated α-synuclein-immunolabeled neuronal cytoplasmic inclusions in the spinal ventral horn. Moreover, the numbers of α-synuclein-immunolabeled cytoplasmic inclusions in Schwann cells (24) were very small in all of the patients in the present study. Further studies will be needed to clarify the significance of α-synuclein accumulation in glia and neurons in the context of MSA-related neuronal degeneration.

The patients with MSA-DH exhibited extrapyramidal dysfunction, including dystonia and flexor / extensor-imbalanced muscle rigidity. For example, MSA-DH 1 developed DH accompanied by axial rigidity, and therefore DH was regarded as a dystonic symptom at the beginning of DH, although DH seemed to reflect neck weakness later. It is known that striatonigral degeneration may be responsible for extrapyramidal dysfunction, and dystonia in patients with MSA could be related to neuronal loss in the putamen (25). However, histopathological examination of the brain in all of the present MSA patients revealed no apparent difference in the severity of degeneration in the striatonigral system between the MSA-DH and MSA-nonDH groups. Thus, it seems unlikely that extrapyramidal dysfunction alone would cause DH in patients with MSA.

Because of the retrospective nature of the present study, detailed clinical information on autopsied patients was not always available. Previous clinical studies have reported the prevalence of DH in patients with MSA to be 7.7-42.1% (3, 26-28) whereas in the present study DH was recorded in only 3 (4%) out of 75 autopsied patients. This low incidence might have been due to case selection bias, as only autopsy-proven cases were included. Moreover, in the present study, neither electrophysiological nor histological analysis of neck extensor muscles was possible for any of the patients with MSA-DH. Therefore, we could not rule out the influence of other factors, including dystonia,

flexor / extensor-imbalanced rigidity and myopathy of the extensor muscles.

It seems reasonable to consider that some shrunken, formerly large, neurons may be counted as non-large neurons. Simultaneously, it seems also reasonable to consider that these formerly large neurons might no longer have a significant physiological role. Therefore, in the present study, only large neurons were considered to be possibly functional motor neurons.

One limitation of the present study was its small sample size. Moreover, the present series of patients did not include any with PD who suffered from DH. Therefore, it remains unclear whether loss of upper cervical motor neurons in patients with MSA-DH is much more severe than that in patients with PD-DH.

In conclusion, for patients with MSA, upper cervical motor neuron loss contributes to weakness of the neck extensor muscles, leading to DH. This information could be crucial when considering the treatment and management of DH in patients with MSA.

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FIGURE LEGENDS

Fig. 1

(A) Representative location of lateral and medial nuclear groups (LNG and MNG) in the ventral horn of the fourth segment of the cervical cord (C4) of a control patient (Con 2 in Table 1). The vertical line represents the border between the LNG and the MNG. Klüver-Barrera (K-B) staining. Bar = 200 μ m. (B) Modified digital images of some MNG neurons. A captured digital image is converted to a gray scale image (*left*), and then depending on the area, each neuron is classified as a large neuron (colored *red*; ≥408.0 μ m^2) or a small neuron (*blue*; ≥100.0 μ m^2 - <408.0 μ m^2) (*right*). Bar = 50 μ m. (C) Area-frequency histograms of neurons. Histograms of three individual control cases and the Control, MSA-DH, MSA-nonDH, ALS-DH and PD-nonDH groups (n = 3 for each group).

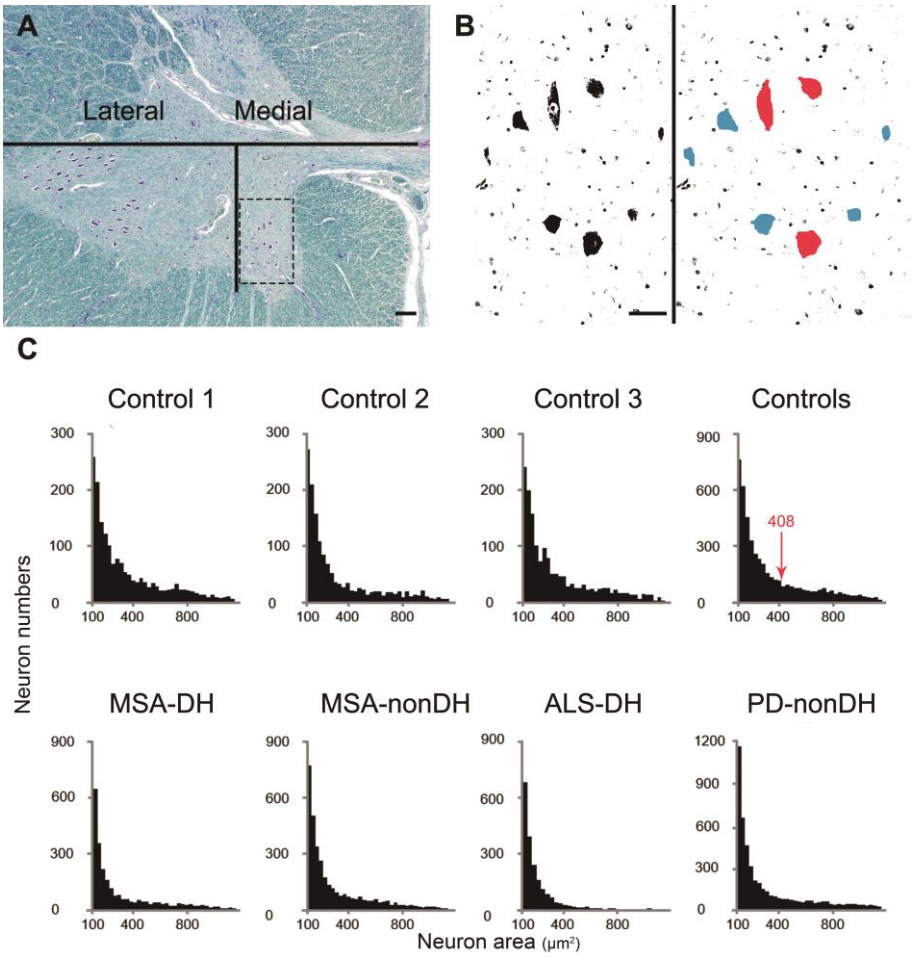
Fig. 2

(A) Photomicrographs of the medial side of the ventral horn in the Control, MSA-DH, MSA-nonDH, ALS-DH, and PD-nonDH groups (n = 3 for each group). K-B staining. Bar = 50 μ m. Schematic representation of the distribution of neurons in the ventral horn of a single case from each group (*right*). Each *red* dot and *blue* dot represents the approximate location of a large neuron and a small neuron, respectively. A vertical line represents the border between the LNG and the MNG. Note the apparent loss of MNG neurons in the MSA-DH, MSA-nonDH, and ALS-DH groups. (B) Mean (± SD) numbers of total neurons and large neurons in the LNG and MNG. For the MNG, the counts of both

total and large neurons in MSA group are significantly smaller than those in controls. Moreover, the corresponding counts in the MSA-DH group are significantly smaller than those in the MSA-nonDH group. On the other hand, for the LNG, there are no differences in the counts of both total and large neurons between MSA-DH and MSA-nonDH group.

$P = 0.0053$, * $P = 0.020$, ** $P = 0.019$, ‡ $P = 0.0030$, ‡ ‡ $P = 0.048$, ‡ ‡ ‡ $P = 0.015$, † $P = 0.020$, †† $P = 0.016$, and ††† $P = 0.046$.

Fig. 1


A
Lateral
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Control 1
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Controls
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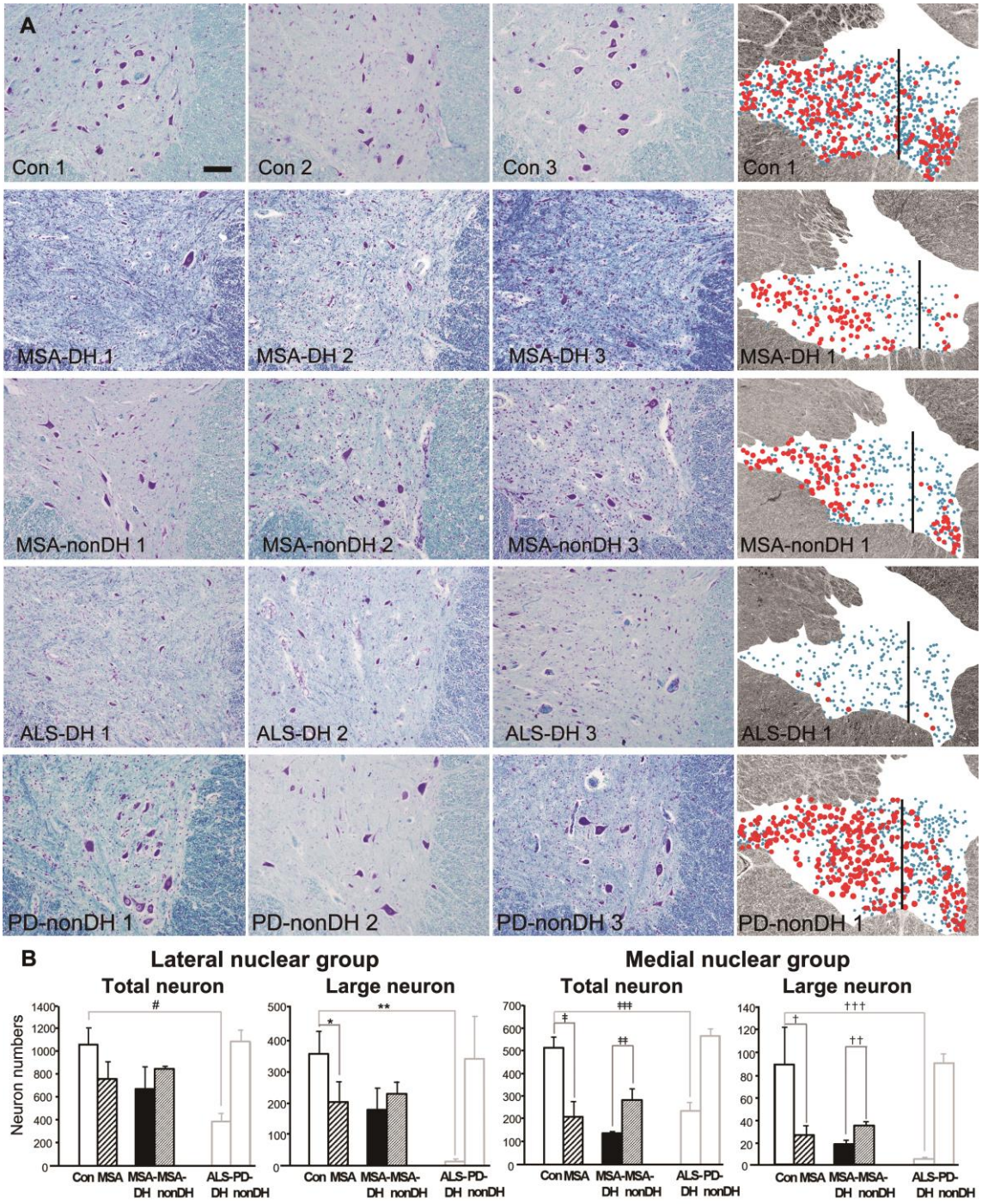
MSA-DH
MSA-nonDH
ALS-DH
PD-nonDH
Neuron numbers
Neuron area (μm²)

Fig. 2


A
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ALS-DH 1
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ALS-DH 1
PD-nonDH 1
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PD-nonDH 3
PD-nonDH 1
B
Lateral nuclear group
Medial nuclear group
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Large neuron
Total neuron
Large neuron
Neuron numbers
Con MSA MSA-DH MSA-nonDH ALS-PD-DH nonDH

TABLE 1. Patient population

A. Patients with multiple system atrophy

Patient	Age/ sex	Disease duration (years)	Clinical phenotype	Initial symptoms	Time for DH development (years)	Pathological phenotype[14]	Grading of neuronal cell loss[12]
							StrN/OPC system
MSA-DH 1	68/M	5.0	MSA-P	Gait disturbance	1.0	SND type	3/2
MSA-DH 2	62/F	9.0	MSA-P	Tremor	4.0	SND type	3/2
MSA-DH 3	71/M	7.0	MSA-C	Dysarthria, ataxia	2.0	OPCA type	1/3
MSA-nonDH 1	58/M	7.0	MSA-C	NB, impotence	NA	SND type	2/1
MSA-nonDH 2	65/M	7.0	MSA-C	Tremor	NA	OPCA type	1/3
MSA-nonDH 3	62/F	5.0	MSA-P	Gait disturbance, tremor	NA	SND type	3/2

B. Control and disease's control

Patient	Age/ sex	Disease duration (years)	Time for DH development (years)	Pathological diagnosis
Con 1	67/F	NA	NA	Subarachnoid hemorrhage
Con 2	61/F	NA	NA	Pneumonia
Con 3	73/M	NA	NA	Cardiac embolism
ALS-DH 1	65/M	2.0	0.5	ALS
ALS-DH 2	45/F	1.5	1	ALS
ALS-DH 3	58/M	3.0	1	ALS
PD-nonDH 1	54/M	16.0	NA	PD
PD-nonDH 2	75/M	27.0	NA	PD
PD-nonDH 3	79/F	6.0	NA	PD

DH = dropped head; MSA-DH = group of patients with MSA who developed dropped head; MSA-nonDH = group of patients with MSA without dropped head; Con = control; ALS-DH = group of patients with ALS who developed dropped head; PD-nonDH = group of patients with Parkinson's disease without dropped head; NA = non-applicable; NB = neurogenic bladder; SND = striatonigral degeneration; OPCA = olivopontocerebellar atrophy; StrN = striatonigral; OPC = olivopontocerebellar.

TABLE 2. α-Synuclein-immunoreactive areas in the ventral horns and lateral columns at the C4 level and motor cortex in the MSA group.

		α-Synuclein-immunoreactive area ($\mu m^2 \times 10^3$)		
	Case	Ventral horn	Lateral column	Motor cortex
MSA-DH	1	2.26	0.68	23.27
	2	0.66	0.47	12.73
	3	1.67	0.57	6.13
MSA-nDH	1	6.38	2.35	21.89
	2	0.06	0.68	11.84
	3†	4.10	2.60	64.74

†= As no paraffin-embedded blocks of the C4 segment were available for α-synuclein immunostaining, the C6 segment was used for measurement of the α-synuclein-immunoreactive area.

Supplementary TABLE 1. Numbers of C4 ventral horn cells

Medial nuclear group			
Patient	Large	Small	Total
MSA-DH 1	13	131	144
MSA-DH 2	23	123	146
MSA-DH 3	18	111	129
MSA-nonDH 1	35	206	241
MSA-nonDH 2	29	202	231
MSA-nonDH 3	39	355	394
Con 1	56	372	428
Con 2	72	453	525
Con 3	136	475	611
ALS-DH 1	4	284	288
ALS-DH 2	7	264	271
ALS-DH 3	4	158	162
PD-nonDH 1	97	533	630
PD-nonDH 2	96	414	510
PD-nonDH 3	75	508	583

Lateral nuclear group			
Patient	Large	Small	Total
MSA-DH 1	206	659	865
MSA-DH 2	243	484	727
MSA-DH 3	89	282	371
MSA-nonDH 1	277	548	825
MSA-nonDH 2	181	626	807
MSA-nonDH 3	231	613	844
Con 1	454	796	1250
Con 2	300	602	902
Con 3	316	634	950
ALS-DH 1	12	346	358
ALS-DH 2	23	448	471
ALS-DH 3	7	301	308
PD-nonDH 1	207	712	919
PD-nonDH 2	527	608	1135
PD-nonDH 3	290	836	1126

Numbers of large, small, and total neurons counted in the bilateral C4 ventral horns in ten sections from each case are shown.