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Amyotrophic lateral sclerosis of Guam: the nature of the neuropathological findings

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Abstract To elucidate the fundamental differences and similarities of the neuropathological features and etiopathogenesis of the amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia complex (PDC) of Guam, we conducted a topographic, quantitative and histological investigation of tau-containing neurons, neurofibrillary tangles (NFTs), Bunina bodies and ubiquitinated inclusion bodies in 27 non-ALS non-PDC Guamanian subjects, as well as 10 Guam ALS patients, 28 PDC patients, and 5 patients with combined ALS and PDC (ALS-PDC). The topographic distribution of NFTs was basically the same in each disease and also in the non-ALS non-PDC group. There were relatively few, if any, NFTs in non-ALS non-PDC subjects and ALS patients, but there were many, especially in the frontal and temporal cortex, in Guam PDC and ALS-PDC patients. The histological and ultrastructural features of Bunina bodies in Guam ALS and ALS-PDC patients were similar to those reported in classic ALS. The ratio of occurrence of the inclusion in Guam ALS and ALS-PDC patients was similar to that reported so far in classic ALS. Ubiquitinated skein-like inclusion bodies were observed in the spinal anterior horn cells in Guam ALS and ALS-PDC patients. These findings indicate that classic ALS does exist on Guam, that NFTs in Guam ALS patients are merely a background feature widely dispersed in the population, that the mechanism of neuronal degeneration of Guam ALS is basically different from that of PDC, and that Guam ALS occurs initially as classic ALS.

Key words Amyotrophic lateral sclerosis
Parkinsonism-dementia complex · Guam
Neuropathology · Quantitative study

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Introduction

In the central nervous system of patients with amyotrophic lateral sclerosis (ALS) of Guam, neurofibrillary tangles (NFTs) and loss of neurons have been observed extensively, especially in the hippocampus, parahippocampal gyrus, temporal and frontal neocortex, amygdaloid nucleus, basal nucleus of Meynert, hypothalamus, substantia nigra, locus ceruleus, and brain stem raphe nuclei, in addition to the typical neuropathological features of classic ALS [15, 16, 20]. Because the topographic distribution of NFTs, and of the neuronal loss related to them, is similar to that of parkinsonism-dementia complex (PDC) of Guam [12–14], because patients with combined ALS and PDC (ALS-PDC) have been identified clinically and pathologically, and because ALS as well as PDC patients are sometimes admixed within a family, it has been proposed that Guam ALS and PDC are a single disease entity, that Guam ALS is a disease different from classic ALS [16, 20], and that Guam ALS is a disease closely related to the presenile or senile process [25].

However, NFTs, which are a basic feature of Guam ALS and PDC, Bunina bodies and ubiquitinated inclusion bodies have not been investigated precisely or extensively. It is essential for this kind of evaluation to be performed not only in Guam ALS, PDC and ALS-PDC patients, but also in non-ALS non-PDC Guamanian subjects to reveal any significant discrepancy from the background distribution in the population.

To elucidate the fundamental differences and similarities of the neuropathological features and etiopathogenesis of Guam ALS and PDC, we conducted a detailed quantitative, histological and ultrastructural investigation on tau-containing neurons, NFTs, Bunina bodies and ubiquitinated inclusion bodies in non-ALS non-PDC Guamanian subjects, as well as in Guam ALS, PDC and ALS-PDC patients.

Materials and methods

Examined cases and experimental design

For the present study, among 175 autopsies performed by the authors on Guam between 1979 and 1982, 10 patients with ALS, 28 patients with PDC, 5 patients with ALS-PDC, and 27 subjects diagnosed clinically and neuropathologically as having non-ALS non-PDC were available. All of these patients and subjects were Guamanian who had been born and grown up on Guam.

Neurons immunopositive for human-tau or ubiquitin, and neurons containing Bunina bodies were examined in the motor cortex, oculomotor, facial and hypoglossal nuclei, and spinal anterior horn at the level of the cervical and lumbar segments. For these observations, tissues obtained at autopsy from 10 Guam ALS patients (age range, 34–73 years), 28 Guam PDC patients (age range, 51–70 years), 5 ALS-PDC patients (age range, 57–69 years), and 13 non-ALS non-PDC subjects (age range, 41–84 years) were available.

The observations were performed using formalin-fixed, paraffin-embedded motor cortex sliced coronally at the parasagittal portion, midbrain sliced transverselly at a level through the top of the superior colliculus and the outlet of the oculomotor nerve for the oculomotor nucleus, the lower pons at a level through the outlet of the facial nerve for the facial nucleus, the mid-medulla oblongata for the hypoglossal nucleus, and the spinal anterior horn cells at the level of the 7th cervical and 4th lumbar segments. The appearance rate of tau- or ubiquitin-immunopositive neurons, and that of neurons containing Bunina bodies were compared among the groups.

To demonstrate the detailed topographic and quantitative distribution of NFTs, the numbers of neurons containing NFTs and of degenerated neurons exhibited by ghost tangles were counted in 61 anatomical areas of the central nervous system. For this study, tissues from 7 ALS (age range, 47–72 years), 6 PDC (age range, 52–64 years), 3 ALS-PDC patients (age range, 57–66 years), and 20 non-ALS non-PDC subjects (age range, 41–84 years) were available.

Ultrastructural features of NFTs in Ammon's horn and spinal anterior horn cells, and of Bunina bodies in the anterior horn cells were examined. For this part of the study, tissues from 5 ALS (age range, 47–73 years) and 7 PDC patients (age range, 52–68 years) were available.

Immunohistological and quantitative examination of tau- and ubiquitinated inclusion-containing neurons in the motor neuron system

Immunohistochemical staining was performed on formalin-fixed paraffin-embedded 6-μm-thick sections, using rabbit anti-human tau polyclonal antibody (dilution 1:2000 [18]) and rabbit anti-cow ubiquitin polyclonal antibody (dilution 1:150) (DAKOPATTS A/S, Glostrup, Denmark) as primary antibodies. For ubiquitin immunohistochemistry, sections were pretreated with 0.025% trypsin for 15 min at room temperature to increase the antigenicity of the ubiquitin. The ABC method (Vectastain ABC kits, Vector, California, U.S.A.) was used for immunostaining. As antibody controls, the primary antisera were either omitted or replaced with normal rabbit serum. Several neural and non-neural tissues from the patients served as positive and negative tissue controls. The immunostained sections were counterstained with hematoxylin for 1 min, mounted on glass slides and examined using a light microscope.

Quantitative study of Bunina body-containing neurons in the motor neuron system

The neurons containing Bunina bodies were examined in the motor cortex, oculomotor nucleus, facial nucleus, hypoglossal nucleus, and spinal anterior horn at the level of the cervical and lumbar segments. Three formalin fixed, paraffin-embedded 6-μm-thick serial sections were stained with hematoxylin-eosin (HE), phosphotungstic acid-hematoxylin (PTAH), or Masson's trichrome. Bunina bodies were identified as eosinophilic round or spherical inclusions a few micrometers in diameter, and staining purple with PTAH and red with Masson's trichrome. Neurons containing Bunina bodies were examined in the HE preparation.

Detailed topographic and quantitative examination of NFTs

Formalin-fixed, paraffin-embedded 6-μm-thick sections were stained with Bodian. The number of NFTs in 61 anatomical areas in one section was counted at x400 magnification by light microscopy. Statistical evaluation was performed using the Mann-Whitney U test for comparison of the number of NFTs between non-ALS non-PDC subjects and each of PDC, ALS and ALS/PDC patients (Table 1). The nomenclature used for the anatomical areas was basically that of Carpenter and Sutin [2], and for the detailed areas in the brain stem, that of Olszewski and Baxter [23].

Ultrastructural investigation of NFTs and Bunina bodies

Tissue blocks of the Sommer sector of Guam ALS and PDC patients were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer solution (PBS) (pH 7.3) and then fixed in 1% osmium tetroxide, followed by dehydration through a graded ethanol series and embedding in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate.

Five anterior horn cells containing NFTs from Guam PDC patients, and 6 anterior horn cells containing Bunina bodies from ALS patients were examined. Selected HE-stained paraffin-embedded sections of the spinal cord were recycled and immersed in xylene until separation of the cover glass. The sections were rehydrated in a graded ethanol series and then washed with 0.1 M PBS. Following post-fixation with 1% osmium tetroxide in PBS, the sections were dehydrated in a graded ethanol series up to absolute ethanol. Epon 812 in a capsule was placed on each section and then polymerized at 60 °C. After the Epon block had been removed from the glass slide, ultrathin sections were cut from the area containing NFTs or Bunina bodies, and stained with uranyl acetate and lead citrate. Observation was performed using an electron microscope (Hitachi H-7100) at 75 kV.

Results

Tau-immunopositive neurons in the motor neuron system (Fig. 1a)

All PDC patients and ALS-PDC patients, about 70% of Guam ALS patients, and 75% of non-ALS non-PDC subjects had immunopositive neurons in the motor cortex. In the oculomotor nucleus, 50% of Guam PDC patients, 66% of PDC-ALS patients, 14% of ALS patients, and 25% of non-ALS non-PDC subjects showed immunopositive neurons. In the facial nucleus, 45% of the PDC patients and 33% of ALS-PDC patients showed immunopositive neurons; however, none of the neurons from ALS patients and non-ALS non-PDC subjects were immunopositive. In the hypoglossal nucleus, immunopositive neurons were identified in 10% of the patients with ALS and PDC, but in none of the ALS-PDC patients or non-ALS non-PDC subjects. In the spinal anterior horn, immunopositive neurons were observed in 20% of ALS-PDC patients, 15% of PDC patients, and 10% of non-ALS non-PDC subjects, but were absent in ALS patients.

Detailed topographic and quantitative examination of NFTs (Table 1)

Among the 20 subjects with non-ALS non-PDC, 14 showed NFTs. The youngest subject with NFTs was aged 42 years, and the oldest subject without NFTs was aged 84 years. There was no difference in the number of NFTs between males and females. An evident fixed pattern of progression of NFTs from non-ALS non-PDC subjects to PDC patients was revealed. NFTs appeared first in the amygdaloid nucleus, or parahippocampal gyrus, and then in the substantia innominata, locus ceruleus and substantia nigra. Thereafter, NFTs appeared hypothalamus, and finally in the temporal and frontal neocortex. This topographic distribution of NFTs was quite similar to that in the PDC patients. However, in the frontal and temporal neocortex, PDC patients showed larger numbers of NFTs than in non-ALS non-PDC subjects. The number and topographic distribution of NFTs in the patients with ALS were the same as those in non-ALS non-PDC subjects, and those in ALS-PDC patients were almost the same as those in PDC patients. Some of the Guam ALS patients did have no NFTs.

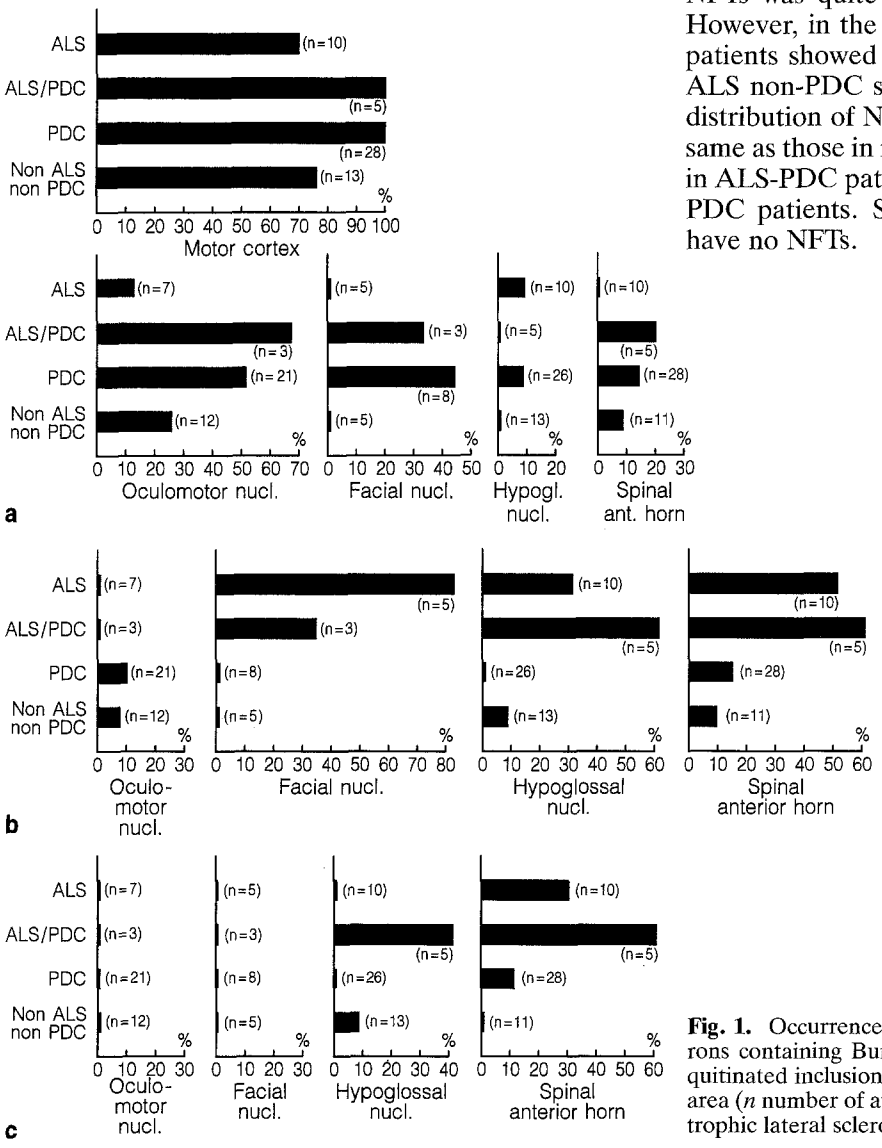

Fig. 1. Occurrence rate of tau-immunopositive neurons (**a**), neurons containing Bunina bodies (**b**), and neurons containing ubiquitinated inclusion bodies (**c**) in each group at every anatomical area (*n* number of available cases for the examination, *ALS* amyotrophic lateral sclerosis, *PDC* parkinsonism-dementia complex).

Table 1 Age and number of NFTs of examined cases. The ranges and means ± S. D. of the values are indicated. The locations within brackets show the levels examined. Statistical evaluation was performed using the Mann-Whitney U test for comparison of the number of NFTs between non-ALS non PDC subjects and each of PDC, ALS and ALS-PDC patients. (*n* number of examined cases; *NFT* neurofibrillary tangle, *ALS* amyotrophic lateral sclerosis, *PDC* parkinsonism-dementia complex)

	Non-ALS non-PDC	PDC	ALS	ALS-PDC
Age (year)	41– 84; 61.3 ± 12.6 (n=20)	52– 64; 58,7 ± 5.8 (n=6)	47– 72; 58.6 ± 10.1 (n=7)	57– 66; 60.3 ± 4.9 (n=3)
Superior frontal gyrus	0– 12; 1.0 ± 2.8 (n=20)	60–1248; 471.7 ± 454.6* (n=6)	0– 12; 4.7 ± 4.9 (n=7)	56–1120; 450.0 ± 583.2* (n=3)
Middle frontal gyrus	0– 11; 0.8 ± 2.5 (n=19)	19– 333; 142.3 ± 120.1* (n=6)	0– 6; 2.1 ± 2.3** (n=7)	15– 236; 100.0 ± 119.0* (n=3)
Inferior frontal gyrus	0– 15; 1.4 ± 3.6 (n=18)	6– 777; 241.5 ± 303.3* (n=6)	0– 10; 2.3 ± 3.6 (n=7)	17– 90; 60.7 ± 38.6* (n=3)
Lateral orbital gyrus	0– 28; 2.3 ± 6.5 (n=19)	22– 707; 366.7 ± 293.4* (n=6)	0– 17; 5.8 ± 6.6 (n=6)	51– 266; 137.7 ± 113.4* (n=3)
Gyrus rectus	0– 9; 0.6 ± 2.0 (n=20)	12– 253; 97.3 ± 91.2* (n=6)	0– 5; 1.3 ± 2.0 (n=6)	10– 180; 71.7 ± 94.1* (n=3)
Cingulate gyrus	0– 21; 2.1 ± 5.0 (n=20)	24–1225; 399.2 ± 489.9* (n=6)	0– 8; 2.9 ± 3.5 (n=7)	44– 875; 335.3 ± 467.9* (n=3)
Long insular gyri	0–230; 19.9 ± 59.4 (n=17)	250– 673; 492.0 ± 165.3* (n=5)	0–123; 20.6 ± 45.4 (n=7)	50– 594; 314.3 ± 272.3* (n=3)
Superior temporal gyrus	0– 8; 0.9 ± 2.2 (n=19)	16– 422; 156.0 ± 146.3* (n=6)	0– 8; 2.9 ± 3.7 (n=7)	16– 325; 119.7 ± 177.8* (n=3)
Middle temporal gyrus	0– 21; 2.5 ± 6.1 (n=19)	54–1749; 507.0 ± 630.4* (n=6)	0–115; 17.9 ± 42.9 (n=7)	36– 952; 347.7 ± 523.5* (n=3)
Inferior temporal gyrus	0– 31; 4.2 ± 7.8 (n=20)	81– 726; 374.3 ± 237.3* (n=6)	0–152; 29.6 ± 56.8 (n=7)	94– 703; 310.0 ± 340.9* (n=3)
Occipitotemporal gyrus	0–123; 16.7 ± 33.9 (n=20)	194–1290; 596.8 ± 421.5* (n=6)	0–111; 23.9 ± 39.4 (n=7)	382– 931; 565.3 ± 316.7* (n=3)
Parahippocampal gyrus	0–478; 87.3 ± 152.1 (n=20)	238–1158; 637.2 ± 418.8* (n=6)	0–264; 103.6 ± 107.1 (n=7)	226–1291; 662.0 ± 558.1* (n=3)
Hippocampus	0–340; 59.7 ± 91.4 (n=20)	15– 459; 257.7 ± 174.7* (n=6)	0–382; 96.7 ± 134.1 (n=7)	32– 803; 439.3 ± 387.4** (n=3)
Dentate gyrus	0– 8; 1.1 ± 2.1 (n=20)	3– 12; 6.3 ± 3.6* (n=6)	0– 3; 0.9 ± 1.1 (n=7)	1– 18; 7.0 ± 9.5 (n=3)
Amygdaloid nucleus	0–120; 10.9 ± 30.6 (n=15)	54–1115; 693.8 ± 468.1* (n=4)	0–208; 59.7 ± 87.0 (n=7)	49– 478; 286.0 ± 218.0* (n=3)
Septal nucleus	0– 1; 0.1 ± 0.3 (n=13)	2– 8; 4.8 ± 3.2* (n=4)	0– 1; 0.2 ± 0.5 (n=5)	14; 14.0 ± 0.0 (n=1)
Diagonal band of Broca	0– 4; 0.7 ± 1.4 (n=12)	2– 34; 12.4 ± 14,2* (n=5)	0– 2; 1.0 ± 0.9 (n=6)	6– 19; 12.5 ± 9.2** (n=2)
Nucleus accumbens septi	0– 8; 0.8 ± 2.2 (n=16)	3– 56; 29.5 ± 37.5 (n=2)	0– 4; 1.2 ± 1.6 (n=5)	18– 19; 18.5 ± 0.7** (n=2)
Medial olfactory stria	0–484; 37.0 ± 116.1 (n=19)	15– 337; 149.2 ± 154.8* (n=5)	0– 98; 21.3 ± 38.7 (n=6)	216– 883; 549.5 ± 471.6** (n=2)
Caudate nucleus head	0– 2; 0.2 ± 0.5 (n=19)	1– 23; 10.2 ± 9.3* (n=6)	0– 3; 1.0 ± 1.3 (n=6)	4 – 14; 10.0 ± 5.3* (n=3)
body	0– 1; 0.1 ± 0.3 (n=19)	0– 7; 3.2 ± 3.1 (n=5)	0– 2; 0.4 ± 0.8 (n=7)	0– 6; 3.0 ± 3.0 (n=3)
Putamen (optic chiasm)	0– 2; 0.3 ± 0.7 (n=19)	1– 21; 7.0 ± 7.3* (n=6)	0– 2; 0.3 ± 0.8 (n=6)	3– 5; 3.7 ± 1.2* (n=3)
(mammillary body)	0– 4; 0.6 ± 1.3 (n=19)	0– 19; 6.3 ± 6.7* (n=6)	0– 2; 0.6 – 1.0 (n=7)	3– 4; 3.7 ± 0.6** (n=3)
Claustrum	0– 6; 0.5 ± 1.4 (n=19)	2– 27; 10.7 ± 8.8* (n=6)	0; 0.0 ± 0.0 (n=7)	0– 16; 6.0 ± 8.7 (n=3)
Substantia innominata	0– 64; 7.4 ± 14.9 (n=20)	6– 250; 59.8 ± 94.5* (n=6)	0– 18; 5.7 ± 6.8 (n=7)	2– 71; 27.0 ± 38.2 (n=3)
Globus pallidus lateral segment	0– 1; 0.1 ± 0.2 (n=19)	1– 9; 4.7 ± 3.7* (n=6)	0– 2; 0.7 ± 0.8** (n=7)	0– 3; 1.5 ± 2.1 (n=2)
medial segment	0– 1; 0.1 ± 0.3 (n=19)	0– 3; 4.5 ± 8.6** (n=6)	0– 2; 0.6 ± 0.8 (n=7)	2– 9; 5.5 ± 5.0* (n=2)
Thalamus (mammillary body)	0– 18; 1.4 ± 4.5 (n=18)	9– 100; 33.8 ± 35.1* (n=6)	0– 20; 4.3 ± 7.5 (n=7)	9– 46; 22.0 ± 20.8** (n=3)
(subthalamic nucl.)	0– 7; 0.7 ± 2.1 (n=19)	6– 65; 34.2 ± 23.1* (n=6)	0– 15; 3.0 ± 5.8 (n=7)	10– 46; 22.7 ± 20.2* (n=3)
Hypothalamus	0– 42; 4.8 ± 11.1 (n=20)	7– 61; 21.0 ± 20.6* (n=6)	0– 18; 4.1 ± 6.3 (n=7)	3– 14; 8.0 ± 5.6 (n=3)
Mammillary body	0– 1; 0.1 ± 0.2 (n=18)	0– 19; 6.3 ± 6.6* (n=6)	0– 4; 1.2 ± 1.8 (n=6)	0– 8; 3.3 ± 4.2 (n=3)
Subthalamic nucleus	0– 2; 0.2 ± 0.5 (n=16)	9– 29; 18.3 ± 10.1* (n=3)	0– 1; 0.3 ± 0.5 (n=4)	3– 13; 7.0 ± 5.3* (n=3)
Precentral gyrus	0– 2; 0.2 ± 0.7 (n=18)	0– 95; 38.8 ± 39.1* (n=6)	0– 23; 3.9 ± 8.6 (n=7)	2– 65; 37.7 ± 32.3* (n=3)
Inferior parietal lobule	0– 5; 0.7 ± 1.4 (n=17)	0– 85; 27.2 ± 34.8** (n=5)	0– 7; 1.5 ± 2.8 (n=6)	3– 23; 11.0 ± 10.6* (n=3)
Striate cortex	0– 5; 0.3 ± 1.1 (n=20)	0– 48; 8.0 ± 19.6 (n=6)	0– 2; 0.3 ± 0.8 (n=7)	0– 4; 1.3 ± 2.3 (n=3)
Substantia nigra	0– 27; 3.5 ± 7.5 (n=20)	12– 46; 27.6 ± 12.5* (n=5)	0– 14; 4.9 ± 6.4 (n=7)	4– 16; 9.3 ± 6.1 (n=3)
Mesencephalic central gray	0– 18; 1.4 ± 4.1 (n=20)	8– 78; 34.0 ± 25.9* (n=6)	0– 30; 8.0 ± 11.0 (n=7)	8– 44; 29.3 ± 18.9* (n=3)
Superior colliculus	0– 2; 0.3 ± 0.7 (n=16)	2– 16; 6.7 ± 4.9* (n=6)	0– 4; 1.2 ± 1.6 (n=6)	2– 16; 10.7 ± 7.6* (n=3)
Interpeduncular nucleus	0– 4; 0.4 ± 1.1 (n=18)	0– 12; 5.0 ± 5.0** (n=4)	0; 0.0 ± 0.0 (n=3)	2; 2.0 ± 0.0 (n=2)
Oculomotor nuclei	0; 0.0 ± 0.0 (n=20)	0– 20; 6.0 ± 8.2** (n=6)	0– 12; 2.3 ± 4.4 (n=7)	4; 4.0 ± 0.0* (n=2)
Mesenceph. reticul. formation	0– 12; 1.3 ± 2.8 (n=20)	6– 42; 19.0 ± 15.7* (n=6)	0– 18; 3.3 ± 6.6 (n=7)	0– 18; 8.7 ± 9.0 (n=3)
Pedunculopontine tegmental nucl.	0– 4; 0.2 ± 0.9 (n=20)	0– 28; 9.0 ± 9.9* (n=5)	0– 10; 1.6 ± 3.7 (n=7)	0– 2; 0.7 ± 1.2 (n=3)
Red nucleus	0; 0.0 ± 0.0 (n=19)	2– 10; 4.5 ± 3.8* (n=4)	0; 0.0 ± 0.0 (n=3)	0– 8; 4.0 ± 5.7 (n=2)
Locus ceruleus	0– 76; 9.1 ± 18.1 (n=20)	1– 44; 13.3 ± 16.4 (n=6)	0– 18; 8.9 ± 7.1 (n=7)	0– 6; 3.0 ± 3.0 (n=3)
Pontine central gray	0– 3; 0.2 ± 0.7 (n=20)	0– 4; 2.7 ± 1.6* (n=6)	0– 4; 0.9 ± 1.6 (n=7)	0– 7; 4.3 ± 3.8 (n=3)
Superior central nucleus	0– 15; 2.0 ± 3.8 (n=20)	0– 26; 6.4 ± 11.2 (n=5)	0– 2; 0.4 ± 0.9 (n=5)	12– 13; 12.5 ± 0.7 (n=2)
Nucl. pontis centralis oralis	0– 5; 0.5 ± 1.2 (n=20)	0– 8; 3.5 ± 3.2** (n=6)	0– 6; 1.4 ± 2.2 (n=7)	0– 12; 5.3 ± 6.1 (n=3)
Nucl. parabrachialis	0– 1; 0.1 ± 0.2 (n=20)	0; 0.0 ± 0.0 (n=6)	0– 1; 0.1 ± 0.4 (n=7)	0– 3; 1.7 ± 1.5 (n=3)
Proc. griseum pont. supralemnisc.	0– 4; 0.2 ± 0.9 (n=19)	0– 4; 1.8 ± 1.8** (n=6)	0; 0.0 ± 0.0 (n=7)	0– 6; 3.0 ± 3.0 (n=3)
Pontine nuclei	0; 0.0 ± 0.0 (n=20)	0– 6; 2.0 ± 3.1 (n=6)	0; 0.0 ± 0.0 (n=7)	0– 12; 4.0 ± 6.9 (n=3)
Nucl. pontis centralis caudalis	0; 0.0 ± 0.0 (n=20)	0– 8; 2.0 ± 3.1 (n=6)	0; 0.0 ± 0.0 (n=6)	0– 2; 1.3 ± 1.2 (n=3)
Nucl. gigantocellularis, pons	0– 2; 0.1 ± 0.5 (n=18)	0– 20; 4.2 ± 7.9 (n=6)	0; 0.0 ± 0.0 (n=6)	0– 6; 3.3 ± 3.1 (n=3)
Dorsal motor nucleus of vagus	0– 2; 0.2 ± 0.5 (n=20)	0– 3; 1.0 ± 1.4 (n=5)	0– 1; 0.3 ± 0.5 (n=7)	0; 0.0 ± 0.0 (n=2)
Hypoglossal nucleus	0; 0.0 ± 0.0 (n=20)	0; 0.0 ± 0.0 (n=5)	0; 0.0 ± 0.0 (n=7)	0; 0.0 ± 0.0 (n=2)
Nucleus solitarius	0; 0.0 ± 0.0 (n=18)	0; 0.0 ± 0.0 (n=4)	0; 0.0 ± 0.0 (n=6)	0; 0.0 ± 0.0 (n=2)
Inferior central nucleus	0; 0.0 ± 0.0 (n=20)	0– 2; 0.8 ± 0.8 (n=5)	0; 0.0 ± 0.0 (n=7)	0; 0.0 ± 0.0 (n=3)
Nucl. medul. oblongata centralis	0– 1; 0.1 ± 0.3 (n=19)	0– 20; 4.6 ± 8.6 (n=5)	0; 0.0 ± 0.0 (n=7)	0– 1; 0.7 ± 0.6 (n=3)
Nucl. medul. oblongata subtrigemin.	0; 0.0 ± 0.0 (n=16)	0– 2; 1.5 ± 1.0** (n=4)	0; 0.0 ± 0.0 (n=7)	0– 3; 1.5 ± 2.1 (n=2)
Inferior olivary nucleus	0; 0.0 ± 0.0 (n=20)	0– 6; 1.5 ± 3.0 (n=4)	0; 0.0 ± 0.0 (n=7)	0; 0.0 ± 0.0 (n=3)
Dentate nucleus	0– 16; 0.9 ± 3.6 (n=20)	0– 5; 1.8 ± 2.1** (n=6)	0; 0.0 ± 0.0 (n=7)	0– 10; 4.0 ± 5.3 (n=3)
Cerebellar hemisphere	0; 0.0 ± 0.0 (n=20)	0; 0.0 ± 0.0 (n=6)	0; 0.0 ± 0.0 (n=7)	0; 0.0 ± 0.0 (n=3)

* $P < 0.01$; ** $P < 0.05$

Quantitative examination of Bunina body-containing neurons in the motor neuron system (Fig. 1b)

In the present series, 70% of Guam ALS patients, 80% of ALS-PDC patients, 7% of PDC patients, and 10% of non-ALS non-PDC subjects showed Bunina bodies in the motor neuron system. In the motor cortex, no Bunina bodies were evident in any disease group. Strangely, in the oculomotor nucleus, about 10% of the PDC patients and non-ALS non-PDC subjects showed Bunina bodies, whereas none were evident in ALS and ALS-PDC patients. In the facial nucleus, 80% of ALS and 33% of ALS-PDC patients showed Bunina bodies, but none were evident in PDC patients or non-ALS non-PDC subjects. Also 30% of ALS patients, 60% of ALS-PDC patients, and 8% of non-ALS non-PDC subjects had Bunina bodies in the hypoglossal nucleus, but none were evident in PDC patients. In the spinal anterior horn cells, about 50% of ALS and 60% of ALS-PDC patients showed Bunina bodies. Interestingly, 15% of PDC patients and 9% of non-ALS non-PDC subjects also possessed the bodies.

Quantitative study of ubiquitinated inclusion body-containing neurons in the motor neuron system (Fig 1c)

No immunopositive neurons were observed in the motor cortex, or oculomotor and facial nuclei in any of the patients and subjects. In the hypoglossal nucleus, 40% of ALS-PDC patients and 8% of non-ALS non-PDC subjects showed ubiquitinated inclusions. In the spinal anterior horn, about 30% of ALS patients revealed typical skein-like inclusions, and 60% of ALS-PDC patients and 10% of PDC patients showed positive staining. No inclusion was observed in non-ALS non-PDC subjects.

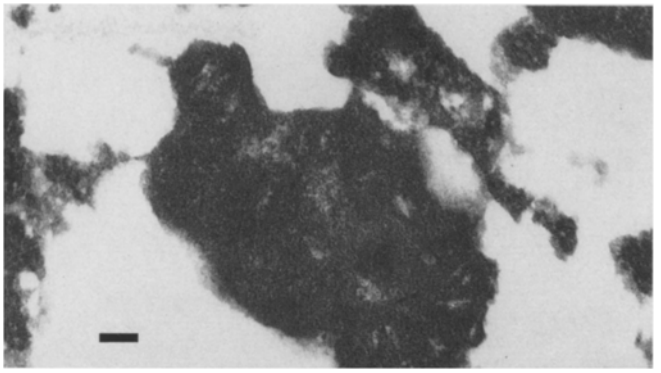

Fig. 3. Electron micrograph of a Bunina body in an anterior horn cell in paraffin-embedded cervical spinal cord in a Guam ALS patient. Uranyl acetate-lead citrate. *Bar* = 100 nm.

Ultrastructural examination of NFTs and Bunina bodies (Figs. 2, 3)

NFTs in the Sommer sector in both ALS (Fig. 2a) and PDC patients were composed primarily of paired helical filaments (PHF), measuring 12 to 27 nm in diameter and showing constrictions regularly spaced at intervals of 58 to 75 nm. A small number of straight tubules, measuring 14 to 16 nm in diameter, were admixed in the PHF.

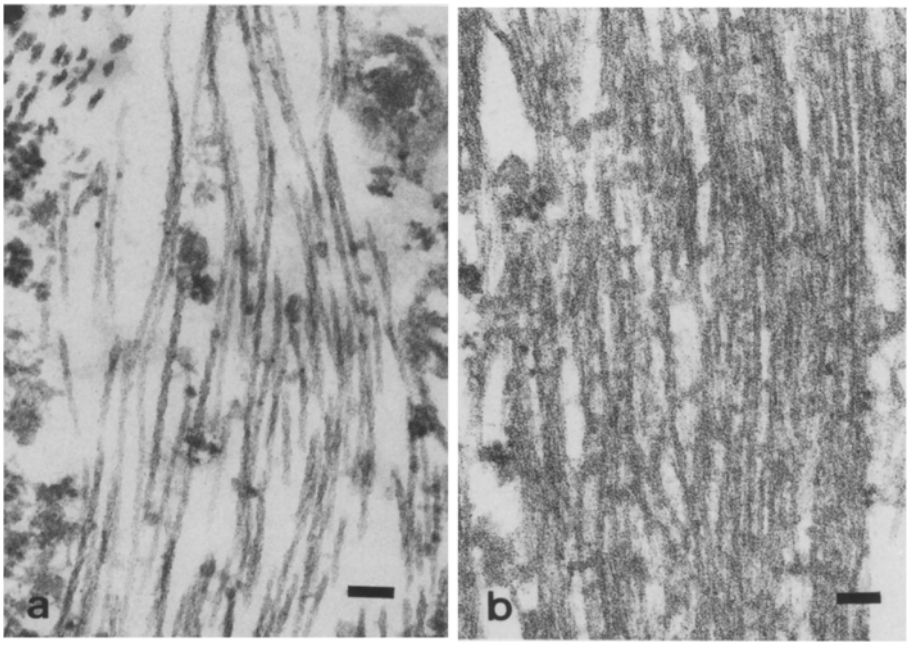

Fig. 2. Electron micrographs of NFTs in pyramidal neurons in the Sommer sector of a Guam ALS patient (**a**), and in an anterior horn cell in paraffin-embedded cervical spinal cord of a PDC patient (**b**). The NFTs are composed mainly of 12- to 27-nm-wide paired helical filaments with a periodicity of 58 to 75 nm in pyramidal neurons in Sommer sector in ALS, but those in the anterior horn cells are composed of 13- to 20-nm-wide straight tubules in PDC. Uranyl acetate-lead citrate. (*NFT* neurofibrillary tangle). *Bars* = 100 nm.

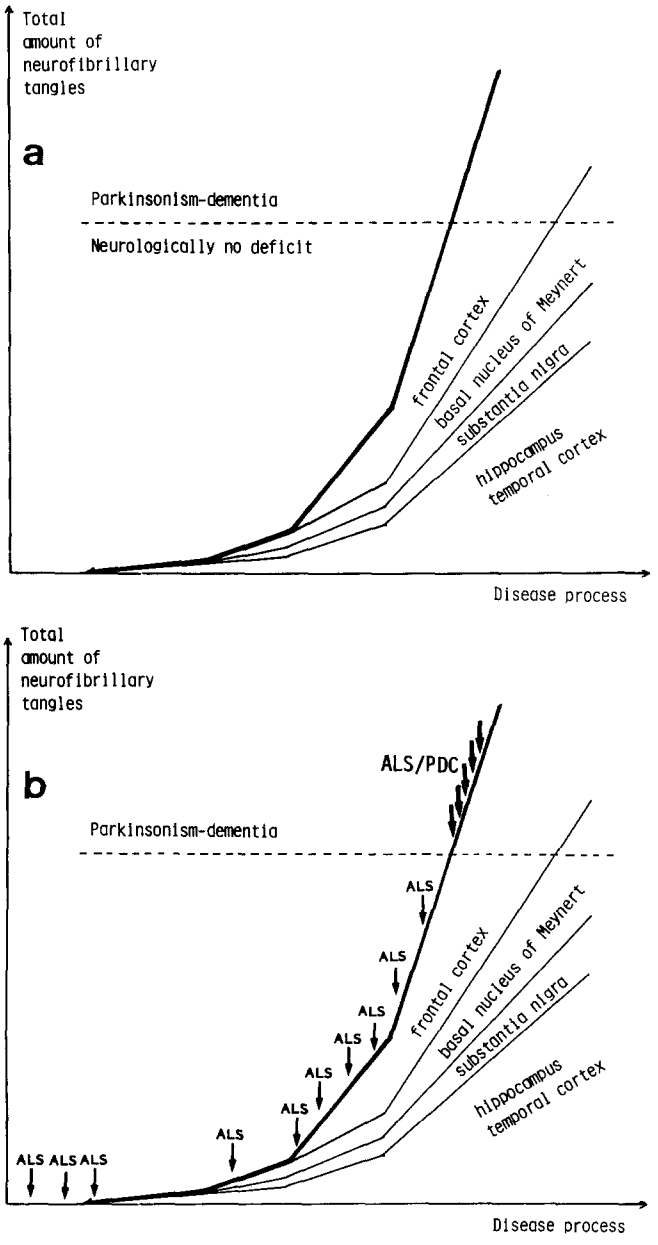

Fig. 4. Progression pattern of NFTs in the brains of non-ALS non-PDC Guamanians and of PDC patients (**a**). NFTs in ALS patients are quantitatively and topographically the same as those in non-ALS non-PDC subjects, but those in ALS-PDC patients are the same as those in PDC patients (**b**).

The NFTs in the spinal anterior horn cells of PDC patients were composed of 13- to 20-nm-wide straight tubules admixed with a fairly small number of paired helical filaments with irregular periodicity (Fig. 2b).

The Bunina bodies in the spinal anterior horn of ALS patients consisted of dense granular or homogeneous material containing hollows, and were irregular in shape. Their margins were uneven and no limiting membranes were evident (Fig. 3).

Discussion

Because of the lack of neurological data on non-ALS non-PDC subjects, and since parkinsonism or dementia might be masked by the amyotrophy in ALS patients, there may be unreliability in the grouping of patients and subjects. In addition, it has been reported that NFTs are present in the brain of not only Guam ALS and PDC patients but also non-ALS non-PDC subjects [1, 6]. However, the present study showed that, whereas the number of NFTs was small in non-ALS non-PDC subjects, they were more frequent in patients with Guam PDC and ALS-PDC, especially in the frontal and temporal cortex. The number of NFTs in ALS patients was the same as that in non-ALS non-PDC subjects, although the number of NFTs in ALS-PDC patients was similar to that in PDC patients. Thus non-ALS non-PDC subjects and ALS patients were clearly differentiated from ALS-PDC and PDC patients by the number of NFTs present (Table 1). The number of NFTs in the subjects in their 40's was smaller than that seen in their 50's and 60's. This finding indicates that NFT formation is closely linked with aging.

The topographic distribution of NFTs was basically the same for all disease groups and the non-ALS non-PDC group. Among non-ALS non-PDC and ALS, there were several subjects and patients without NFTs, as reported previously [8]. Progression of the NFTs showed a fixed pattern. NFTs appeared first in the amygdaloid nucleus or parahippocampal gyrus, and subsequently progressed to the substantia innominata, locus ceruleus and substantia nigra, and then the hypothalamus; finally the temporal and frontal neocortex was affected. In Guam ALS-PDC and PDC patients, numerous NFTs were observed in these areas, especially in the frontal and temporal cortex (Table 1, Fig. 4a).

The occurrence rate of tau-immunopositive neurons in the motor cortex, oculomotor nucleus, and facial nucleus was almost the same between Guam PDC and ALS-PDC patients, and between Guam ALS patients and non-ALS non-PDC subjects. In the hypoglossal nucleus, a small number of tau-immunopositive neurons were observed in Guam ALS and PDC, but in the spinal anterior horn, no tau-immunopositive neurons were evident in ALS patients, although a few tau-immunopositive neurons were observed in Guam PDC and ALS-PDC patients and non-ALS non-PDC subjects.

These findings indicate that the presence of NFTs in Guam ALS patients is merely a background feature which affects Guamanians in general (Fig. 4b). Motor weakness or oculomotor palsy observed in PDC patients [3, 13] may be due to NFTs in the anterior horn cells or oculomotor nucleus. In ALS-PDC patients, these symptoms may be accelerated by overlapping of NFT formation and motor neuron degeneration in the ALS disease process.

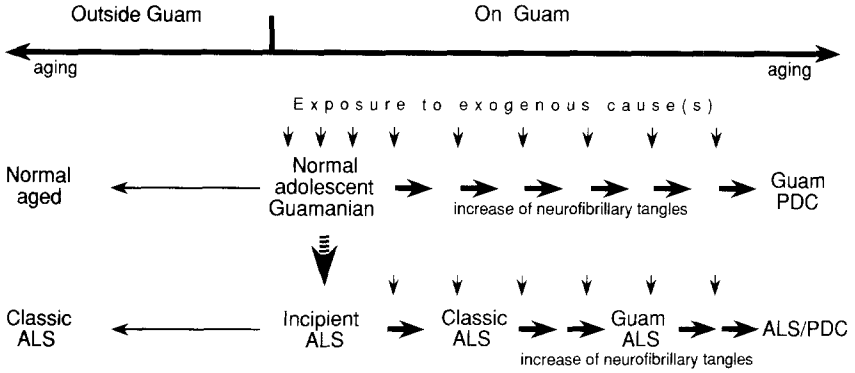

Fig. 5. Schematic demonstration of possible disease processes of ALS, PDC and ALS-PDC on Guam. The authors think that Guam ALS is a disease predestined by exposure to some exogenous factor(s) during the patients' childhood or adolescence, and that its symptoms appear after middle age, independently of the patients' life thereafter. In contrast, Guam PDC is a disease whose expression requires continuous exposure to the factor(s) for a long period in middle age. If designated incipient ALS patients are continuously exposed to the causative factor(s) until middle or old age, some will become ALS-PDC patients. If young individuals with incipient ALS emigrate from Guam, then they will show classic ALS. The authors consider that the true onset of Guam ALS occurs in the patients' childhood or adolescence.

It has been reported that there are similarities in the antigenic composition of NFTs between Guam ALS, PDC and Alzheimer's disease [11, 24]. NFTs in the Sommer sector were composed mainly of PHFs in both Guam ALS and PDC, while the NFTs in the anterior horn cells of Guam PDC were composed mostly of straight tubules, as reported previously [17, 19]. The reason why NFTs show different ultrastructural features in disparate areas awaits further clarification.

The histological and ultrastructural features of Bunina bodies in the spinal anterior horn cells in Guam ALS, PDC, and ALS-PDC patients, and non-ALS non-PDC Guamanians were similar to those reported in classic ALS [26]. In the present series, 70% of the patients with Guam ALS and 80% of the patients with ALS-PDC showed Bunina bodies. These ratios are similar to those reported previously for classic ALS [7, 22]. In addition, the fact that 7% of PDC patients and 10% of non-ALS non-PDC subjects showed Bunina bodies may indicate that the ALS disease process prevails widely in the population.

Ubiquitinated skein-like inclusions were observed in the anterior horn cells in Guam ALS patients, as reported previously [21]. The frequent occurrence of the inclusions in ALS-PDC patients seemed to be due to overlap of NFTs and skein-like inclusions.

The foregoing findings suggest that classic ALS does, in fact, exist on Guam, that the presence of NFTs in patients with Guam ALS is merely a background feature widely dispersed among the population of the island, that the disease process in Guam ALS is basically different from that in PDC, and that Guam ALS initially occurs as the classic ALS (Figs. 4b, 5)

Guam ALS = Classic ALS + neurofibrillary degeneration?

From a survey of Guamanian migrants from Guam who showed a high mortality rate from ALS, it was reported that the minimum exposure time to environmental variables on Guam was 18 years, and that all patients had spent their childhood and adolescence on Guam [9]. In contrast, the minimum exposure time for Guam PDC is 13 years in their middle age [4, 5, 10].

The authors believe that Guam ALS is a disease predestined by exposure to some exogenous factor(s) during the patients' childhood or adolescence, and that its symptoms appear after middle age, independently of the patients' life thereafter. In contrast, Guam PDC is a disease whose expression requires continuous exposure to the factor(s) for a long period in middle age. If designated incipient ALS patients are continuously exposed to the causative factor(s) until middle or old age, some will become ALS-PDC patients. If young individuals with incipient ALS emigrate from Guam, then they will show classic ALS (Fig. 5). Indeed, Guamanian migrants from Guam showed a high incidence of ALS, but ALS-PDC was never established among them [9]. The authors, therefore, consider that the true onset of Guam ALS occurs in the patients' childhood or adolescence, and thus may be classic ALS.

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