Thalamic involvement in a spinocerebellar ataxia type 2 (SCA2) and a spinocerebellar ataxia type 3 (SCA3) patient, and its clinical relevance

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Summary
In spite of the considerable progress in clinical and molecular research, knowledge regarding brain damage in spinocerebellar ataxia type 2 (SCA2) and type 3 (SCA3) still is limited and the extent to which the thalamus is involved in both diseases is uncertain. Accordingly, we performed a pathoanatomical analysis on serial thick sections stained for lipofuscin granules and Nissl substance through the thalami of two genetically confirmed cases: one an SCA2 patient, the other an SCA3 patient. During this systematic study, we detected severe destruction of the reticular (RT), fasciculus (FA), ventral anterior (VA), ventral lateral (VL), ventral posterior lateral (VPL), ventral posterior medial (VPM), cucullar (CU) and mediodorsal thalamic nuclei (MD), the lateral geniculate body (LGB) and inferior nucleus of the pulvinar (PU i) in the SCA2 case, and a severe neuronal loss in the RT, FA, VA and PU i of the SCA3 case. In the SCA2 patient, additional obvious neuronal loss was observed in all nuclei of the anterior and rostral intralaminar groups, in the lateral posterior nucleus (LP), the lateral (PU l) and the medial subnuclei of the pulvinar (PU m), whereas in the SCA3 patient only two of the nuclei that belong to the anterior thalamic group, the VL, VPL, VPM, LP, LGB, PU l and PU m, displayed marked neurodegeneration. These novel findings indicate that thalamic involvement in SCA2 and SCA3 patients has been underestimated in the past. In view of what is known about the functions of the affected thalamic nuclei, the present findings provide an appropriate pathoanatomical explanation for some of the disease-related symptoms seen in both of our and other SCA2 and SCA3 patients: gait, stance, truncal and limb ataxia, dysarthria or anarthria, falls, dysdiadochokinesia and bradykinesia, problems with writing, somatosensory deficits, saccadic dysfunctions, executive dysfunctions and abnormalities of visual evoked potentials.

Keywords: Machado–Joseph disease; pathoanatomy; polyglutamine diseases; spinocerebellar ataxias; thalamus

Abbreviations: AD = anterodorsal nucleus; AP = anteroprinicipal nucleus; CAG = cytosine, adenine, guanine trinucleotide sequence; CEM = central medial nucleus; CL = central lateral nucleus; CM = centromedian nucleus; CU = cucullar nucleus; FA = fasciculus nucleus; GFAP = glial fibrillary acidic protein; LD = laterodorsal nucleus; LGB = lateral geniculate body; LI–SG = limitans–suprageniculate complex; LP = lateral posterior nucleus; MD = mediodorsal nucleus; MGB = medial geniculate body; MJD = Machado–Joseph disease; PC = paracentral nucleus; PF = parafascicular nucleus; PT = parataenial nucleus; PU a = pulvinar, anterior nucleus; PU i = pulvinar, inferior nucleus; PU l = pulvinar, lateral nucleus; PU m = pulvinar, medial nucleus; PV = paraventricular nucleus; RT = reticular nucleus; SCA = spinocerebellar ataxia (type indicated by following number); SPF = subparafascicular nucleus; VA = ventral anterior nucleus; VL = ventral lateral nucleus; VPL = ventral posterior lateral nucleus; VPM = ventral posterior medial nucleus; VPMpc = ventral posterior medial nucleus, parvocellular part
Introduction

Spino cerebellar ataxia type 2 (SCA2) and type 3 (SCA3 or Machado–Joseph disease, MJD) represent autosomal dominantly inherited ataxic disorders that are assigned to a group of untreatable, and ultimately, fatal neurological disorders known as CAG repeat or polyglutamine diseases. Other CAG repeat disorders currently include Huntington’s disease, spinobulbar muscular atrophy, dentatorubro-pallidolysian atrophy, SCA1, SCA6, SCA7 and SCA17. All of these diseases commonly begin in adulthood, and the onset of clinical symptoms tends to begin earlier in the following generation. Furthermore, they become chronic and take a progressive course that extends over a 10–30 year period (Kawaguchi et al., 1994; Schöls et al., 1995; Trottier et al., 1995; Imbert et al., 1996; Pulst et al., 1996; Paulson et al., 1997; Klockgether and Evert, 1998; Huynh et al., 1999; Klockgether et al., 2000; Paulson, 2000; Nakamura et al., 2001; Paulson and Ammache, 2001). Molecular biologically, this group of diseases is characterized by meiotic unstable expansions of (CAG)n trinucleotide repeat sequences at specific gene loci that encode aberrantly long glutamine stretches in the disease proteins. The expanded CAG repeat in SCA2 resides on chromosome 12q23–24.1 and, in SCA3, on chromosome 14q24.3–32.2, whereby the normal SCA2 allele comprises 14–31 and the allele for SCA3 12–40 CAG triplets. The expanded CAG repeat disorders currently include Huntington’s disease, spinobulbar muscular atrophy, dentatorubro-pallidolysian atrophy, SCA1, SCA6, SCA7 and SCA17. All of these diseases commonly begin in adulthood, and the onset of clinical symptoms tends to begin earlier in the following generation. Furthermore, they become chronic and take a progressive course that extends over a 10–30 year period (Kawaguchi et al., 1994; Schöls et al., 1995; Trottier et al., 1995; Imbert et al., 1996; Pulst et al., 1996; Paulson et al., 1997; Klockgether and Evert, 1998; Huynh et al., 1999; Klockgether et al., 2000; Paulson, 2000; Nakamura et al., 2001; Paulson and Ammache, 2001). Molecular biologically, this group of diseases is characterized by meiotic unstable expansions of (CAG)n trinucleotide repeat sequences at specific gene loci that encode aberrantly long glutamine stretches in the disease proteins. The expanded CAG repeat in SCA2 resides on chromosome 12q23–24.1 and, in SCA3, on chromosome 14q24.3–32.2, whereby the normal SCA2 allele comprises 14–31 and the allele for SCA3 12–40 CAG triplets.

In SCA2 patients, the mutated allele is expanded by 35–59 CAG repeats, and, in SCA3 patients, to a sequence of ~56–84 CAG triplets (Kawaguchi et al., 1994; Gispert et al., 1995; Hernández et al., 1995; Schöls et al., 1995, 1997; Stevanin et al., 1995; Imbert et al., 1996; Nechiporuk et al., 1996; Pulst et al., 1996; Lorenzetti et al., 1997; Paulson et al., 1997; Klockgether et al., 2000; Paulson, 2000; Tang et al., 2000; Paulson and Ammache, 2001). Widely expressed in both neural and non-neural human tissue, the products of the SCA2 and SCA3 genes, ataxin-2 and ataxin-3, are proteins with unknown physiological functions that share no homologies with the gene products of other known polyglutamine diseases (Pulst et al., 1996; Cancel et al., 1997; Lorenzetti et al., 1997; Paulson et al., 1997; Schmidt et al., 1998; Huynh et al., 1999; Paulson and Ammache, 2001).

Although further data regarding the pathogenesis of SCA2 and SCA3 are expected to emerge from animal models (Pulst et al., 1996; Schmidt et al., 1998; Paulson, 2000), their scientific value can only be evaluated on the basis of reliable knowledge of the full topographical extent of damage in SCA2 and SCA3 brains. Owing to a dearth of systematic post-mortem analyses, however, such a basis currently is unavailable, and recent clinical studies hypothesize that the degenerative process that underlies SCA2 and SCA3 may not be restricted to known predilection sites, but may also involve re-entrant prefrontal cognitive circuits (Maruff et al., 1996; Pulst et al., 1996; Geschwind et al., 1997; Gambardella et al., 1998; Bürk et al., 1999; Storey et al., 1999; Klockgether et al., 2000; Le Pira et al., 2002; Pang et al., 2002; Zawacki et al., 2002). Thalamic integrity is crucial for the efficient performance of re-entrant prefrontal, somatomotor and oculomotor basal ganglia–thalamocortical and cerebellothalamocortical circuits. The thalamus additionally is integrated into elementary and higher order processing sensory and limbic circuits (Jones, 1985; Alexander et al., 1990; Ghez, 1991; Alexander and DeLong, 1992; Gilman, 1992; Robinson and Petersen, 1992; Bentivoglio et al., 1993; Groenewegen and Berends, 1994; Grieve et al., 2000; Nakano et al., 2000) and is known to be involved in Huntington’s disease (Dom et al., 1976; Heinsen et al., 1996, 1999). Reports regarding the status of the thalamus in SCA2 and SCA3 are not unanimous, and the results range from no involvement (Rosenberg et al., 1976, 1979; Romanul et al., 1977; Kanda et al., 1989; Dürr et al., 1995) to slight (Iwabuchi et al., 1999; Pang et al., 2002) or moderate involvement (Iwabuchi et al., 1999). Encouraged by the latest clinical studies cited above, we performed a pathoanatomical analysis of the thalamus in one clinically diagnosed and genetically confirmed SCA2 and one SCA3 case to see whether, and to what extent, thalamic nuclei had undergone degeneration. We now can provide new insights into thalamic involvement in SCA2 and SCA3 together with its possible relevance for the clinical picture.

Patients and methods

Patients

SCA2 patient

The female patient was descended from a German SCA2 family and received treatment in the Department of Neurology of the University Hospital Düsseldorf, Germany. She showed initial difficulties with walking and writing at the age of 6 years, and developed the first signs of SCA2 (Table 1) >30 years prior to her father’s age of onset, who likewise was affected by the disease. When she was 10 years old, gait and stance ataxia as well as dysarthria were observed and, as a 13 year old, she additionally developed dysmetria and ataxia of the upper limbs. MRI investigation at that point revealed no signs of cerebellar atrophy. At the age of 15 years, fasciculation-like movements of the tongue, facial hypokinesia, ingestive malfunctions leading to dysphagia, absence of myotatic reflexes of the upper and lower extremities, and diffuse myoclonia compounded the clinical picture. Upon neurological examination at age 20 years, the patient, along with severe stance, gait, truncal and upper limb ataxia, presented with dysarthria, bradykinesia, diffuse myoclonia, intention tremor and extinguished myotatic reflexes, rigidity, dysdiadochokinesia and impaired coordination of the upper and lower limbs, decreased vibration sense and impaired thermal discrimination in the lower limbs. In addition, truncal and head tremor, double vision, dysmetrical and slowed horizontal saccades and a pallor of the optic disk were present. Progressive worsening of the disease symptoms forced the patient into a wheelchair at the age of 17 years. By the age of 23 years, she was bedridden. Two years later, the
patient died from the results of a central respiratory paralysis in a poor state of general health and undernourished (Table 1). Multiple fractures resulting from falls and aspiration pneumonias related to dysphagia repeatedly necessitated in-patient treatment. At no point during the course of disease were indications of a general cognitive decline or mnestic dysfunctions observable.

SCA3 patient
This female patient was a member of a Dutch SCA3/MJD family from the northern part of The Netherlands, who was admitted to the Department of Neurology of the University Hospital Groningen, The Netherlands. Other family members known to be affected by SCA3 included the mother and the patient’s two sisters. Gait ataxia in this patient began around the age of 47 years (Table 1). At 51 years of age, neuroradiological investigation by means of a CT scan revealed a slight dilation of the fourth ventricle. Subsequently, at age 52 years, the patient resigned from her job in a hotel because she had often dropped the crockery. During the initial neurological examination at the age of 55 years, the dysarthric patient mentioned frequent falls in the past, serious difficulties with climbing stairs, loss of muscular strength, and lack of coordination in the upper and lower limbs. Investigation of eye movements revealed gaze-evoked nystagmus, abnormal saccadic smooth pursuits and slowed horizontal saccades. Patellar tendon reflexes were brisk, Achilles tendon reflexes were absent and, apart from a slight dilation of the fourth ventricle, a CT scan showed no other intracranial pathology. Patellar tendon reflexes were brisk, Achilles tendon reflexes were absent and, apart from a slight dilation of the fourth ventricle, a CT scan showed no other intracranial pathology. Following the gradual exacerbation of gait and stance ataxia as well as imbalance, the patient became wheelchair-bound at the age of 68 years and required assistance with the essential activities of daily living. During a second neurological examination at age 70 years, the patient displayed anarthria, gait and stance ataxia, upper limb ataxia, bradykinesia and generalized motor weakness. Her tongue was slightly atrophic, her facial muscles weak, and the muscularity of her arms and hands were atrophic. She had problems with writing, and dysdiadochokinesia was present in the lower and upper limbs. Patellar tendon and Achilles tendon reflexes were extinguished, and the perception of vibration was reduced in her legs. Oculomotor dysfunctions included horizontal and vertical gaze palsy, gaze-evoked nystagmus and an abducens paresis. Further deterioration of her general health resulted in a growing loss of independence and necessitated nursing home care. At the age of 75 years, the seriously handicapped patient died from aspiration pneumonia (Table 1). During the course of the disease, she occasionally reported visual hallucinations. States of confusion and disorientation were also observed periodically, and moderate cognitive decline was noticed by her care givers and relatives during the 5 years preceding her death.

Genetic analysis
SCA2 patient
At age 18 years, genomic DNA isolated from peripheral leucocytes by polymerase chain reaction amplification and application of the oligonucleotide primers SCA2A and SCA2B was investigated to assess the length of the CAG repeat sequence in the SCA2 gene on chromosome 12q23–24.1 (Imbert et al., 1996; Pulst et al., 1996). Genetic analysis confirmed the clinical diagnosis SCA2 by showing the presence of 22 CAG triplets in the normal allele and a sequence of 52 CAG repeats in the mutated allele (Table 1).

SCA3 patient
At age 74 years, genetic diagnosis was performed by genotyping the DNA extracted from peripheral lymphocytes with polymorphic dinucleotide repeat sequences that flank the MJD1 gene locus on chromosome 14q24.3–32.2 (Verschuuren-Bemelmans et al., 1995). This investigation revealed a sequence of 28 CAG repeats in the normal allele.
and 68 CAG repeats in the diseased allele (Table 1), thereby identifying the patient as a carrier of the mutated SCA3 gene.

Tissue treatment
After fixation of both patients’ brains and those from five additional individuals without medical histories of neurological or psychiatric diseases (two females, three males; mean age 52.8 ± 19.5 years; Table 1) in a 4% non-buffered, aqueous formaldehyde solution, the brainstems together with the cerebella were severed perpendicular to the long axis at the level of the inferior colliculus. Thereafter, the cerebella were separated by dissecting upwards through the cerebellar peduncles bilaterally and then divided by a mediosagittal cut. The left cerebral hemispheres and cerebella of the two patients and the five control individuals as well as the brainstems of the SCA2 patient and the control cases were embedded in polyethylene glycol (PEG 1000, Merck,
Degeneration of the thalamus in SCA2 and SCA3

Darmstadt, Germany) (Braak and Braak, 1991). This embedded tissue was sectioned as follows: (i) the cerebral hemispheres into uninterrupted series of equidistant 100 μm thick frontal sections; (ii) the cerebella into uninterrupted series of equidistant 100 μm thick sagittal sections; and (iii) the SCA2 brainstem into uninterrupted series of equidistant 100 μm thick horizontal sections. The brainstem from the SCA3 patient was embedded in paraffin and cut into 6 μm uninterrupted horizontal serial sections.

In all cases studied, a collection of the first, eleventh, twenty-first, etc. of the 100 μm hemispherical sections containing the thalamus underwent staining for lipofuscin pigment (aldehydefuchsin) and Nissl material (Darrow red) (Braak and Braak, 1991; Braak et al., 2003). The same numerical collection of sections through the cerebellum and brainstems of the control cases and the SCA2 patient also were pigment Nissl stained, while 15 equidistant 6 μm sections through the pons and medulla oblongata of the SCA3 patient were Nissl stained with gallocyanin (Heinsen and Heinsen, 1991). These pigment Nissl- and Nissl-stained sections were employed for pathoanatomical assessment of neurodegeneration in the thalamus. Using a stereomicroscope at a final magnification of 10 : 1, the nuclei of this diencephalic complex were localized as recommended by the Michigan School of Anatomy (Jones, 1985; Hirai and Jones, 1989) as well as previous authors (Hassler, 1982; Heinsen et al., 1996, 1999; Morel et al., 1997), and were categorized (Table 2) in functional groups on the basis of their preferred cortical and subcortical projections (Mesulam, 1985). In the SCA2 and SCA3 cases and control individuals, the collection of the second, twelfth, twenty-second, etc. of the 100 μm hemispherical sections through the thalamus was treated with a rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP) (1 : 500, Dako, Glostrup, Denmark) to visualize reactive astrocytes.

As in a previous SCA2 study (Pang et al., 2002), the extent of nerve cell loss and reactive astrogliosis in the thalamic nuclei of the SCA2 and SCA3 brains was assessed semiquantitatively and scored as not discernible (−), obvious (+) or severe (+++) (Table 2). In the same manner, we evaluated the occurrence and density of extracellular lipofuscin granules (− not discernible; + moderate; ++ high) in the pigment Nissl-stained sections through the thalamus. Normally found in the cytoplasm of both nerve and glial cells, these granules are structurally stable post-mortem and remain unaltered even when fixation is delayed or suboptimal. Owing to the low affinity of glial and the high affinity of neuronal lipofuscin granules for the aldehydefuchsin stain, extracellularly visible lipofuscin granules serve to mark the position of lipofuscin-containing nerve cells that have perished (Braak et al., 2003).

In addition, representative tissue sections from the neo- and allocortex of the two patients and all control individuals were stained with a modified Gallyas silver iodide technique to show Alzheimer’s disease-related neurofibrillary changes, and an advanced silver pyridine Campbell–Switzer method was applied to identify β-amyloid deposits (Braak and Braak, 1991). Neuropathological classification of the Alzheimer’s disease-related cortical neurofibrillary pathology and β-amyloidosis was performed on these sections according to the acknowledged Braak and Braak staging system that distinguishes six stages of cortical neurofibrillary changes (I–
VI) and three stages (A–C) of cortical $\beta$-amyloidosis (Braak and Braak, 1991) (Table 1).

**Routine neuropathology**

**SCA2 patient**

During routine neuropathological investigation, a brain weight of 960 g was determined. Macroscopic examination revealed a marked atrophy of the cerebellum and the brainstem, a moderate atrophy of the temporal, occipital and frontal lobes, together with a reduction of cerebral and cerebellar white matter. Microscopic investigation showed remarkable neuronal loss accompanied by the presence of reactive astrogliosis in the pallidum, in the substantia nigra, in the red, subthalamic and pontine nuclei, and in the inferior olive. The cerebellum was severely atrophic with thinning of the foliae and reduction of white matter, moderate neuronal loss in the dentate nucleus and serious neuronal drop-out together with a marked Bergmann gliosis in its Purkinje cell layer.

**SCA3 patient**

Routine neuropathological investigation revealed a brain weight of 1256 g and macroscopically visible atrophy of the brainstem. Upon microscopic examination, conspicuous neuronal loss together with astrogliosis were seen in the internal segment of the pallidum, in the subthalamic nucleus, substantia nigra, the red, pontine, abducens, facial and vestibular nuclei, and in the hypoglossal and lateral reticular nuclei. In addition, there was a slight reduction of the number of Purkinje cells within the cerebellar cortex and a severe loss of nerve cells accompanied by grumose degeneration of the remaining neurons in the deep cerebellar nuclei.

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**Table 2** Extent of semi-quantitative assessed neuronal loss, reactive astrogliosis and extraneuronal lipofuscin deposition in the thalamic nuclei of the SCA2 and SCA3 patients studied

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<td>Pulvinar, anterior nucleus</td>
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N = neuronal loss; A = reactive astrogliosis; L = lipofuscin deposition; – = not discernible; + = obvious; ++ = severe.
Results

Degeneration of the thalamus in the SCA2 patient

The thalamus displayed widespread degeneration, whereby its nuclei differed with respect to their proclivities to sustain neuronal loss (Table 2). A severe reduction of nerve cells was seen in the reticular (RT) (Figs 1A–H, 2A–D, and 5B and D), fasciculosisus (FA) and ventral anterior nuclei (VA) (Figs 1B–D, and 2A and C), in the ventral lateral (VL), the ventral posterior lateral (VPL) and ventral posterior medial nuclei (VPM) (Figs 1D–F, 2B and D, and 4A and C), in all magno- and parvocellular laminae of the visual lateral geniculate body (LGB), and in the caudolaterally located inferior nucleus of the pulvinar (PU i) (Figs 1F–H, 4B and D, and 5A and C; Table 2). The cucullar nucleus (CU) (Figs 1E, and 3B and D; Table 2) and the lateral portions of the mediodorsal nuclei (MD) were completely obliterated, and the medial MD portions displayed obvious neuronal loss (Figs 1D–F, 3A and C, and 4A and C; Table 2).

Obvious neuronal loss occurred in the following thalamic nuclei: (i) all of the nuclei of the anterior group [anterodorsal nucleus (AD); anteroprincipal nucleus (AP); laterodorsal nucleus (LD)] (Fig. 1C–F; Table 2); (ii) the central medial (CEM), paracentral (PC) and central lateral (CL) nuclei of the rostral intralaminar group (Figs 1D–G, and 3A and C; Table 2); and (iii) the lateral posterior nucleus (LP) (Fig. 1F and G), as well as the medial (PU m) and lateral (PU l) nuclei of the pulvinar (Figs 1F–H, and 5A–D; Table 2).
The paraventricular (PV) and parataenial nuclei (PT) that are adjacent to the wall of the third ventricle (Figs 1B–E, and 3A and C), the centromedian (CM), parafascicular (PF) and subparafascicular nuclei (SPF) (Figs 1E and F, and 4A and C), the anterior nucleus of the pulvinar (PU a), the gustatory parvocellular part of the ventral posterior medial nucleus (VPmPc) (Figs 1F, and 4A and C), the auditory medial geniculate body (MGB), and the limital–supragenulate complex (LI-SG) that forms the thalamic boundary to the pretectum were unremarkable (Figs 1G, and 5A and C; Table 2).

Extraneuronal lipofuscin deposits were confined to those nuclei that had become involved in the disease process (Table 2). The extent of this lipofuscin deposition was considerable in the nuclei that normally harbour richly pigmented nerve cells and that had sustained severe damage (RT, FA, MD, CU, VL and LGB) (Table 2), whereas moderate amounts of extraneuronal lipofuscin granules were detected in the severely damaged but less strongly pigmented thalamic nuclei (VA, VPL, VPM and PU i) and in all nuclei displaying obvious neuronal loss (AD, AP, CEM, LD, PC, LP, PU l, CL and PU m) (Table 2).

With the exception of the severely degenerated VA and VL (Fig. 6C and E), GFAP-positive reactive astrocytes were present in all of the patient's thalamic nuclei, even in those where no neuronal destruction was observable (Fig. 6G; Table 2). The extent of the reactive astrogliosis in the severely damaged thalamic nuclei varied from slight (MD,
VPL, LGB and PU i) (Fig. 6G; Table 2) to severe (RT, FA and CU) (Fig. 6A; Table 2). Finally, an abundance of reactive astrocytes was seen in the AD, CEM, LD, PU l and PU m (Table 2), whereas the AP, PC, VPM, LP and CL were moderately involved (Table 2).

Degeneration of the thalamus in SCA3 patient

The thalamus was subject to neurodegeneration, whereby the distribution pattern of nerve cell loss showed similarities to, as well as differences from, that observed in the previous...
case. Whereas the four caudal intralaminar nuclei (CM, PF, SPF and LI-SG) of both SCA patients appeared intact, and the involvement of the RT, the sensory thalamic nuclei (VPL, VPM, VPMpc, LGB and MGB), the nuclei assigned to the lateral basal thalamic group (VA, VL and LP) and the four subnuclei of the pulvinar were comparable, in the SCA3 case a larger number of nuclei assigned to the limbic system (i.e. AP, PV, CEM, CU, MD, PC, LD and CL) obviously resisted damage to nerve cells (Table 2).

As in the SCA2 case, the RT, FA and VA (Figs 1A–D, and 2A, E, B, F and 5B, F) together with the PU i (Figs 1F–H, and 5A and E) sustained major destruction (Table 2), whereas obvious neuronal loss was present in the AD, LD, VL (Figs 1C–F, and 2B and F), VPL, VPM, LP (Figs 1F and G, and 4A and E), PU i and PU m, and in the deep magnocellular laminae of the LGB (Figs 1F–H, 4B and F, and 5A, B, E and F; Table 2).

Extracellular lipofuscin granules were extraordinarily numerous in the severely depleted and normally well pigmented RT and FA. The severely degenerated and less strongly pigmented VA and PU i together with the mildly involved AD, VL, LD, VPL, VPM, LP, LGB, PU i and PU m displayed comparatively smaller amounts of these markers for lost lipofuscin-laden nerve cells (Table 2).

As in the thalamus of the SCA2 patient, even the apparently well-preserved thalamic nuclei seen here exhibited GFAP-positive astrocytes (AP, PV, PT, CEM, MD, CU, PC, CM, PF, SPF, VPMPc, PU a, MGB, CL and LI-
SG) (Fig. 6F; Table 2). The severely degenerated thalamic nuclei of this SCA3 patient either were devoid of reactive astrocytes (PU i) (Fig. 6H), displayed only occasional GFAP-positive astrocytes (RT) or showed numerous such astrocytes (FA and VA) (Figs 6B and D; Table 2). Finally, in the thalamic nuclei of the SCA3 patient in which obvious destruction of nerve cells had taken place, the extent of the GFAP-positive astrogliosis ranged from moderate (AD, LD, VPL, VPM, LP and LGB) to severe (VL, PU l and PU m) (Table 2).
Discussion

Here, we describe the thalamic involvement in terminal and genetically confirmed SCA2 and SCA3 patients. Although a greater number of thalamic nuclei in the SCA2 case, in particular those assigned to the limbic system (i.e. AD, AP, LD, FA, CM, PC, MD, CU and CL) (Hassler, 1982; Jones, 1985; Bentivoglio et al., 1993; Groenewegen and Berendse, 1994; Morel et al., 1997), displayed an obvious or severe degree of neuronal loss than those in the SCA3 patient, the extent of thalamic neurodegeneration seen in both individuals was more widespread and advanced than reported or estimated in previous studies (Rosenberg et al., 1976, 1979; Romanul et al., 1977; Kanda et al., 1989; Dür Racing et al., 1995; Iwabuchi et al., 1999; Pang et al., 2002). Inasmuch as for the purposes of this study only tissue sections from these two patients were at our disposal, the present results represent an initial step toward clarifying the extent of thalamic involvement in SCA2 and SCA3 autopsy brains. They cannot, on the other hand, provide evidence for the potential impact of epidemiological factors, e.g. age of disease onset or age at death, duration of the illness, or evidence for the impact of the numbers of expanded CAG repeats on the thalamic degeneration. Nor does the present study contain cross-sectional data that pertain to the order or possible sequence in which thalamic nuclei become involved in the disease process. It is hoped that future studies will show whether the human thalamus represents a consistent target of the pathological process in SCA2 and SCA3.

Despite progress in molecular biological research, the pathomechanisms that underlie SCA2 and SCA3 remain enigmatic. As in other CAG repeat diseases, intense efforts are underway to generate appropriate animal models (Bates et al., 1997; Burright et al., 1997; Cemal et al., 1999; Klement et al., 1999; Huynh et al., 2000; Schilling et al., 2001; Yvert et al., 2001), intended to reflect the lesional pattern seen in the brains of SCA2 and SCA3 patients. To evaluate and appreciate the results obtained from such animal models, however, exact anatomical knowledge of the extent of brain damage that is to be reproduced in the species of animal selected is an indispensable prerequisite. Here, insight into the thalamic damage that exists in SCA2 and SCA3 is provided with the aim of extending previous findings of neurodegeneration in SCA2 and SCA3 brains. In so doing, molecular biologists and animal researchers may receive support in their efforts to design and provide realistic animal models.

Since astrocytes become reactive, migrate and subsequently proliferate in response to almost every type of brain injury or disease so as to phagocytose nerve cell debris and form a glial scar, they are regarded as very sensitive indicators of any abnormality in the CNS (Duchen, 1984; Eng and Ghirnirak, 1994; Eng et al., 2000). Although SCA2 and SCA3 studies report the presence of reactive astrocytes in degenerated CNS components (Rosenberg et al., 1976, 1979; Romanul et al., 1977; Kanda et al., 1989; Dür Racing et al., 1995, 1996; Estrada et al., 1999; Huynh et al., 1999; Iwabuchi, 1999), they do not consider astrocytic involvement in detail. For example, they do not provide empirical data as to at which stage of ongoing neurodegeneration astrocytes possibly become activated, nor do they raise the question of whether the migration to and subsequent proliferation at damaged brain sites may be the sole means of astrocytic involvement in SCA2 and SCA3. Our findings in the thalamus with respect to astrocytic reactivity differed from earlier reported findings in SCA2 and SCA3 patients. To our surprise, GFAP-positive reactive astrocytes in both of our cases were present even in thalamic nuclei that did not undergo neurodegeneration, whereas some of the most severely damaged thalamic nuclei exhibited reactive astrocytes only occasionally or were devoid of them. In view of the broad consensus among neuropathologists that reactive astrocytes are among the best indicators of any kind of abnormal process within the CNS, the occurrence of reactive astrocytes even in virtually intact thalamic nuclei indicates that the degenerative process in our SCA brains was not solely confined to areas that had sustained severe neuronal damage or loss. Rather, the disease process presumably was active already in apparently well-preserved thalamic nuclei and was capable of provoking early astrocytic migration and proliferation thereafter. Furthermore, the nearly complete absence of reactive astrocytes in some of the thalamic nuclei with very severe neuronal destruction suggests that reactive astrocytes may have vanished from the tissue at the height of the disease process for the following reasons: first, exhaustion of their functional capacities may have terminated their resistance to the stronger forces of neurodegeneration, and/or secondly, sooner or later, they themselves may have fallen victim to the degenerative process and undergone cell death. Further studies are needed to explore these hypotheses and the role of astrocytes in SCA2 and SCA3.

The thalamic degeneration seen in this study provides plausible explanations for a variety of somatomotor, oculomotor, sensory and neuropsychological symptoms, as well as electrophysiological findings in these and other SCA2 and SCA3 patients. By relaying pallidal and cerebellar output to the cortical motor fields (primary, premotor and supplementary motor cortices), the VA and VL thalamic nuclei represent major components of the re-entrant motor cerebellothalamocortical and basal ganglia–thalamocortical circuits (Jones, 1985; Alexander et al., 1990; Alheid et al., 1990; Ghez, 1991; Alexander and DeLong, 1992; Gilman, 1992; Steriade, 1995; McFarland and Haber, 2000; Nakano et al., 2000). Damage to the motor basal ganglia–thalamocortical circuit may be associated with brady- or akinesia and postural instability (Alexander and DeLong, 1992; Marsden, 1992), whereas impairment of the motor cerebellothalamocortical circuit may cause gait, stance, limb and truncal ataxia, decomposition of movements, dystarthria or anarthria, and dystadiachokinesia (Ghez, 1991; Gilman, 1992; Alexander and DeLong, 1992). Because the activity of the latter circuit depends heavily on proprioceptive input from the limbs and trunk which feed into this circuit via the projections of the VL.
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The thalamic nucleus (Ghez, 1991; Alexander and Delong, 1992; Gilman, 1992; Steriade, 1995), lack (or reduction) of such input may contribute substantially to gait, stance, limb and trunk ataxia, difficulties with handwriting and coordination of limb and/or truncal movements, and may hinder anticipatory and compensatory postural mechanisms that normally prevent falls (Ghez, 1991; Asbury and Bird, 1992; Lindblom and Ochoa, 1992; Said and Thomas, 1992). Thus, damage to the VA and VL thalamic nuclei together with that to other components of the motor basal ganglia–thalamocortical (pallidum and subthalamic nucleus) and cerebellothalamocortical circuits (pontine nuclei, deep cerebellar nuclei and cerebellar cortex) suffices to explain, from the pathoanatomical view, why both of the SCA cases investigated here developed brady- and dysdiadochokinesia, dysarthria or anarthria, problems with handwriting, and repeated falls, in addition to gait, stance, limb and truncal ataxia. Moreover, both patients displayed decreased thermal discrimination and/or vibration sense in their legs. Inasmuch as the VPL thalamic nucleus, among others, is an important relay station for transmission of vibration- and temperature-related data from the lower limbs to the primary somatosensory cortex (Jones, 1985; Kaas, 1990), the involvement of this thalamic nucleus most probably contributed to the pathogenesis of the somatosensory deficits in our SCA patients.

The circuits of the limbic system play an important role in cognitive function and decline, whereby a decline of cognitive capacities may result from selective or combined damage to a variety of the cortical and subcortical components of these circuits (Devinsky and Luciano, 1993; Markesbery, 1998). Since the limbic thalamic nuclei of the SCA2 case showed more severe degeneration than those in the SCA3 case, thalamic involvement per se cannot account for the absence of overall cognitive decline in the former or its presence in the latter patient. Nevertheless, our study is well suited to verify the previously hypothesized involvement of the prefrontal cognitive circuits in SCA2 and SCA3 patients (Maruff et al., 1996; Bürk et al., 1999; Storey et al., 1999; Le Pira et al., 2002; Zawacki et al., 2002). These circuits are known to be associated with the generation of cognitive processes termed ‘executive functions’ (Alexander et al., 1990; Alheid et al., 1990; Funahashi, 2001; Fuster, 2001). By applying highly sensitive tests, neuropsychological studies recently have shown the presence of such dysfunctions in SCA2 and SCA3 patients: deficient abstraction, visual attentional deficits, impaired verbal fluency, inability to form cognitive sets, deficits in cognitive switching and inflexibility in shifting attentional behaviour (Maruff et al., 1996; Gambardella et al., 1998; Bürk et al., 1999; Storey et al., 1999; Le Pira et al., 2002; Zawacki et al., 2002). The MD and VA thalamic nuclei, along with the subthalamic nucleus and pallidum, are important relay stations within the prefrontal basal ganglia–thalamocortical circuits involved in generating executive functions. In addition, they are integrated into the oculomotor basal ganglia–thalamocortical circuit that is concerned with saccades (Alexander et al., 1990; Alheid et al., 1990). Accordingly, the involvement of the MD and/or VA nuclei together with that of the subthalamic nucleus and pallidum not only contributed to the saccadic alterations in our SCA patients, but also interrupted their re-entrant prefrontal cognitive circuits, which reportedly underlie the executive dysfunctions described previously in SCA2 and SCA3 patients (Maruff et al., 1996; Gambardella et al., 1998; Bürk et al., 1999; Storey et al., 1999; Le Pira et al., 2002; Zawacki et al., 2002). The Pu I and PU i, aside from the aforementioned thalamic nuclei, are associated with the generation of saccades. Together with the Pu m, they are integrated into the networks of the human brain that are responsible for generating and sustaining visual attention (Robinson and Petersen, 1992; Grieve et al., 2000).

In view of their pathological involvement in the patients studied, the role of these pulvinar nuclei in the pathogenesis of saccadic dysfunctions and visual attentional deficits in SCA2 and SCA3 should not be neglected.

The LGB showed degenerative changes in our SCA3 case and was severely involved in our SCA2 case. Because visual processing in this laminated thalamic complex is thought to be related to the P100 wave of visual evoked potentials (White et al., 1983), the severe affection of the LGB in our and a SCA2 patient studied by Pang et al. (2002) offers, for the first time, a plausible explanation for the decreased amplitudes or complete absence of the P100 wave of the visual evoked potentials in SCA2 patients (Peretti et al., 1996; Abele et al., 1997; Schöls et al., 1997). Although severe damage to the LGB is known to cause extensive deficits in the visual field (Helgason et al., 1986; Kosmorsky and Lancione, 1998), none were recorded in the clinical protocols of our SCA2 patient. Since she was examined neuroophthalmologically on a regular basis only up to the age of 20 years by clinically experienced neurologists, we think it is less likely that they overlooked any visual field defect. Rather, were such a defect to have emerged at all in this instance, it is likely to have been subsequent to the last systematic neurological investigation that took place when the patient was 20 years old.

The RT of the thalamus along with collaterals from corticothalamic fibres receives collaterals from cortical projections of the motor, sensory, higher order processing and limbic nuclei of the thalamus. In turn, it sends inhibitory GABAergic axons back to these thalamic nuclei. Although the thalamic RT has been implicated in various attentional mechanisms, its exact role in the transmission of information through the thalamus to the cerebral cortex and the consequences of its destruction in humans are still under discussion (Guillery et al., 1998; Ilinsky et al., 1999). Given the concomitant pathology seen in interconnected thalamic nuclei of the SCA patients under consideration here, our post-mortem study cannot provide further insights into as yet ill-defined functions of the RT. However, inasmuch as loss of GABAergic inhibitory nerve cells in the RT is advantageous for thalamocortical data flow (Guillery et al., 1998), the severe neuronal loss seen throughout the entire extent of the
RT in both of the SCA cases studied may have compensated, in part, for the functional consequences of the lesions in interconnected thalamic nuclei. Disruption of the activity of the inhibitory GABAergic neurons of the RT of the thalamus is a known risk factor for myoclonia (Matsumoto et al., 2000), and a recent Creutzfeld–Jakob disease study has established a correlation between neuronal loss in the thalamic RT and the occurrence of myoclonia (Tschampa et al., 2002). The fact that myoclonia was mentioned only in the clinical records of the SCA2 patient suggests, however, that damage to the RT is not a compelling prerequisite for the occurrence of myoclonia, and supports the viewpoint that the pathoanatomical basis of these hyperkinetic motor dysfunctions is heterogeneous (Vercueil and Krieger, 2001).

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