

Robust autophagy in optic nerves of experimental Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker disease

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Abstract

We report here autophagy in the optic nerve in experimental Gerstmann-Sträussler-Scheinker disease (GSS) (Fujisaki-1) in mice and experimental Creutzfeldt-Jakob disease (CJD) (Echigo-1) in hamsters. Lesions of both experimental GSS in mice and experimental CJD in hamsters were practically indistinguishable. Briefly, they consisted of widespread Wallerian degeneration, spongiform change and a glial reaction. Numerous axonal swellings were seen. The latter were filled with numerous mitochondria and lysosomal electron-dense bodies. Autophagic vacuoles defined as structures bound in double membranes were readily found in many neuronal processes. The following description is organized as a sequence; however, the changes were all observed in the same specimens. First several empty double membrane-bound autophagic vacuoles were seen. In several of those vacuoles, the inner membrane was separated from the outer membrane and enclosed cargo. At the final stage, a mixture of empty autophagic vacuoles and electron-dense lysosomal vesicles was seen. Dystrophic neurites filled with a mixture of mitochondria, empty autophagic vacuoles and electron-dense lysosomal vesicles were interpreted as the final stage of autophagy. Of note, several areas were replaced with dense astrocytic gliosis.

Key words: autophagy, prion diseases, optic nerve.

Introduction

Prion diseases include kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and fatal familial insomnia (FFI) in humans; scrapie in sheep, goats and mouflons; transmissible mink encephalopathy in ranch-reared mink; bovine spongiform encephalopathy in cattle and antelopes or domestic cats, cheetah and tigers in zoological gardens; and chronic wasting disease in mule deer [2,5,12]. They are caused by a unique agent believed by the majority of investigators to be “prions” – proteinaceous infectious

particles compose entirely of the misfolded isoform (PrP^{Sc}) of a normal cellular isoform (PrP^C) of prion protein [1].

The pathological changes in prion diseases are confined to the central nervous system. They include spongiform change, astro- and microglial proliferation, amyloid plaques in a proportion of cases and neuronal drop-out [20]. Neurons degenerate and die by either apoptosis or autophagy, or both [6,7,14,15]. A novel prion disease with autonomic disturbances and diarrhea was recently described [20].

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The optic nerve, being in reality not a nerve but an extension of the central nervous system (CNS), is a unique model system to gain insight into what is going on within the CNS. We pioneered use of the optic nerve in electron microscopic study of transmissible spongiform encephalopathies [8,16,18]. We found, typical for CNS lesions, spongiform change, astrocytosis and a robust microglial/macrophage reaction. Recently, the optic nerve was used to study mechanisms of acute axonal degeneration following the crush of the nerve. Surprisingly, the major lesion was widespread autophagy. We thus returned to our archival material of the optic nerves from experimental CJD and GSS to find robust autophagy as well.

Material and methods

Creutzfeldt-Jakob disease – strain, animals, incubation period of illness

Outbred 6-week-old golden Syrian hamsters (Medical University of Lodz, Department of Oncology, Lodz, Poland) were inoculated intracerebrally with 0.05 ml of a 10% (w/v) centrifugation-clarified hamster brain suspension containing the Echigo-1 strain of the CJD agent. Control animals were sham inoculated intracerebrally with the same volume of saline. We used five hamsters for each experiment; the control group consisted of two hamsters. In the first passage in our laboratory (7th serial passage after initial isolation), the incubation period was approximately six months. The experiment was repeated twice, with similar results. Two uninfected hamsters were perfused identically.

Gerstmann-Sträussler-Scheinker disease – strain, animals, incubation period of illness

Five weanling 4- to 6-week-old NIH Swiss mice (Animal Production Area, Frederick Cancer Research Facility, Frederick, MD) were each inoculated intracerebrally with 0.03 ml of a 10% (w/v) clarified brain suspension of the Fujisaki strain of GSS (titer, 3.1×10^4 LD₅₀ per 0.03 ml, by intracerebral route). The incubation period ranged from 16 to 18 weeks. Two control animals were sham-inoculated with saline.

Electron microscopy

Hamsters in the terminal stage of CJD and control sham-inoculated hamsters at the same interval after inoculation were anaesthetized with ketamine. They

were perfused by intracardiac injection with saline followed by 150 ml of 1.25% glutaraldehyde and 1% paraformaldehyde prepared in cacodylate buffer (pH 7.4) and then by 50 ml of 5% glutaraldehyde and 4% paraformaldehyde.

Five CJD- and two sham-inoculated mice were sacrificed by intracardiac perfusion using 180 ml of 1.5% glutaraldehyde and 1% paraformaldehyde prepared in phosphate buffer (pH 7.4). The differences resulted from experiments being performed at different times, but the quality of tissue preservation was excellent in both cases. From perfused animals, optic nerves were dissected and divided into three or four samples which were postfixed in 1% osmium tetroxide for 1 to 2 hours, dehydrated through a series of graded ethanols and propylene oxide, and embedded in Embed 812 (Electron Microscopy Sciences, Ft. Washington, PA).

Results

The optic nerves from control animals were unremarkable and consisted of innumerable axons containing no pathological structures. Lesions of both experimental GSS (Fujisaki-1) in mice and experimental CJD (Echigo-1) in hamsters were practically indistinguishable and an outline was published before [17,19]. Briefly, they consisted of widespread Wallerian degeneration, spongiform change and a glial reaction (Fig. 1). It must be stressed that dystrophic neurites were relatively rare – one or two per section (Fig. 2). The latter were filled with numerous mitochondria and lysosomal electron-dense bodies. Autophagic vacuoles defined as structures bound in double membranes were readily found in many neuronal processes (see the companion paper). The following description is organized as a sequence; however, the changes were all observed in the same specimens. First several empty double membrane-bound autophagic vacuoles were seen (Figs. 3-5). In several of those vacuoles, the inner membrane was separated from the outer membrane and enveloped cargo (Figs. 3 and 4). At the final stage, a mixture of empty autophagic vacuoles and electron-dense lysosomal vesicles was seen (Figs. 6A-B). Dystrophic neurites filled with a mixture of mitochondria, empty autophagic vacuoles and electron-dense lysosomal vesicles were interpreted as the final stage of autophagy (Fig. 7). We attempted to count autophagic vacuoles and autophagolysosomes but the results were ambiguous. In the majority of neurites during counting, we found lysosomal dense bodies as predominant structures; however, the reverse rela-

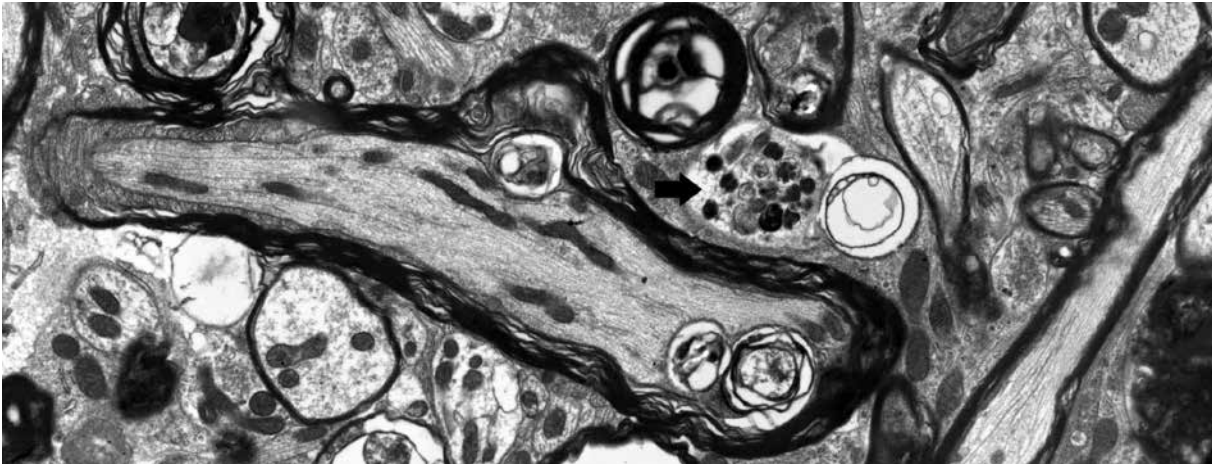


Fig. 1. General view of the optic nerve from a mouse infected with the Fujisaki-1 strain of Gerstmann-Sträussler-Scheinker disease. A dystrophic neurite is marked with an arrow. Original magnification, $\times 12\ 000$.

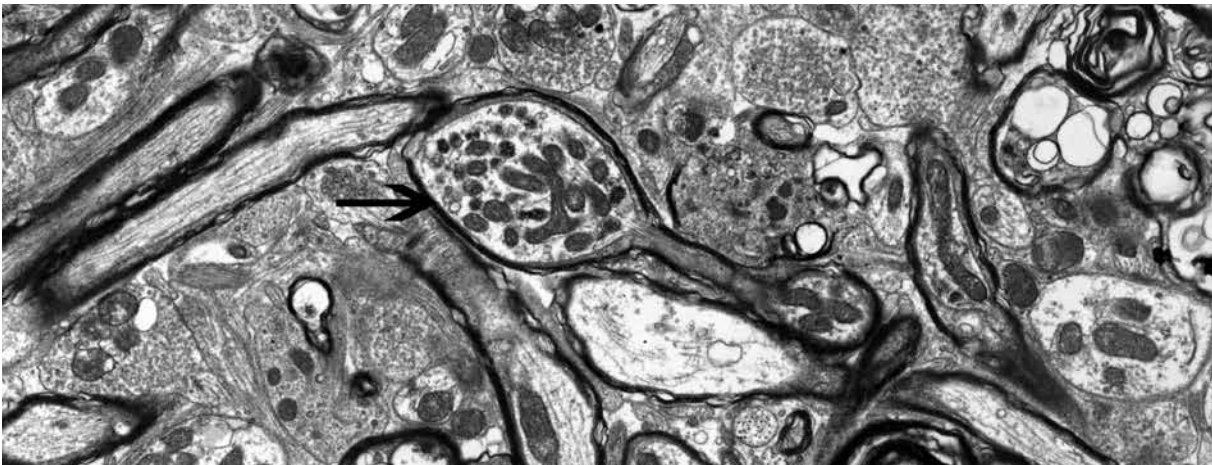


Fig. 2. Longitudinal section through the axon showing bullous swelling (arrow), a mouse infected with the Fujisaki-1 strain of Gerstmann-Sträussler-Scheinker disease. Original magnification, $\times 12\ 000$.



Fig. 3. Transverse section through the axonal process from a mouse infected with the Fujisaki-1 strain of Gerstmann-Sträussler-Scheinker disease. Numerous mitochondria are seen (arrows). Original magnification, $\times 12\ 000$.

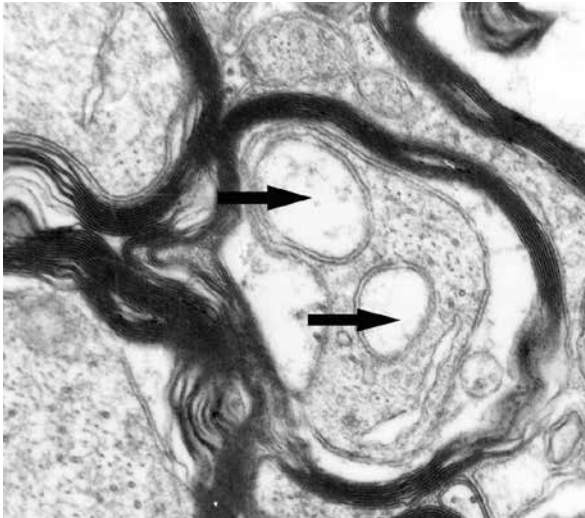


Fig. 4. Non-myelinated process from a mouse infected with the Fujisaki-1 strain of Gerstmann-Sträussler-Scheinker disease. Two empty vacuoles (arrows). Original magnification, $\times 12\ 000$.

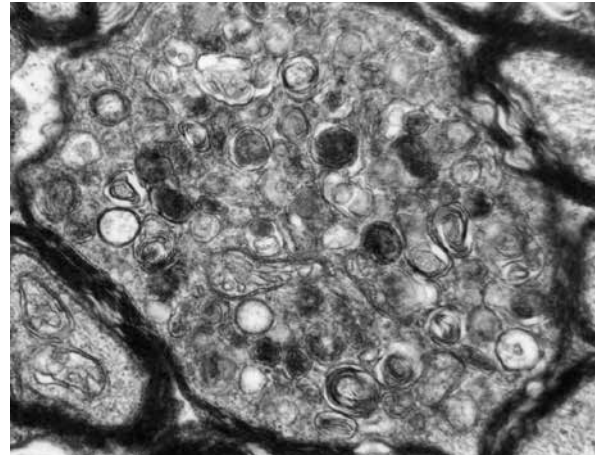


Fig. 5. Myelinated process from an optic nerve of a mouse infected with the Fujisaki strain of Gerstmann-Sträussler-Scheinker disease. The dystrophic neurites are completely filled with mitochondria and autophagic vacuoles. Original magnification, $\times 12\ 000$.

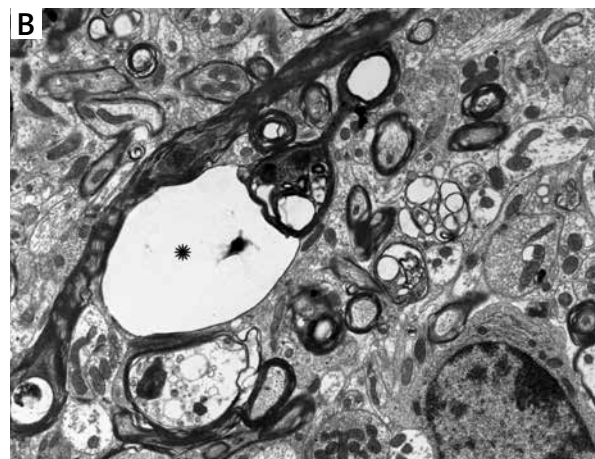
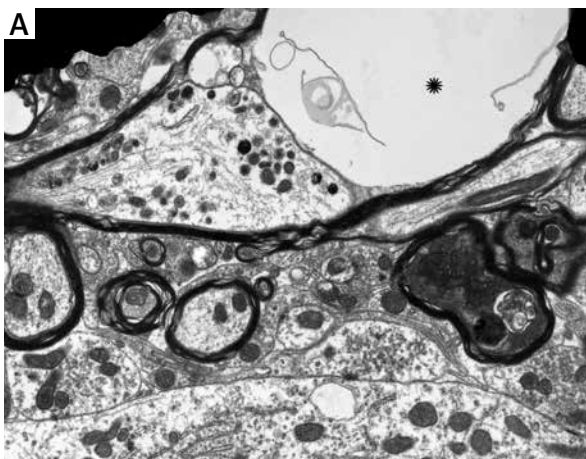


Fig. 6. A) Myelinated process from an optic nerve of a mouse infected with the Fujisaki strain of Gerstmann-Sträussler-Scheinker disease (GSS). Note myelinated vacuole attached to a neurite (star). Original magnification, $\times 12\ 000$. **B)** A myelinated process from an optic nerve of a mouse infected with the Fujisaki strain of GSS. Note myelinated vacuole attached to a neurite (star). Original magnification, $\times 12\ 000$.

tionship was also observed. These results were probably dependent on the plane of sections and the stage of degeneration. Of note, several areas were replaced with dense astrocytic gliosis.

Discussion

We pioneered the work on the optic nerves in prion diseases [8,17-19] and demonstrated widespread myelinated axon damage, Wallerian degeneration and

astrocytic and macrophagic reaction. It must be remembered that the optic nerve is not a nerve in the sense of a peripheral nerve; it is an extension of the CNS formed by and composed of retinal ganglion cells. The classification of dystrophic neurites is also variable [11; supplementary data]. The study of optic nerve injury may also have clinical significance with regard to Leber's optic nerve atrophy, glaucoma and trauma. Knöferle *et al.* [11] distinguished several types of spheroid formation: 1) acute,

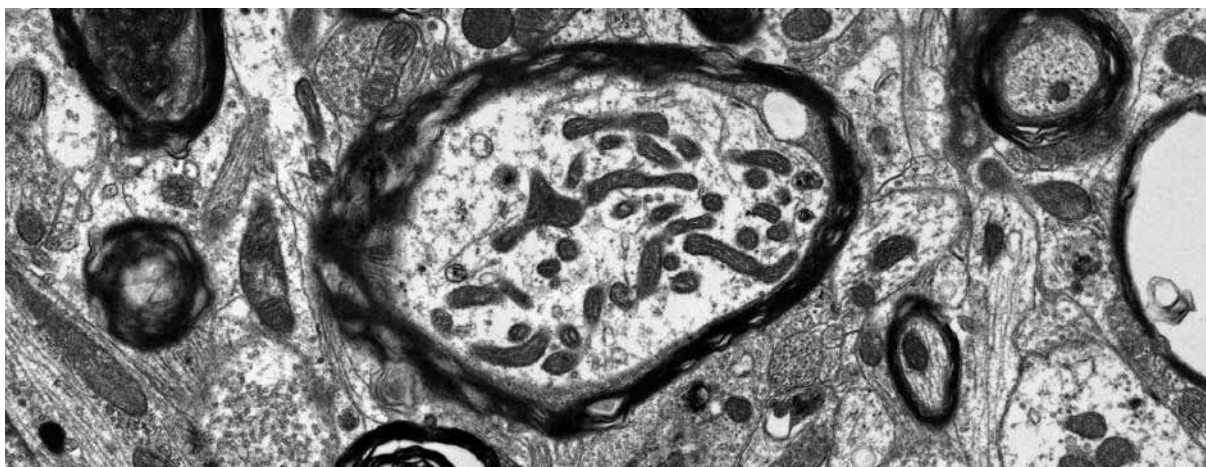


Fig. 7. Myelinated process from an optic nerve of a hamster infected with the Echigo-1 strain of Creutzfeldt-Jakob disease. Original magnification, $\times 12\,000$.

corresponding to those classically reported by Lampert [4], which is the most typical; neurites contain diverse subcellular organelles such as degenerating mitochondria and lysosomes; 2) those with “sparse axoplasm without regularly structured microtubules” and those transforming into electron lucent vacuoles. The latter may seem indistinguishable from typical “spongiform change” that is a hallmark of the whole group of prion diseases. Here we reported that, additionally, axons were packed with autophagic vacuoles. The observed changes varied and we plan now to extend our observations using a sequential study. It must also be stressed that axonal changes after damage to the optic nerve appear relatively early, some 30 minutes after transection, and this means that our observation taken at the terminal stage of disease may not be whole representative.

Those changes in the optic nerve are observed in many different conditions, for example in transgenic mice expressing mutant (P301L) human tau [9] and hereditary diffuse leukoencephalopathy with spheroids [10,13], to mention just two, and the appearance of dystrophic neurites in every condition is practically identical [3].

In conclusion, we observed widespread autophagy in optic nerves in experimental CJD and GSS. The pathogenic role of this phenomenon is unknown.

Disclosure

Authors report no conflict of interest.

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