Iron is an essential element for most life on Earth. In the human brain, iron plays a critical role in oxygen utilization, enzymatic systems, and especially neural development. In contrast, the excess accumulation of iron in nerve cells has been reported to cause neurodegenerative diseases, such as Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis. In this study, we analyzed the distribution of iron and other elements in nerve cells in the brains of patients with Parkinson’s disease and in those of age-matched controls to investigate the pathogenesis of Parkinson’s disease.

Transmission electron microscopy (TEM) combined with energy-dispersive X-ray spectroscopy (EDS) is usually applied to demonstrate elements in biological samples. However, it is impossible to analyze the localization of Fe by TEM-EDS analysis, because a high peak of the Fe spectrum emerges from components of the transmission electron microscope. Therefore, we developed a new method to demonstrate Fe using scanning electron microscopy (SEM) combined with EDS.

Brain tissues (substantia nigra pars compacta) were taken after autopsy, fixed with 3% glutaraldehyde and 2.5% potassium dichromate, dehydrated, and embedded in Epon. Semi-thin sections (0.4 to 1-μm thick) were cut using a microtome equipped with a diamond knife, mounted on copper mesh, placed on a sample holder made of carbon, and used as samples for SEM-EDS analysis.

<Control brains> A large number of electron-dense neuromelanin granules were present in the cytoplasm of nerve cells on SEM observation (Fig. 1A). When elements contained in neuromelanin granules (Fig. 1A, arrow) were analyzed by EDS, a high peak of Fe was detected (Fig. 1B, arrow). Spectra of Al, P, S, K, and Ca were also demonstrated in nerve cells. The highest peak of the Fe spectrum in nerve cells was detected in neuromelanin granules.

<Parkinson’s disease brains> The number of neuromelanin granules reduced markedly in nerve cells. In some nerve cells, neuromelanin granules lost their electron density (Fig. 2A, arrow), and levels of Fe in these granules showed a marked decrease on EDS (Fig. 2B, arrow). It is likely that the levels of Fe in neuromelanin granules decrease markedly during the development of Parkinson’s disease. We also conclude that SEM-EDS analysis is a method capable of demonstrating iron in biological samples.
Fig. 1: Control brain. Nerve cell observed by SEM (Fig. 1A) and spectra taken by EDS (Fig. 1B). High levels of Fe are demonstrated in neuromelanin granules.

Fig. 2: Parkinson’s disease brain. Neuromelanin granules lost their electron density (Fig. 2A arrow), and levels of Fe in these granules showed a marked decrease (Fig. 2B).