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Parkinson's: Why are these brain cells dying?
Elemental maps of iron accumulation within the brain

Parkinson‘s: Why are these brain cells dying?

The actual causes of Parkinson‘s disease are not clarified as of yet. For example, what is the role of iron accumulating in the midbrain of Parkinson‘s patients? Researchers (Prof. N. Nagai et al.) at Okayama University (Japan) have been investigating ultrathin sections of diseased brain tissue via electron microscope. Using the iTEM Solution EEFTEM by Olympus Soft Imaging Solutions they have the iron distribution visualized and measure it systematically.

Prof. Noriyuki Nagai and Dr. Noriyuki Nagaoka, Department of Oral Pathology and Medicine, Graduate School of Medicine and Dentistry, Okayama University, Japan
Dr. Peter Stallknecht, Olympus Soft Imaging Solutions GmbH, Germany

Hands trembling and a mask-like face

Parkinson is a disease that affects the central nervous system and progresses slowly. It usually begins between 40 and 60 years of age. The three primary symptoms are: uncontrollable shaking, stiff muscles and slowed movements. Patients in the early stages complain of trembling hands, tiring quickly, pains and an increasing lack of coordination (eg, when writing). A person‘s posture when walking is bent over, the steps taken become shorter. As the disease progresses, the shaking increases and movement becomes more and more difficult. Keeping one‘s balance or concentrating on something becomes a problem. Patients speak quietly and in a monotone voice. Their faces lose the capacity to show expression such that they appear to have a mask-like face (referred to as the „Parkinson‘s mask“). The capacity to feel joy or fear may also be disturbed and depression is not unusual. Today‘s medicines can alleviate these symptoms and significantly improve the patients‘ quality of life. However, they cannot arrest the disease, let alone heal it. Medical research on its actual causes continues.

1

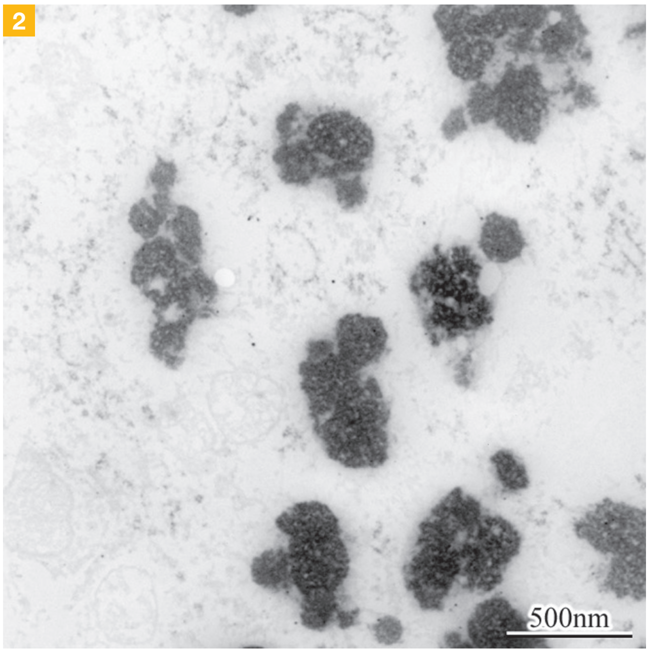
Not enough dopamine

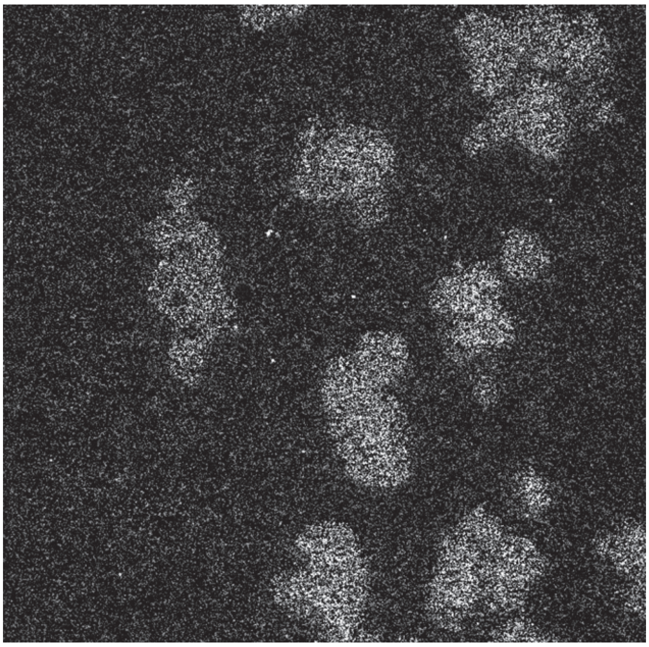
Parkinson‘s is named after the London doctor and pharmacist James Parkinson (1755-1824). He was the first one to describe its symptoms. In 1817 he wrote „An Essay on the Shaking Palsy“, referring to the disease as „shaking palsy“. He suspected its origins to be in the cervical spine. It was a century later before it was discovered that pathological alterations in the substantia nigra of the midbrain are responsible for the symptoms. The standard theory today is that in Parkinson‘s patients certain nerve cells in the substantia nigra and in the locus coeruleus have degenerated. These are nerve cells that usually produce dopamine, a neurotransmitter. The result is that too little dopamine is produced. At the same time, other neurotransmitters are present in overabundant quantities. The transmission impulses of excitation and inhibition to the muscles is thus disturbed to a very significant extent. And this is what leads to the characteristic symptoms.

Accumulation of metals

Why the nerve cells degenerate and die has not yet been satisfactorily clarified. The relevant interrelationships are possibly more complex than had been assumed. Several research groups are investigating the role certain metals play. Their publications are being followed with great interest. Their measurements show that in the affected regions of the brains of Parkinson‘s patients significantly more metal is accumulating than in the brains of healthy persons. An unusually high amount of iron, copper and zinc was detected in the neuromelanin released by greatly degenerated or already dead nerve cells.

2





Iron apparently plays an important role concerning Parkinson's disease. Via electron microscope, it is demonstrated that the iron concentration in the diseased part of the midbrain is greatly increased. The image on the left is an electron-spectroscopical image of an ultrathin section taken from the substantia nigra of a Parkinson's patient (inverted display). The dark areas are the neuromelanin. The image on the right is the elemental map of iron computed using the 3-window method. The light areas are where iron has accumulated. The quantitative evaluation in the example shown above shows an iron concentration that is three times as high as what it would be in the brain of a healthy person. A LEO 912 AB EFTEM was used (acceleration voltage of 120 kV, electron energy loss of 725 eV) along with Olympus Soft Imaging Solutions iTEM Solution EFTEM software. (by Prof. N. Nagai and Dr. N. Nagaoka).

Digital image analysis in Okayama

An Okayama University research project is hot on the trail of iron in particular. In the Department of Oral Pathology (Prof. N. Nagai and Dr. N. Nagaoka), Graduate School of Medicine and Dentistry a LEO 912 AB energy-filtering transmission electron microscope (EFTEM) and Olympus Soft Imaging Solutions TEM Imaging platform iTEM with the extension iTEM solution EFTEM are used. Ultrathin sections of nerve tissue from the substantia nigra of people with and without Parkinson's are what are examined and compared. The tissue is fixed in a solution of 2% formaldehyde and 2% paraformaldehyde and without any further osmium fixing, is conventionally embedded in epon resin. An ultramicrotome takes care of the 30 nm thin sections. These sections are then evaluated with the electron microscope at an acceleration voltage of 120 kV..

Making the iron visible

Figure 2 shows how the iron in the degenerated nerve cells is distributed within the substantia nigra of a Parkinson's patient. At first glance, the distribution looks just like that in the healthy nerve cells: iron has accumulated within the neuromelanin. The difference becomes apparent only once a quantitative evaluation has been conducted. Via the imaging EELS method and with the aid of the image-analytical software, the energy-loss spectrum of the beam electrons can be calculated. A good measure for the number of iron atoms the neuromelanin contains is the area beneath the peak of the Fe (iron) L2,3 edge. Comparing the areas of different energy-loss spectra provides a clear and unambiguous result for this experiment series. The neuromelanin of a Parkinson's patient contains three times as much iron as the neuromelanin of a healthy person.

New medications?

Quantitative image analyses of this nature can help to shed light in the as yet obscure interrelationships between the accumulation of metals and cell degeneration. Improved methods of treatment may result from a deeper understanding of these interrelationships. It is conceivable that medications will be developed containing iron-binding substances. These would be able to protect nerve cells from excessive accumulation of iron.

Working the digital way

When clarifying interrelationships between production conditions, the microstructure, and the physical properties of pyrocarbon, doing things ‚digitally' helps a great deal. Being able to acquire light-microscope images digitally, analyzing them interactively and in an automated fashion and archiving them in a well-structured way makes image evaluation and management much easier. It's also a big help being able to use the same software environment for the electron-microscope investigation. The images and spectra are acquired, the microscope is controlled, and results are displayed graphically – all via the software. All images, spectra, data and results are placed into a central database and are available for later investigation at any time.

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Text written in close collaboration with:

Prof. Noriyuki NAGAI and Dr. Noriyuki Nagaoka, Department of Oral Pathology and Medicine, Graduate School of Medicine and Dentistry, Okayama University

Image source: Okayama University, Japan; Carl Zeiss SMT. Germany

How is an elemental map of iron generated?

EFTEM stands for energy-filtering transmission electron microscope. The electron beam of the EFTEM is transmitted through the sample (the ultrathin section of the brain tissue) and generates a magnified image of the sample in the imaging plane. The built-in energy filter makes it possible to generate electron-spectroscopic images (ESI). If the energy filter is set to 725 eV, for example, only those electrons reach the imaging plane that have an energy loss of 725 eV while going through the sample. The energy filter intercepts all other electrons.

2

An energy-filtering transmission electron microscope. Elemental maps can be generated using its built-in energy filter in conjunction with digital image acquisition and image processing.

725 eV is the characteristic energy loss of an electron from the beam when it ‚flies by' an iron atom really closely and causes a certain inner-shell transition. The energy filter will allow such an electron to pass through. Then it contributes to image generation in the imaging plane. Unfortunately, other electrons manage to make it through, influencing the image. These electrons have also coincidentally lost 725 eV due to various sorts of multiple impacts that occurred while passing through the sample. Without these other ‚bothersome' electrons, the ESI image at 725 eV would be an elemental map of iron: ie, this would mean that it would be possible to see how iron is distributed throughout the sample.

The elemental map can be calculated using multiple ESI images. The iTEM Solution EFTEM software package is used in the oral pathology department of Okayama University. The software remote controls the electron microscope and acquires a series of ESI images at various energy losses using a digital camera. Because ESI images have a very low signal-to-noise ratio, the software optimizes image quality during acquisition directly.

Using what is known as the 3-window method, 3 ESI images are acquired. The first one is at 725 eV, the second and third ones at somewhat lower energy losses. The second and third images are used to compute a ‚background image'. This is then subtracted from the first image. This is how the ‚bothersome' background is removed from image number one (made up of all the contrast components caused by the ‚bothersome' electrons referred to above). The result is the desired elemental map of iron. This is also suitable for quantitative measurement.

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OLYMPUS

OLYMPUS SOFT IMAGING SOLUTIONS GMBH
Johann-Krane-Weg 39
48149 Münster
Germany
Phone: +49 (251) 7 98 00-0
Fax: +49 (251) 7 98 00-99
info.osis@olympus-sis.com

OLYMPUS SOFT IMAGING SOLUTIONS CORP.
12596 West Bayaud Ave., Suite 300
Lakewood, CO 80228
USA
Phone: +1 (303) 234-9270
Fax: +1 (303) 234-9271
info.osis@olympus-sis.com

OLYMPUS SOFT IMAGING SOLUTIONS PTE.LTD.
No 2 Jurong East St 21 #05-30
IMM Building, Singapore 609601
Singapore
Phone: +65 (6777) 7898
Fax: +65 (6777) 7218
info.osis@olympus-sis.com