IRON ON THE BRAIN

Everyone needs iron, but too much of it in the wrong place in the body can be a very bad thing. Neurodegenerative disorders, such as Alzheimer's, Huntington's, and Parkinson's disease, have all been shown to be closely related to the presence of too much iron in brain tissue, which is the result of disturbances in normal iron metabolism. But discovering how much iron is present in the brain, and in what particular compounds, has proven to be a challenge. The chief methods used at present rely on staining of tissue samples, a technique that provides poor resolution and does not provide any data on the makeup of specific iron compounds. Clearly, a more precise and accurate strategy for locating and identifying iron anomalies in brain tissue is needed. Now, researchers from the University of Florida, the University of Minnesota, Keele University, and Argonne National Laboratory, using the MR-CAT insertion device beamline at the APS, may have found just that strategy.

Using the MR-CAT 10-ID beamline at the APS, the team has developed a technique that combines the technique of x-ray absorption near-edge spectroscopy (XANES) with with iron-edge area scanning. The process not only allows individual, minute iron anomalies to be detected with much greater resolution and sensitivity than ever before, but it also makes possible their identification and specific characterization *in situ*.

The experimenters used tissue samples from the midbrain of a homing pigeon for this initial demonstration of the technique. The samples were fixed and sealed between two sheets of Kapton film containing gridlines of zinc wire for use as an orientation tool. The researchers ensured that the methods and materials used to prepare the samples, including the fixing solutions, Kapton sheets, and slides, were fully cleaned and purified to prevent the detection of extraneous impurities during the experimental procedures. Comparing the x-ray fluorescence of the tissue samples above the iron K absorption edge at 7,112 eV with their fluorescence below the iron absorption edge, the team was able to generate an iron concentration map for each sample (Fig. 1). A detection limit of less than 1 ppm is possible because of the high intensity of the APS microfocused synchrotron x-rays. Because of the extreme sensitivity this affords, a single particle of nanometer scale can be detected in a 500-μm spot.

The tiny areas of iron concentration found were then examined at even higher resolution— down to 5 μm—followed by XANES analysis. The data obtained from the XANES studies were compared with measured standards and published results for magnetite, ferritin, haemosiderin, and haemoglobin. This revealed that the iron anomalies detected in the tissue samples consisted of ferritin, magnetite, and haemoglobin. The researchers also confirmed that the sample preparation techniques did not affect the iron compounds detected, and that any trace metallic contamination introduced during the preparation process can be distinguished from biogenic iron anomalies with XANES.

The research team's work provides a powerful demonstration of the potential of highly focused synchrotron x-ray beams to detect and identify *in situ* iron deposits in brain tissue with very high resolution and with only a few hours of study. The use of the zinc wire gridlines on the sample slides also allows the identification and location of anomalies for comparison study by other techniques, including electron and light microscopy. Other metals that have been tied to neurological abnormalities, such as aluminum and zinc, can also be detected and analyzed via this method. Another interesting potential use of this technique is the study of magnetoreception in animals—how certain species (such as pigeons) may use magnetic fields to orient themselves and navigate over great distances.

The researchers believe that their work is the first step in the development of even more sophisticated techniques to find, map, and precisely characterize abnormal iron and other metallic compounds within brain tissue (they have now demonstrated the technique in human tissue from Alzheimer's patients). This new tool promises to provide crucial information on the specific cells and structures containing such anomalies, and clues about the ways in which these metallic deposits do their neurological dirty work. — *Mark Wolverton*

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Author Affiliations: [1]University of Florida, [2]Argonne National Laboratory, [3]University of Minnesota, [4]Keele University
Correspondence: bea22@keele.ac.uk

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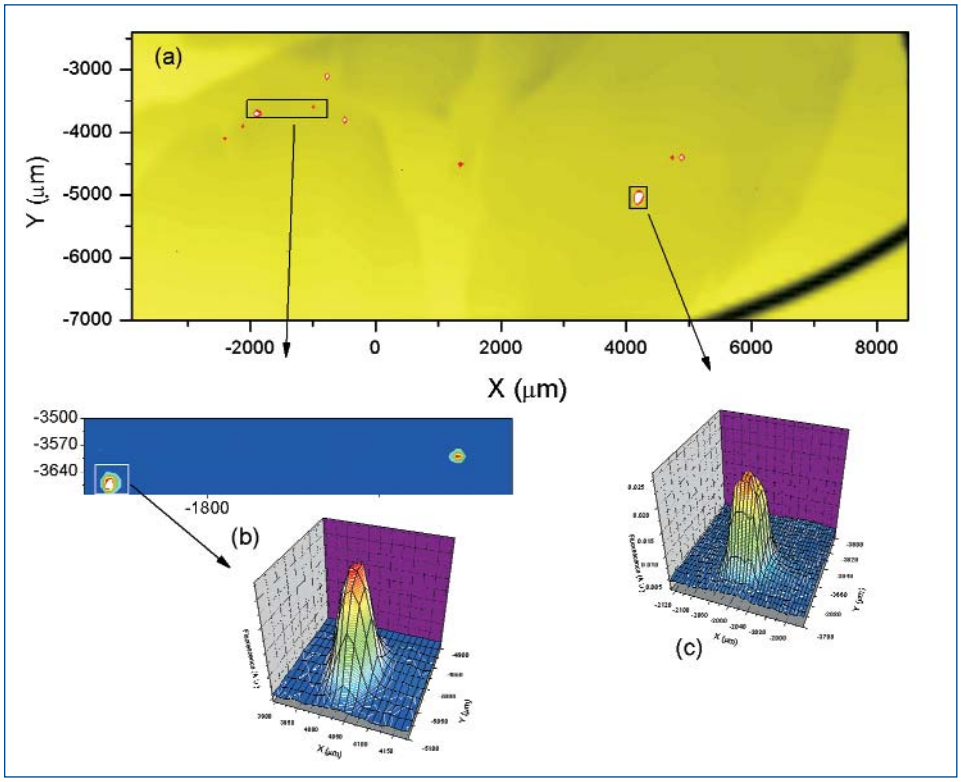

Fig. 1. Maps of the coronal section of a pigeon brain consisting of (a) x-ray transmission map (black–yellow) with superimposed iron fluorescence map (red–white) with 500-μm resolution; (b) iron fluorescence map at 20-μm resolution, with 5-μm resolution wireframe image inset of iron anomaly A consisting primarily of ferritin-like iron compounds; and (c) iron fluorescence wireframe map at 5-μm resolution of iron anomaly D, consisting primarily of magnetite.

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