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Commentary

Response to Comment on "Mapping and Characterization of Iron Compounds in Alzheimer's Tissue"

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We would firstly like to thank Dr. Carmen Quintana Rodriguez for the positive comments on our recent paper. We are also appreciative of the supplementary comments and would like to address the points raised.

In regards to the SIMS technique, it is probably worthwhile pointing out that SIMS is both destructive to the tissue and does not give information on the specific metal compounds present in the sample. It provides only the elemental composition. In addition, nanoSIMS is very sensitive to the matrix in which a given element is bound. The elemental concentrations observed may be significantly affected by the type of structure from which they are obtained, distorting actual concentrations. We do agree, however, that this is a very worthwhile technique, particularly when used in combination with other imaging modalities, and we certainly would not want to imply otherwise.

We are also in agreement with the comments regarding the use of XEDS and EELS. When used in combination with TEM morphological imaging and electron diffraction, structural information on the metal compounds present in the tissue can also be observed. These studies are very valuable in contributing to our understanding of iron and other metals in the brain and in our original paper we made reference to some of the studies employing these techniques.

The main drawback to these techniques is that TEM/EELS images samples on a very small scale. Generally, ultra-thin sections are used which are much smaller in dimension than the thickness of neurons and other brain cells and the grids themselves are only a few millimeters in diameter. This makes mapping specific iron compounds in a large (centimeter-scale) sample of brain tissue akin to looking for a needle in a haystack. In addition, x-ray analysis has the advantage that it can be done with minimal sample preparation [1,6] whereas the fixation and embedding processes required for many of the alternative advanced microscopy techniques are known to interfere with metal compounds and distribution [5,7].

While it is true that at "low" resolution, electron-dense material can be identified within the TEM samples, in order to identify the *specific metal compounds* present, they must be crystalline and each particle also must be characterized by electron diffraction, as EELS and XEDS only give elemental information. ATEM combined with EXAFS and ELNES provides information similar to that obtained with the synchrotron, but again, not for large tissue sections. Therefore it is very time-consuming to produce a map of brain tissue samples identifying specific iron (or other metal) compounds using these techniques and the maximum sample size is still only a few millimeters.

For these reasons, we feel that the synchrotron method is the best way to map and characterize the

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distribution of iron and other metal compounds over a large section of tissue in order to obtain both spatial distribution and speciation information. It also enables scaling of the mapping pixels to the appropriate sample size – i.e., mapping at low resolution can identify iron anomalies that can then be mapped and *identified* at progressively higher resolution in tissue sections. This is also a process which is automated, allowing collection of data over long periods of time. This is critical if we are to relate *changes* in normal iron homeostais and distribution and, consequently, the deposition of abnormal iron compounds, to structures in the brain. However, we certainly agree that in order to examine *individual iron biominerals* from brain tissue, the techniques described by the authors must be employed.

We also would like to thank the author for providing the addition references. We did not mean to imply that the references to the malfunction of the ferritin protein and its potential relationship with magnetite formation were exhaustive. In fact, one of us (JD) had been discussing this possibility with colleagues since the mid-1990s in relation to another well documented biological example of the conversion of ferritin to magnetite – the chiton – a marine mollusk which uses magnetite as a coating for its radula [9].

It is true that the potential role of a ferritin precursor in the formation of biogenic magnetite was discussed in the Quintana et al. [8] paper and there were discussions between our two groups (cited as personal communication in that paper). We also agree that it is worthwhile citing the Quintana et al. [8] work as this excellent paper is one of the first (perhaps the first) to identify abnormal iron phases within the ferritin protein associated with neurodegenerative disease. This fact was highlighted in a subsequent commentary by one of us [2] and its omission in our *Journal of Alzheimer's Disease* paper was an oversight.

Anomalous deposition of iron compounds is associated with virtually all neurodegenerative diseases and unraveling its potential role in these diseases is a critical task. Contributions from a wide variety of techniques are enabling us to make progress towards an understanding of iron's role and this knowledge has the potential to guide future therapies and diagnostic developments [1,3,4]. By combining techniques such as those highlighted with other techniques, including synchrotron x-ray spectroscopy, we should be able to more fully understand the origin of this anomalous iron, the processes leading to its deposition, and whether it is a cause or an effect of these diseases.

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