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Short communication

Iron deposits in multiple sclerosis and Alzheimer's disease brains

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Abstract

Iron may contribute to the pathogenesis of neurological diseases by promoting oxidative damage. The localization of iron in multiple sclerosis (MS) and Alzheimer's disease (AD) brains was investigated to further the understanding of its pathogenic role in these disease states. Earlier studies, utilizing a standard Perls' stain, yielded conflicting reports regarding the distribution of iron deposits in MS brains, and a previous study on AD brains utilized a diaminobenzidine (DAB) enhanced version of this stain. In the present study, a modified version of the DAB-enhanced stain was used; it utilizes sodium borohydride, proteinase K, Triton X-100 and xylenes to increase the accessibility of tissue iron to histochemical reagents. This modified method can reveal iron deposits that are missed by the Perls' or DAB-enhanced Perls' stains. In addition to its normal deposition in oligodendrocytes and myelin, iron was detected in reactive microglia, ameboid microglia and macrophages in MS brains. In AD brains, three types of plaques were stained: dense core, clear core and amorphous plaques. Punctate staining was also observed in neurons in the corticies of AD brains. The structure accounting for punctate labeling may be damaged mitochondria, lipofuscin or amyloid deposits. Dense core plaques, clear plaques and punctate labeling were not detected in the previous AD study which utilized only the DAB-enhanced Perls' stain. The labeling of these additional structures illustrates the benefit of the modified method. In summary, the localization of iron deposition in MS and AD brains indicates potential sites where iron could promote oxidative damage in these disease states. © 1997 Elsevier Science B.V.

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Iron is thought to promote oxidative damage in pathological states by catalyzing the formation of hydroxyl radical [10,11] and by causing secondary initiation of lipid peroxidation [4,5]. Oxidative damage has been implicated in several neurological diseases, e.g., Parkinson's disease, multiple sclerosis (MS) and Alzheimer's disease (AD). There is mounting evidence suggesting that iron participates in the pathogenesis of Parkinson's disease [2,3,17,18], but its role in oxidative damage in MS and AD is unresolved. The goal of this study was to examine the localization of iron in MS and AD brains in order to gain insights about its potential role in these diseases.

There have been conflicting reports about the presence of iron deposits in MS brains. In 1982, Craelius and colleagues identified iron deposits adjacent to demyelinating plaques in five out of five MS brains but not in any control brains [8]. Based on these results, they suggested that abnormal iron metabolism is related to the MS condition. A subsequent study by Walton and Kaufmann failed to confirm the findings by Craelius and colleagues; they found iron deposits associated with plaques in cerebral white matter in only one of 13 MS brains [19]. A third study was performed by Adams on a larger sample size; he found iron deposits in 21 out of 70 MS brains and in four out of 64 control brains [1]. The collective data from these three studies does not settle the question whether there is abnormal deposition of iron in MS brains. In each study, paraffin sections were used with a standard Perls' potassium ferrocyanide technique to detect non-heme iron.

In 1980, Nguyen-Legros and colleagues [16] developed a procedure to enhance the signal from the standard Perls' stain, called the diaminobenzidine (DAB)-enhanced Perls' stain. They demonstrated that the ferric ferrocyanide product can be intensified by allowing it to catalyze the oxidation of DAB in the presence of hydrogen peroxide. A more recent addition to this technique employed several permeabilization steps to improve the penetration of the histochemical reagents into the tissue so that the reagents could readily access iron [14]. This development was particularly important for staining of white matter regions, because the

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compact structure of myelin can exclude staining reagents [13]. This modified DAB-enhanced method was used to demonstrate that iron is enriched in oligodendrocytes and myelin throughout cerebral white matter tracks of the mouse [9,14,15].

The poor sensitivity and the poor penetration of the standard Perls' technique suggest that iron deposits in MS brains could have been missed in the three earlier studies. In the present study, the localization of iron deposits in MS brain tissue was reexamined using the modified DAB-enhanced Perls' stain.

In addition to MS, brains from AD patients were used to examine the localization of iron deposits in this disease. A previous study on AD brains [6] used a DAB-enhanced Perls' stain without any of the additional steps employed in the modified method. Since the modified method makes use of sodium borohydride, proteinase K, Triton X-100 and xylenes, it was of interest to determine if there were additional sites of iron deposition in AD brains that were not revealed in the earlier study.

The previously described modified DAB-enhanced Perls' stain [14] was followed except for a few modifica-

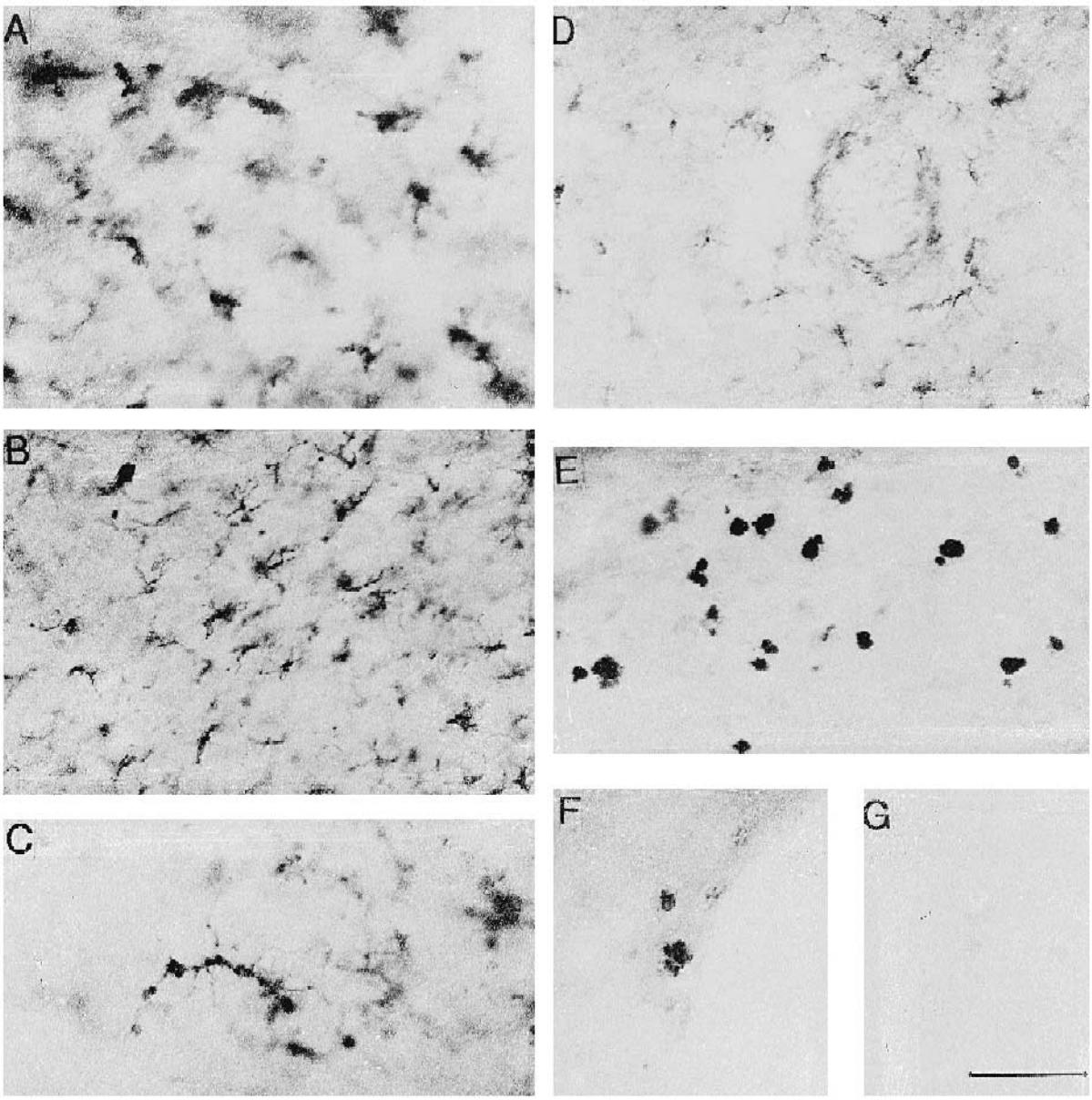

Fig. 1. MS brain tissue. A: ameboid microglia are present in the subcortical white matter. B: in sections with ameboid microglia, highly branched reactive microglia were generally present adjacent to them. C: a high magnification of a reactive microglial cell located at the edge of a region containing a high concentration of reactive microglia. D: reactive microglia are present around blood vessel. E: round macrophages are intensely labeled. These were located in a region that was devoid of ameboid or reactive microglia. F: round macrophages were often observed in association with a blood vessel (lower right corner). G: a control section shows very light labeling of macrophages (center), which is clearly differentiated from specific labeling. Bar = 20 μm (C) or = 50 μm (A,B,E,F,G) or 100 μm (D).

tions. Brain tissue was immersion fixed in formalin and 100 μm thick Vibratome sections were prepared. The final DAB step was stopped after 2, 6 or 12 min of incubation in order to optimize the labeling and contrast of structures. Besides exercising caution with the use of DAB, sodium borohydride was not dissolved in a closed container, because it can generate excessive pressure. The staining procedure should be carried out in a fume hood.

Control sections were incubated in potassium chloride/Triton X-100/HCl in place of potassium ferrocyanide/Triton X-100/HCl [14], and they were exposed to the DAB solution for the full 12 min incubation.

Iron deposits were observed in macrophages in white matter from each of the five MS brains examined. In three brains, ameboid microglia/macrophages (Fig. 1A) and reactive microglia (Fig. 1B,C) (microglia with highly branched processes) were stained in white matter and in the inner cortex adjacent to the white matter. The reactive microglia were located at the edges of the areas containing ameboid microglia/macrophages, around vessels (Fig. 1D), and/or isolated from ameboid microglia/macrophages. Round macrophages, typically with high concentrations of iron, were present in four out of five MS brains; they were found adjacent to labeled microglia, in white matter devoid of other labeled microglia (Fig. 1E) and/or adjacent to vessels (Fig. 1F).

Iron-enriched ameboid cells have been identified in white matter of the developing rat brain [7,12]. Thus, it is possible that ameboid cells contain high levels of endogenous iron, and this is what accounts for their staining in the present study. The source of the concentrated iron within round macrophages in MS brains could be iron from oligodendrocytes and/or myelin that have undergone destruction, since both the oligodendrocyte and myelin sheath contain iron [9,14,15]. Another source could be the extravasation of blood into the brain [8].

In each of the brains, oligodendrocytes and/or myelin (Fig. 2A–C) were stained to varying degrees, which is a consequence of the variable nature of the iron histochemistry procedure and fixation [9,14]. Astrocytes were not labeled. In one MS brain, there was labeling of some straight or twisted axons which were observed near a heavily damaged area of white matter (Fig. 2D,E). Axonal labeling in damaged MS white matter was observed in the study by Crealius and colleagues [8]. In discrete regions of the corticies of three MS brains, there was punctate stain-

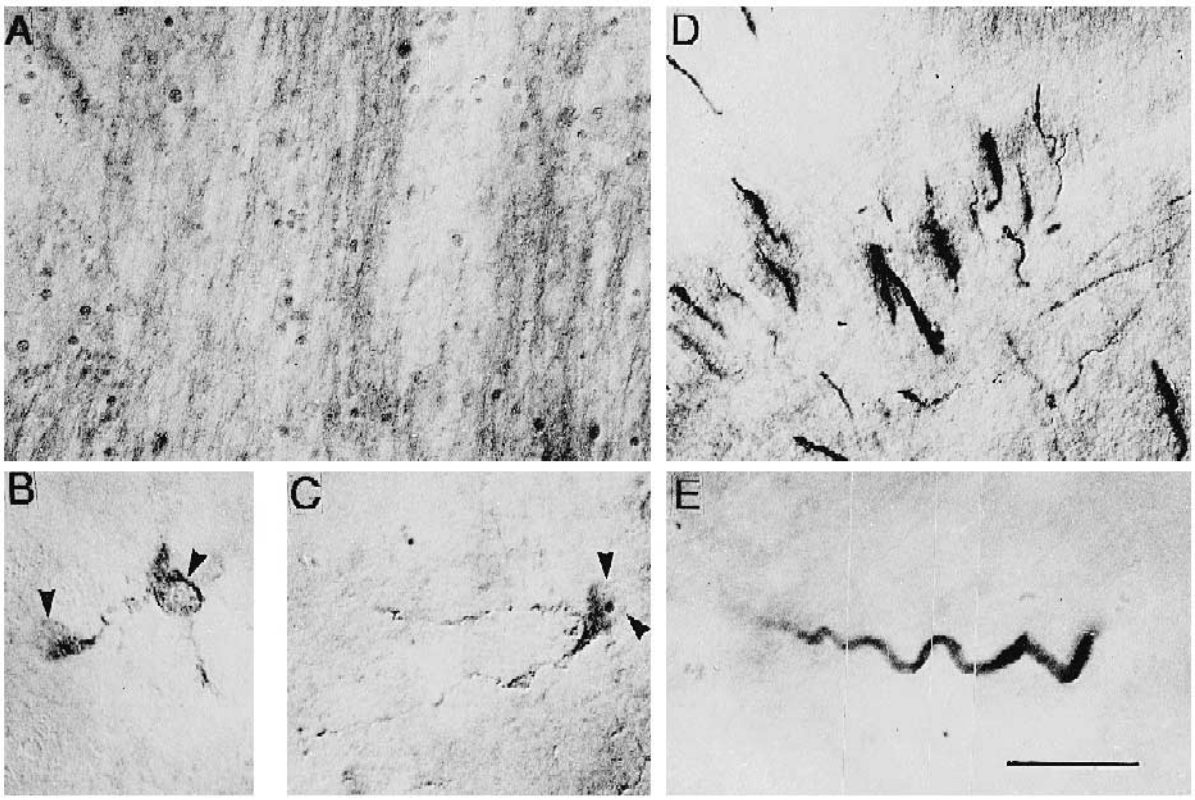

Fig. 2. MS brain tissue. A: discrete staining of myelin was infrequent and it often appeared light. Myelin is seen together with oligodendrocytes. B,C: a high-power view of oligodendrocytes in white matter. Often only the soma of the oligodendrocyte was stained, but in some regions oligodendrocyte processes also could be seen (arrowheads point to the somas of oligodendrocytes). D: in one MS brain, some densely labeled axons were observed in a region of damaged white matter. These axons could be straight or twisted, and sometime very long profiles of these axons were present within the section. E. A montage of higher magnification images at different focal planes of a labeled twisted axon. The axon has a corkscrew appearance. Bar = 20 μm (B,C,E) or = 100 μm (A,D).

ing observed in some neurons (Fig. 3I). There was staining of Alzheimer-like plaques in the corticies of two of these brains.

Large tissue areas or different brain regions had to be sampled before some of the MS brains revealed the staining features (e.g., round macrophages, punctate staining) described above.

Plaques were labeled in AD brains. The plaques could generally be categorized in one of three types: a dense core plaque (Fig. 3A–C), a diffuse amorphous plaque (Fig. 3D), or a empty core plaque (Fig. 3E). The dense core plaque often had a halo of staining surrounding it with an unstained area between the dense core and the halo. Sometimes cellular processes emanated from the plaque. The amorphous plaque had a consistent pattern of staining throughout its width, and the empty core plaque had a central core that was unstained. It is possible that the three types of plaques are really one in the same with the plane

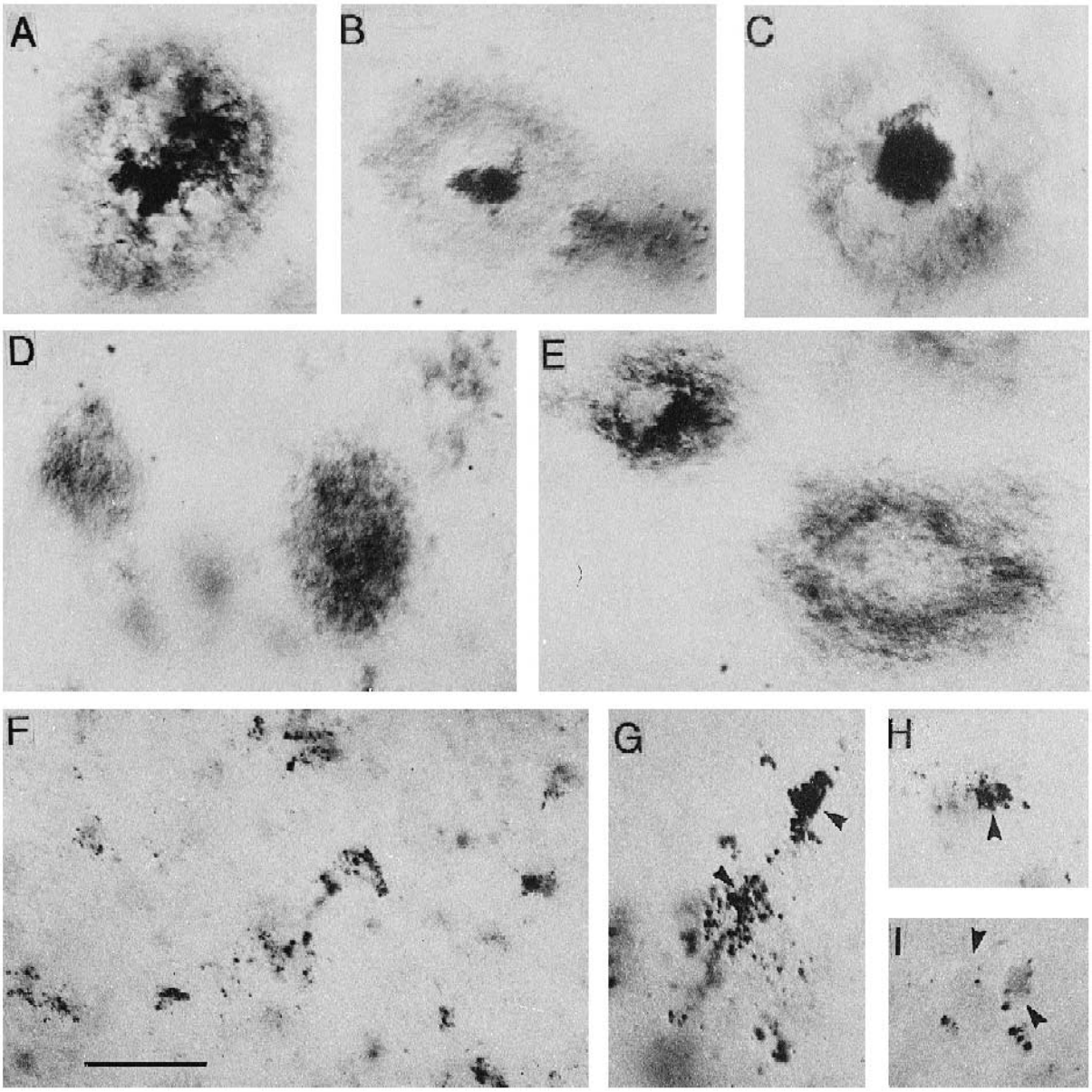

Fig. 3. AD brain tissue (A–H) and MS brain tissue (I). A–C: dense core plaques. The dense cores had a large variety of shapes; only some are depicted here. An unstained region generally surrounded the dense core, and a halo of amorphous material was present in a ring around the unstained region. D: diffusely stained, amorphous plaques has a similar staining quality to the ring of staining seen in the dense core plaques. E: a clear core plaque (right) and a plaque with its core half clear and half dense (left). The latter plaque suggests that all three types of plaques could be of the same type, and it is the plane of sectioning that accounts for their different appearances. F: punctate staining is present in some neurons and neuropil. G: a high magnification of some neurons with a high concentration of punctate labeling (arrowheads). H: an isolated neuron with a high concentration of punctate labeling (arrowhead). I: cortical cells with a low density of punctate labeling in a MS brain (arrowheads, Normarski optics outlines the edges of the cells). Bar = 20 μm (A–E, G–I) or 50 μm (F).

of sectioning determining the stained appearance. For example, if the plane of sectioning caught the area between the halo and core, but missed the core, an empty core plaque would be observed. If the plane of sectioning caught the edge of the plaque, missing both the clear area and the core, then it would be an amorphous plaque. In a previous study that just used the DAB-enhanced Perls' stain, the dense core or empty core plaques were not described in AD brains [6]. Thus, the sodium borohydride, proteinase K, Triton X-100 and/or xylenes used in the modified method are likely responsible for unveiling the dense core site of iron concentration.

In addition to plaque staining, punctate labeling was observed in neuronal cell bodies and in neuropil of the corticies of AD brains (Fig. 3F–H). The labeled neurons could be isolated or in large clusters, and the punctate staining within them could vary from punctate structures at a low density to large bodies filling the cytoplasm. The structures accounting for the punctate labeling are not known, but it is possible they are damaged mitochondria, lipofuscin or amyloid deposits. Punctate staining was also observed in neurons confined to small cortical regions of a normal brain and three MS brains (Fig. 3I). The normal brain and two of the three MS brains also displayed plaques. The number of cells with punctate labeling and the density of punctate staining within these cells were generally far below that observed for diseased cortical areas in AD brains. The profile of punctate staining suggests that when a neuron becomes stressed or damaged it acquires punctate staining, which continues to increase in density as the degeneration of the neuron progresses. The presence of punctate iron deposits in neurons indicates that this iron could partake in oxidative events that promote damage in neurons. Cells with punctate staining appear to precede plaque staining since high concentrations of these cells could be observed in the absence and/or presence plaque staining. It should be noted that not all cortical regions of AD brains displayed punctate and/or plaque staining.

In a previous study using just the DAB-enhanced Perls' stain, punctate staining of neurons was not described in AD brains [6]. Thus, the additional steps used in the modified method are likely responsible for unveiling this site of iron accumulation.

The failure of the DAB-enhanced method to reveal two features of iron deposition in AD brains, punctate staining in neurons and dense core staining of plaques, argues that the modified method should be used in place of, or together with, other methods in future histochemical explorations of iron localization in brain diseases.

Cytoplasmic punctate staining of iron deposits has been described in normal and diseased oligodendrocytes in the mouse utilizing the modified DAB-enhanced Perls' stain [14,15]. A small number of punctate structures was also observed in some oligodendrocytes in the present study when the tissue was examined at high magnification (not shown). Also, in a rare oligodendrocyte from a MS brain, the concentration of these structures was increased from that in other cells. The punctate structures in oligodendrocytes were smaller and less concentrated than those observed in neurons in the diseased corticies of AD brains.

Control tissues revealed very light staining of round macrophages (Fig. 1G) that was clearly differentiated from the staining in macrophages from tissue that was exposed potassium ferrocyanide. Control tissues also had light labeling of red blood cells and some gritty staining around some large vessels or torn tissue areas.

In summary, the sites of iron accumulation in MS and AD brains are plausible locations where iron could partake in reactions that contribute to oxidative damage in these disease states.

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