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Copper accumulation in rodent brain astrocytes: A species difference

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ABSTRACT

Changes in Cu homeostasis have been implicated in multiple neurodegenerative diseases. Factors controlling and regulating the distribution of Cu in the brain remain largely unknown. We have previously reported that a sub-set of astrocytes in the subventricular zone (SVZ) contain Cu-rich aggregates. Here we expand previous studies with detailed X-ray fluorescent imaging (XRF) analysis of the additional brain areas of hippocampus (HP) and rostral migratory stream (RMS). We also use conventional DAB (3,3′-diaminobenzidine) staining which accesses both peroxidase and pseudo-peroxidase activities. Both the HP and RMS support neurogenesis while the latter also serves as a migratory pathway for neuronal precursors. Some variations in neurogenic activities have been noticed between species (such as mice and rats). We report here that in rats, the HP, rostral migratory stream (RMS) and third ventricle contain glia which stain positively for DAB and contain copper-rich aggregates as measured by XRF. In contrast, mice hippocampi and RMS display neither DAB+ aggregates nor Cu-rich accumulations *via* XRF. DAB+ aggregates were not induced in the HP of mice transgenic for human amyloid precursor protein (APP) and presenilin, suggesting that accumulations positively stained for DAB are not directly caused by APP. These observed critical differences suggest different properties of the astrocytes in two species. Results suggest that the rat model may have important advantages over the mouse model for the study of hippocampal aging and neurodegeneration.

1. Introduction

Owing to its extreme metabolic demands and the wide range of biochemistry facilitated by Cu, the brain is capable of accumulating incredible amounts of Cu with concentrations second only to the liver [1]. The storage and uptake/export capacity of astrocytes have made them prime candidates for *in vivo* uptake of Cu into the brain where they are thought to act as "depots" for Cu sequestration in the brain [2,3]. Previous studies using energy dispersive spectroscopy (EDS) in neuroproliferative zones have reported metal-rich aggregates to be present in astrocytes, finding both Fe and Cu rich aggregates that exhibit peroxidase (or pseudoperoxidase) activity in rats [4–6]. Our more recent work with quantitative X-ray fluorescence (XRF) microscopy found Cu-rich aggregates in slow-dividing astrocytes of the subventricular zone (SVZ) of both rats and mice where Cu concentrations increase with age [7]. Given the prevalence of transgenic mice in researching Cu-related diseases such as Menkes disease [8,9], Alzheimer's disease [10], and prion disorders [11], it is important to determine if regions that form Cu aggregates in rats also do so in mice to further validate these models.

Using X-ray fluorescence microscopy, it is demonstrated that the rat but not mouse dentate gyrus and rostral migratory stream (RMS) – additional neuroproliferative zone in adults – contain Cu-rich aggregates. Both rats and mice exhibit Cu aggregates in regions of the habenula and hypothalamus adjacent to the third ventricle. These results are mirrored by DAB (3,3′-diaminobenzidine) staining which accesses peroxidase or pseudoperoxidase activity. DAB staining, however, lacks elemental specificity and has generated some ambiguity as to whether Cu or Fe aggregates were responsible for DAB+ staining in these regions. Using combined DAB and XRF imaging, we report that both Cu and Fe aggregates are responsible for a subset of DAB+ staining in these regions.

The results presented here show that Cu is distributed differently in rat and mouse brains and that subsets of astrocytes display different Cu accumulation properties. While the role of these Cu

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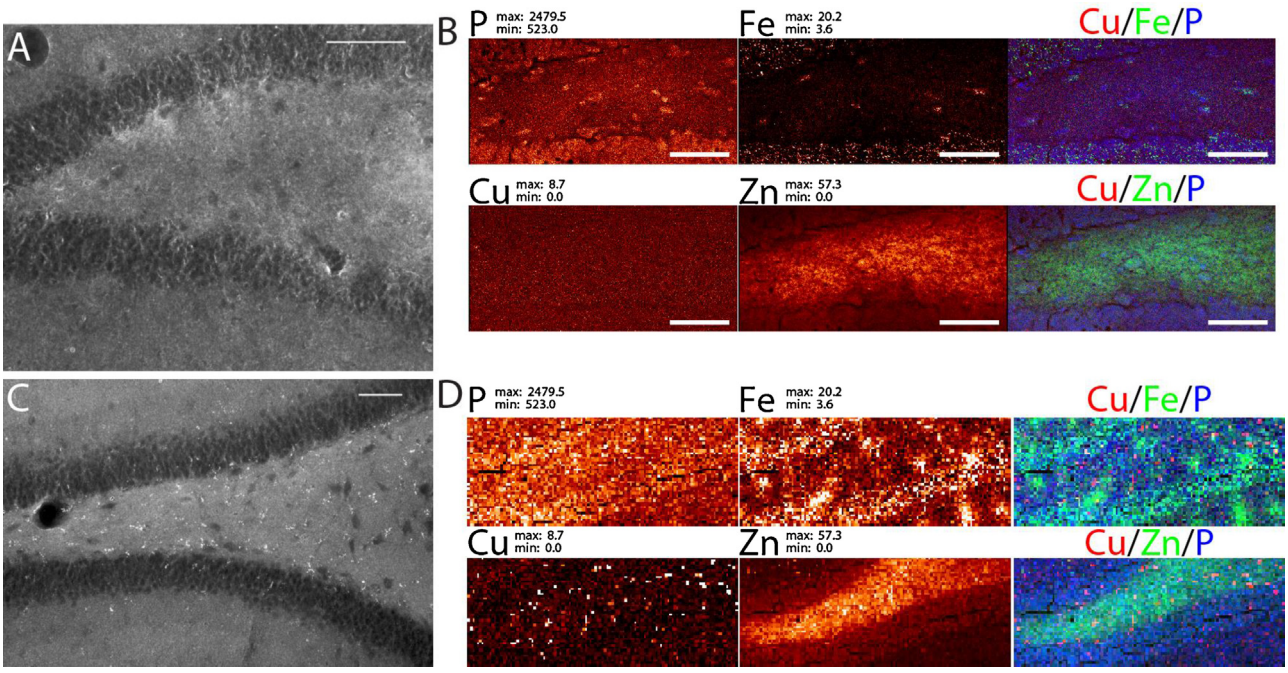

Fig. 1. Distribution of Cu aggregates in the hippocampus. **A**. Dark field micrograph of a DAB-stained mouse section shows no aggregates. **B**. X-ray fluorescence maps of a mouse hippocampus also shows no Cu accumulations, confirming that the Cu aggregates present in rats and in the SVZ of both species are not present. **C**. Dark field micrograph of a DAB-stained rat section reveals positively-stained aggregates in the dentate gyrus. **D**. Similarly, elemental maps show dense Cu aggregates in dentate gyrus of the rat hippocampus. Scale bars: 100 μm. Display ranges are in mM through a 10 μm thick section.

Table 1
Rodents and figures.

	129S1/SvImJ Mouse	C57BL/6J† mouse	C57BL/6J × SvImJ mouse	CD-1 mouse	Sprague-Dawley rat
Number of Rodents	3 (9 weeks)	6 (16 week), 2 wild-type (28 week), 2 transgenic (28 week)	3 (9 weeks)	3 (12 weeks), 6 (32 weeks)	6 (12 weeks)
Habenula	Cu+	Cu + [k], DAB+	Not Imaged	DAB+[f]	DAB+[e,l]
Hippocampus	Cu-	DAB-	Cu-[h]	DAB-, Cu-[g]	DAB+, Cu + [i]
Hypothalamus	DAB+	DAB+, Cu+	Not Imaged	DAB+[d], Cu+	DAB+[c], Cu+
SVZ	Cu + [j]	Not Imaged	Cu+	Cu+[b]	Cu+[a]
RMS	No Imaged	Cu-	Not Imaged	Cu-[b]	Cu + [a]

[a] Fig. 1A,C; [b] Fig. 1B,D; Fig. 4C–E; [c] Fig. 3B; [d] Fig. 3C; [e] Fig. 3E; [f] Fig. 3D; [g] Fig. 2A; [h] Fig. 2B; [i] Fig. 2C, D; [j] Fig. 5; [k] Fig. 4F; [l] Fig. 4G.
† Both wild-type and transgenic overexpression of amyloid precursor protein and presinilin-1.

aggregates remains elusive, the present work serves as a baseline to characterize species differences. Given the importance of astrocytes in metal distribution and brain function, detected differences may have important consequences in choosing rodent models for studying neurogenesis, aging, metal homeostasis, and neurodegeneration.

2. Materials and methods

2.1. Animals

For XRF, 7–8 weeks old Male Sprague–Dawley rats from Harlan Laboratories were purchased and were housed in a temperature-controlled, 12/12 light/dark room, and allowed free access to pelleted rat chow (Purina rodent chow 5001) and distilled, deionized water. Three nine-week old 129S1/SvImJ mice were purchased from the Jackson Laboratory, three CD-1 mice from Charles River Laboratories and three female C57BL/6J × SvImJ from Jackson Laboratory. At the age given in Table 1, the animals were sacrificed and the brains were dissected and frozen directly on dry ice. For histochemical labeling, 4 male and 2 female mice (C57bl/6j strain) about 16 weeks old and Sprague-Dawley rats were used initially. To confirm that observed differences in mice were not strain-specific, brains from four 7-month old mice of the C57bl strain were examined. Two of these mice were wild type, control mice, and two additional mice were transgenic for human amyloid precursor protein and presenilin-1 [12] as amyloid precursor protein exerts damaging, oxidative stress upon astrocyte mitochondria and thus could possibly contribute to the development of the astrocytic pathology in the hippocampus (HP) [13]. Additionally, brains from six 8-month old retired breeder males of the albino CD-1 strain were obtained. All experiments complied with appropriate institutional animal use and care committees.

2.2. X-ray fluorescence microscopy

Brains for XRF were stored at −80 °C prior to sectioning. The night before sectioning, brains were warmed to −20 °C and ultimately to −12 °C for sectioning. With the exception of DAB + XRF co-localization, brains were not chemically treated in any way (*e.g.* fixation or perfusion) to avoid metal redistribution. To identify planes containing the SVZ and HP, cresyl violet stains were performed on sections adjacent to regions before and after those taken for imaging. Planes taken contained the SVZ or HP (coronal sections) or the SVZ, HP, and RMS (sagittal sections.) Sections for XRF imaging were thawed onto 4 μm thick polypropylene film stretched on