Sample preparation with sucrose cryoprotection dramatically alters Zn distribution in the rodent hippocampus, as revealed by elemental mapping – Supporting Information

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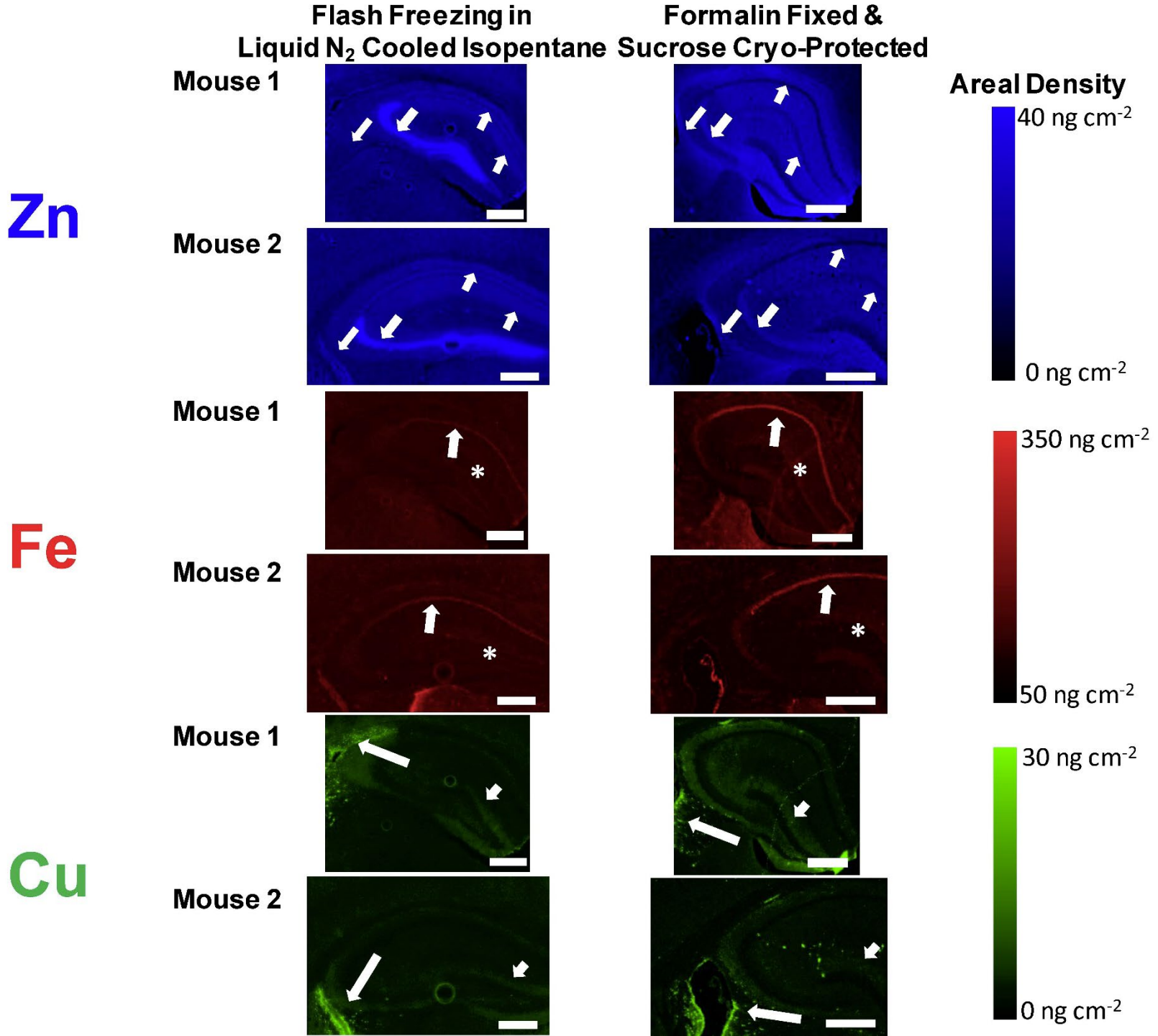
Rationale for experimental design

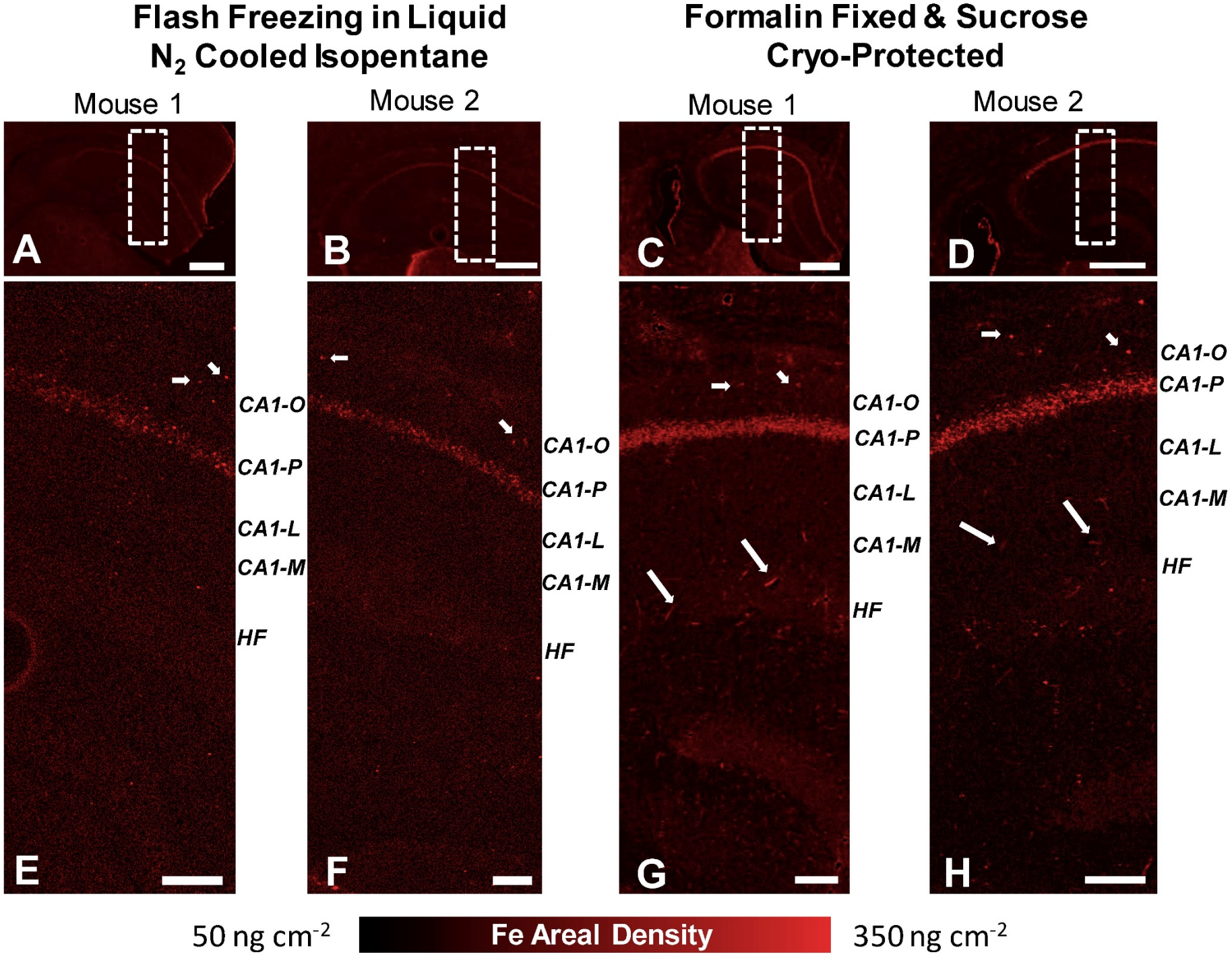
In this study we report elemental distributions of mouse and rat hippocampus, from brain tissue prepared via either rapid plunge freezing (RPF) in liquid nitrogen-cooled isopentane, or sucrose cryo-protection (SCP). Data analysis has focussed on qualitative assessment of the pattern of Zn distribution in mouse and rat hippocampal tissue and how it differs between the two sample preparation methods. We focus on qualitative assessment rather than quantitative assessment because different elemental mapping facilities or research groups typically cut tissue sections at different thicknesses, and therefore direct quantitative comparison of areal densities is not possible without first back-calculating to concentration based on tissue thickness, but this still leaves experiment-dependent quantitative differences which are not so straight-forward to account for when comparing inherently different experimental setups. The variation in tissue thickness affects self-absorption and X-ray attenuation, further complicating direct quantitative comparison across different studies. Further, the absolute concentration of Zn in the hippocampus is not static and changes during development, adult age, and during senescence, and is also influenced by factors such as diet. Comparison of absolute Zn concentration between different animal studies is therefore difficult. However, the relative distribution of hippocampal Zn is highly reproducible and well conserved between different mouse and rat strains, simplifying its visual qualitative assessment.

We have focussed this study on hippocampal Zn, as opposed to other metal ions (*e.g.*, Ca, Fe, or Cu) for two reasons: 1) the hippocampus contains a large pool of labile Zn that is prone to redistribution, and is therefore an excellent "positive control" to assess the extent of redistribution that can occur during SCP; and 2) there is an abundance of literature using an additional analytical method, sensitive to labile Zn (histochemistry) that supports the findings of this study. The elemental maps for Fe and Cu that were also obtained are presented in Supporting Information, but are not the focus of this study.

In this study, analyses have been carried out in duplicate, with highly reproducible results observed across the duplicate measurements. For each animal, the brain was cut into two sagittal hemispheres, with the left hemisphere prepared via RPF, and the right hemisphere prepared via SCP (as described in the methods). The intra-animal experimental design used in this study helps reduce biological variation, and thus a larger number of animal replicates have not been analysed.


Flash Freezing in Liquid N_2 Cooled Isopentane
Formalin Fixed & Sucrose Cryo-Protected
Areal Density
Zn
Mouse 1
Mouse 2
40 ng cm^{-2}
0 ng cm^{-2}
Fe
Mouse 1
Mouse 2
350 ng cm^{-2}
50 ng cm^{-2}
Cu
Mouse 1
Mouse 2
30 ng cm^{-2}
0 ng cm^{-2}

Supporting Information Figure 1: Transition metal ion (Zn, Fe, Cu) distributions in the hippocampus of mice, from tissues prepared via rapid plunge freezing in liquid-nitrogen-cooled-isopentane (RPF), or sucrose cryo-protection (SCP). Images are shown from two duplicate animals. White arrows indicate hippocamp sub-regions that display localised metal enrichment. Zn was observed to be redistributed as a consequence of SCP however, there was no obvious visual redistribution of Fe and Cu from the hippocampal regions naturally enriched in these metals. The hippocampal fissure (asterisk in Fe maps) did display regions of locally elevated Fe (highlighted in Supporting Information Figure 2), which were only visually observed in tissues prepared via SCP, but not RPF. Scale bar = 500μm.


Flash Freezing in Liquid
N2 Cooled Isopentane
Formalin Fixed & Sucrose
Cryo-Protected
Mouse 1
Mouse 2
Mouse 1
Mouse 2
A
B
C
D
CA1-O
CA1-P
CA1-L
CA1-M
HF
E
F
G
H
50 ng cm-2
Fe Areal Density
350 ng cm-2

Supporting Information Figure 2: Zoomed in view of hippocampal Fe distribution near the hippocampal fissure in mouse tissue prepared via rapid plunge freezing in liquid-nitrogen-cooled-isopentane (RPF), or sucrose cryo-protection (SCP). (**A-D**) Overview Fe images, showing the region of interest centred on the hippocampal fissure (white dashed box). (**E-F**) Fe distribution around the hippocampal fissure in (**E, F**) tissue prepared via RPF, and (**G, H**) tissue prepared via SCP. All samples displated Fe enriched cells (small white arrows), however elongated Fe rich regions (possibly blood capillaries) were only observed in tissues prepared via SCP (large arrows).
Scale bar in B, C = 500μm, and D-H = 100 μm.
CA1 – O = CA1 oriens layer
CA1 – P = CA1 pyramidal layer
CA1 – L = CA1 stratum radiatum
CA1 M = CA1 stratum lacunosum moleculare
HF = Hippocampal Fissure