Using MEMRI and Evans Blue to compare calcium uptake between mdx and Black 10 mice brains

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Introduction

•**Aim:** to compare specific functional features in the brains of Black 10 mice and mdx mice.

•In Duchenne Muscular Dystrophy (DMD), muscle cells have an increased intracellular concentration of calcium. Therefore, our plan was to see if this is reflected in the brain

•Use manganese uptake as a marker for calcium (as they are potentially taken up in the same way) and to compare this uptake between the two models.

Methods

•Anaesthetized mice were scanned on a 7 Tesla Varian micro-imaging system

•T1 weighted images were acquired of the brains of the BL10 and mdx mice.

•Prior to the scan, the mice were injected with Evans Blue dye (25ml/10g of body weight).

•The mice were also infused intravenously with a manganese solution

•Scans were taken at baseline and five minute intervals.

•After scanning, the brains were dissected, cut with a cryostat and mounted on microscope slides with samples of 5 different brain areas.

Evans Blue Dye

•Evans Blue dye injected into the mice before scanning can be detected in cryosections.

•The slides were fixed in chilled acetone for 10 minutes, followed by 3 washes in PBS.

•They are then mounted with vectashield containing DAPI.

•The DAPI is a fluorescent marker which stains nuclei blue and helps to locate the tissue on the slide.

•Under the fluorescent microscope, the Evans Blue fluoresces red if it is present.

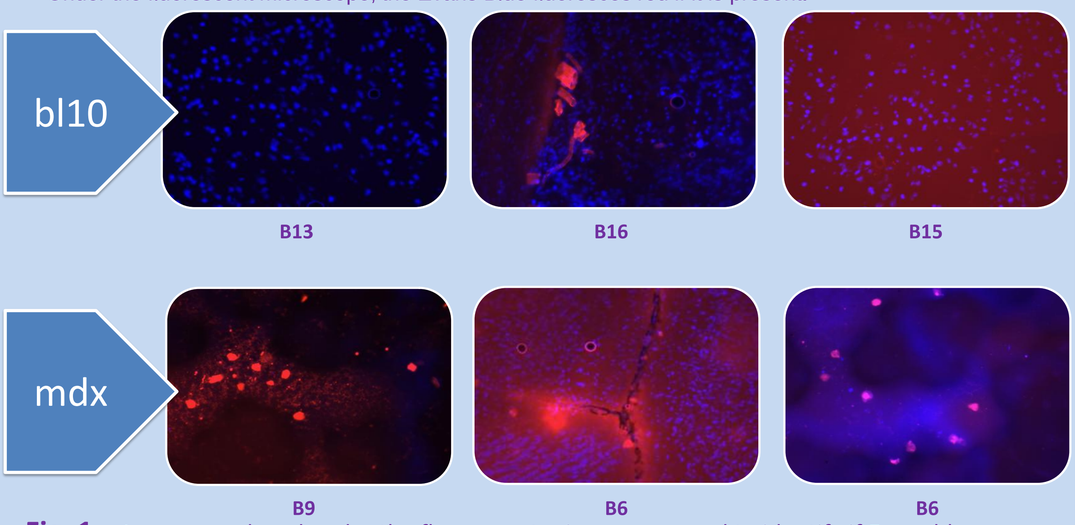

Fig. 1 – Images produced under the fluorescent microscope, used to identify if Evans blue dye is present in the brain section. Blue indicates nuclei, Red indicates Evans blue.

Manganese Uptake

•Using image J, an intensity measurement was taken at 5 minute interval scan images for each brain area

•The manganese shows up as a brighter area on the image, and since it enters the cell potentially via all routes which calcium enters, it acts as a marker for calcium uptake.

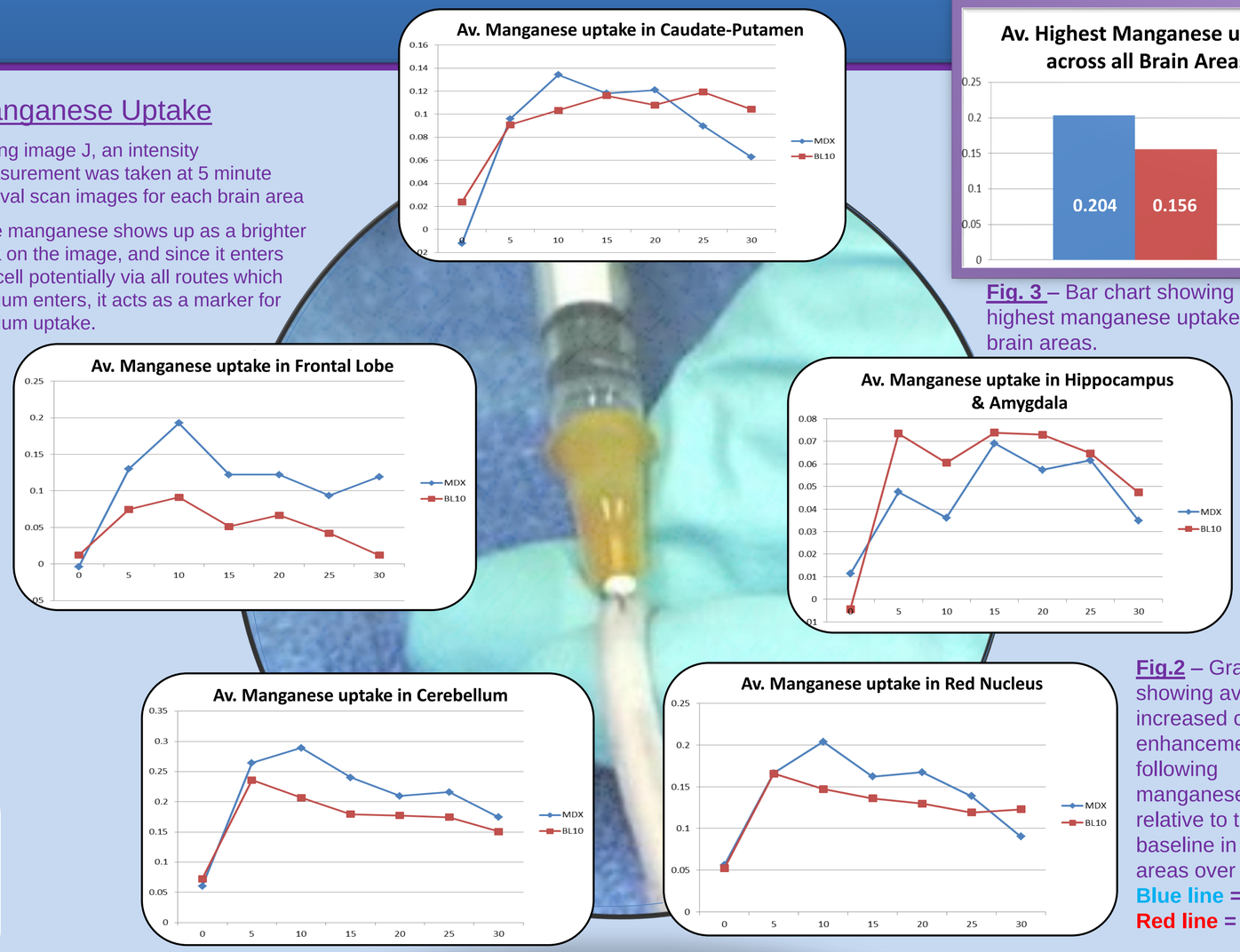

Fig. 3 – Bar chart showing average highest manganese uptake across all brain areas.

Fig.2 – Graphs showing average increased contrast enhancement following manganese relative to the baseline in 5 brain areas over time.
Blue line = mdx
Red line = BL10

Conclusion

Evans Blue Dye

•On the fluorescent microscope images, in the BL10 brains there is no, or very little, evidence of Evans Blue Dye being present.

•However, in the mdx brains, the appearance of red fluorescent patches suggests that the Evans Blue Dye was able to enter these brains with greater ease, and therefore indicates compromised blood-brain barrier integrity.

•Between these two sets of results, the data suggests that in addition to destabilisation of muscle, loss of dystrophin also leads to leaky blood-brain barrier and increased calcium uptake in the brain.

MEMRI Analysis

•Across the 5 brain sections, the manganese intensity readings (and therefore, uptake) were higher in the mdx brains.

•I was using the manganese as a calcium uptake marker, so these results also suggest an increase in calcium in the brain.

Discussion

•This is a non-invasive method that can be used longitudinally and potentially allows researchers to test agents for reducing calcium uptake such as channel blockers or membrane sealants in live animals over time. There is also potential for this to translate into patients (although not with manganese).

References

Nico B, Roncali L, Mangieri D, Ribatti D. Blood-brain barrier alterations in MDX mouse, an animal model of the Duchenne muscular dystrophy. Curr Neurovasc Res. 2005 Jan;2(1):47-54. Review. Erratum in: Curr Neurovasc Res. 2005 Apr;2(2):185. PubMed PMID: 16181099