Measuring the impact of dystrophin deficiency using MRI and histology of the mouse brain

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Introduction

•**Aim:** define key differences in mdx brain vs. control

•MRI (magnetic Resonance Imaging) techniques were coupled with histological staining to identify contrasting features in mdx mouse brain size and cell number counts due to the absence of dystrophin.

Methods

Histology/Staining

•Cresyl Violet is a basic stain that binds to the acidic components of the neuronal cytoplasm which are present in a large numbers on neurons.

•It binds to and highlights the nuclei of the cells in the section. This stain enabled me to identify specific structural areas with my samples and also provided enough contrast to enable me to count the nuclei in a specific area of the mouse brain samples.

Brain Area Measurements

•Subsequent to the mice having brain MRI scans the images were uploaded onto the imageJ software

•Utilising the polygon tool I drew around the mice brain on each MRI slice then used the software to calculate the area

•I then multiplied the calculated areas by the total number of brain slices to get an overall value for mouse brain area.

Cell Counts

•Looking at the Cresyl violet-stained sections down a light microscope, I was able to capture images of a specific part of the cerebral cortex in each brain at a magnification of x20.

•I then used image J to count the number of cells in the area.

Mice Brain Areas

mdx Mice

MOUSE	WEIGHT (g)	Average Brain Volume	RATIO (W/V) 1:
B5	29.7	444.86	14.97845118
B6	30.62	685.67	22.39288047
B7	32.15	443.35	13.79004666
B8	31.89	459.78	14.41768579
B9	31.82	471.53	14.8186675
B10	31.09	674.87	21.70697974
		530.01	17.01745189

Black10 mice (aged matched with mdx)

MOUSE	WEIGHT (g)	Brain Volume (mm3)	RATIO (W/V) 1:
B11	20.8	467.3	22.46634615
B12	21.92	441.48	20.14051095
B13	25.38	441.66	17.40189125
B14	22.71	420.1	18.49845883
B15	25.53	425.2	16.65491579
B16	24.16	449.92	18.62251656
		440.9433333	18.96410659

Fig.1- Illustrates the data collected for the brain areas of a number of mdx and Black10 mice. The brain area calculated is then compared to the weight of the mice in a ratio of weight to brain volume

Cerebellum Cell density

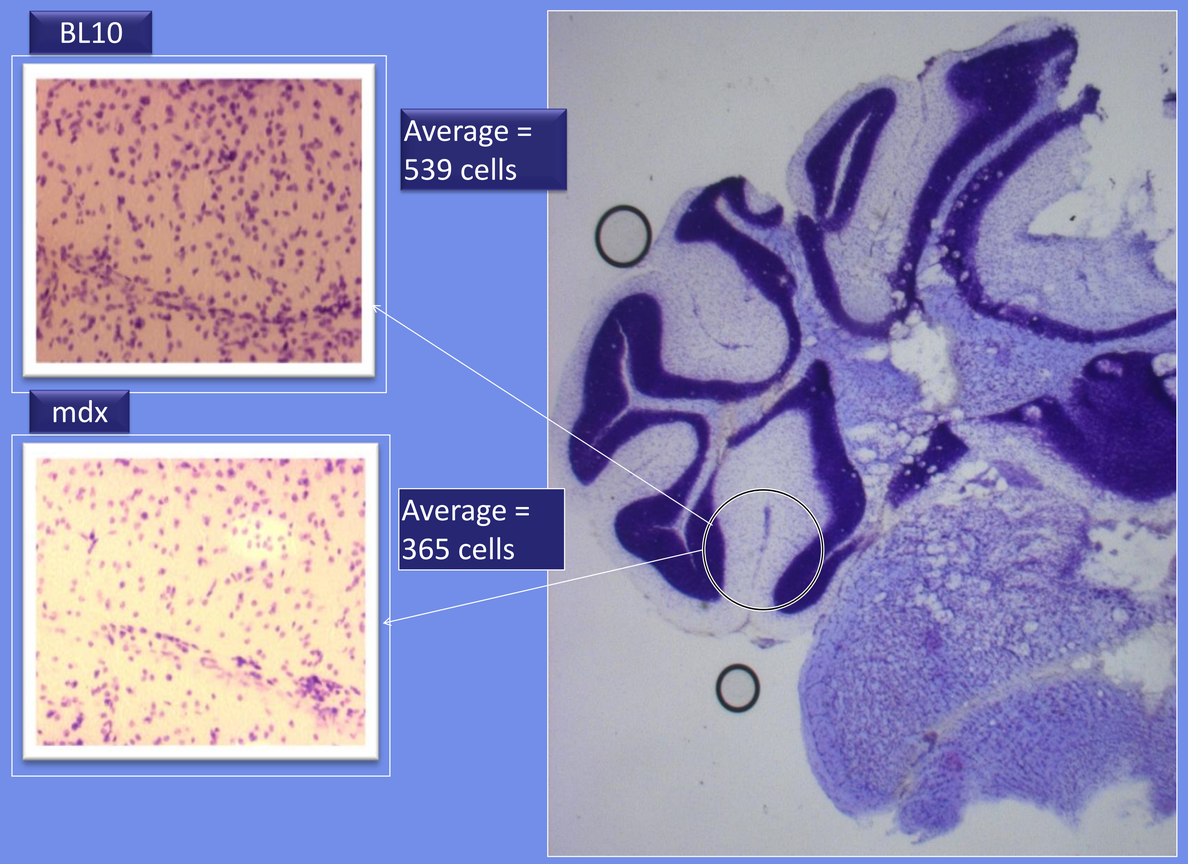

Fig.2 Shows a diagrammatic representation of the cell counts conducted in the cerebellum of the mdx and Black10 mice brains. The image was taken by a light microscope of a sample generated through cryostat techniques and staining utilising the dye Cresyl Violet. The values are an average of the cell counts taken from a number of mice of each type.

Conclusions

•The brain volume measurements illustrated that the mdx mice have, on average, larger brain volumes, although in comparison the Black10 mice have a greater weight to volume ratio. Demonstrated in Fig.1.

•The cell counts conducted in the mice brain cerebellum concluded that on average Black10 mice have a higher cell number (539) then that of the mdx mice (365). Demonstrated in Fig.2.

•The data shows a substantial decrease in the mdx cell numbers which could be due to the lack of cell membrane stability caused by the absence of dystrophin.

•In conclusion the data shows a correlation between the known lack of dystrophin in the mdx mice and reduced stability of cell membranes, which could directly result in reduced cell numbers and an increased brain volume.

References

Anderson JL, Head SI, Rae C, Morley JW. Brain function in Duchenne muscular dystrophy. Brain. 2002 Jan;125(Pt 1):4-13. Review. PubMed PMID: 11834588.