Axonal Transport Deficits Occur Prior to Behavioral Deficits in Response to GM_2 Accumulation in the HexB-/- Mouse Model

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Introduction

Tay-Sachs disease and Sandhoff disease are two developmental diseases that result in the inability to break down GM_2 ganglioside (GM_2) resulting in GM_2 accumulation in lysosomes primarily of neurons. The catabolism of GM_2 in mammalian cells is dependent upon β-hexosaminidase, a lysosomal acidic hydrolase. In humans, β-hexosaminidase (HEX) is comprised of α & β subunits and exists in two major isoforms, HEXA and HEXB[i]. To study these diseases more closely an animal model with a targeted mutation of the HexB-/- locus to produce excess GM_2 storage has been developed and has been used in the present study[ii].

Neurodegeneration in cohort with mental and physical deterioration are classic signs of GM_2 accumulation. However, one of the underlying aspects to many neurodegenerative diseases is whether the neurodegeneration can be detected prior to the loss of behavioral deficits. Here we test this question using manganese enhanced magnetic resonance imaging (MEMRI)[iii] on pre-symptomatic mice at eight weeks of age to measure axonal transport of the HexB-/- compared to control animals in conjunction with the Morris Water Maze (MWM) behavioral test to assess spatial learning at 2 separate ages, 6 weeks (pre-symptomatic age point) and 3 months.

Materials and Methods

$HexB^{+/-}$ breeder pairs on 129/sv background were mated to obtain $HexB^{-/-}$ and $HexB^{+/+}$ littermate animals. **MRI** $HexB^{-/-}$ animals (n=3) and $HexB^{+/+}$ animals (n=6) were imaged at 8 weeks of age. Animals were anesthetized (0.1 ml/kg mouse) with ketamine/xylazine (0.75mg/ml)/(0.5 mg/ml) in phosphate buffered saline, 0.1 ml per 10g body weight, 10 min prior to lavage. A manganese lavage of 4 μl of 0.75 mg/ml $MnCl_2$ was administered 1 hr prior to imaging. Animals recovered prior to imaging and were then induced at 5% isoflurane and maintained with 2% isoflurane in 100% O_2. Images were acquired utilizing a 9.4T, Bruker Avance Biospec Spectrometer, 21 cm bore horizontal scanner with 35 mm volume resonator (Bruker BioSpin, Billerica, MA). The imaging parameters to acquire olfactory multi-spin/multi-echo MEMRI images were as follows: TR = 500 ms; TE = 10.2 ms; FOV = 3.0 cm; slice thickness = 1 mm; matrix = 128 x 128; NEX = 2; number of cycles = 15; each cycle ~2 min. Core temperature was maintained at 37°C during scanning. Data was acquired using Paravision (Bruker BioSpin) and then analyzed using linear regression and two-tailed t-tests with Prism (Graphpad Software, Inc). Region of interest (ROI) was placed on an axial slice 1.5 mm from the posterior of the olfactory bulb (OB). It measured 0.12 X 0.12 mm and was vertically centered on the dorsal olfactory neuronal layer. This ROI was copied for each cycle and each value normalized to the unaffected muscle of the same slice. **MWM** was used to test spatial learning at two different age points, 6 weeks and 3 months with 15 $HexB^{-/-}$ and 1 $HexB^{+/+}$ littermate controls at each age. The maze consists of a circular pool (130 cm in diameter) filled with 25–27°C opaque water and a hidden platform. Patterns on the walls surrounding the pool serve as cues for the animals. Animals were trained for four days consisting of eight (60 s) training trials per day. A different start location was used for each trial. Immediately after the eighth trial on day four, the hidden platform was removed and animals were scored during a 60s probe trial for latency to and crossings over the previous platform location. Each swim was digitally recorded and tracked using Noldus EthoVision System. One-way ANOVA was calculated using Prism (Graphpad Software, Inc).

Results

Figure 1 illustrates the normal $HexB^{-/-}$ learning compared to controls at 6 weeks as measured by the number of platform crossings during the probe trial of the MWM. There is a significant deficit in learning behavior between $HexB^{+/+}$ and $HexB^{-/-}$ animals at 3 months of age indicating that this deficit is progressive and not present at birth. It is important to note that the swimming speed at the 3-month age point does not vary between $HexB^{+/+}$ and $HexB^{-/-}$ (data not shown). This behavioral deficit is preceded by decreased axonal transport rates shown in figure 2. This indicates that axonal deficits precede behavior deficits in the progression of the disease.

Discussion

The data shown here illustrates that neurodegenerative deficits occur early in the $HexB^{-/-}$ animals prior to deficits in learning. If treatments can be applied early enough to halt this neurodegeneration then it is possible that normal learning and memory could be maintained, quality of life improved, and lifespan lengthened as most $HexB^{-/-}$ mice do not live past 4 months of age. For future studies we would like to test whether the treatments that decrease the GM_2 accumulation, such as enzyme replacement, can also maintain normal axonal transport and prolong normal learning and memory.

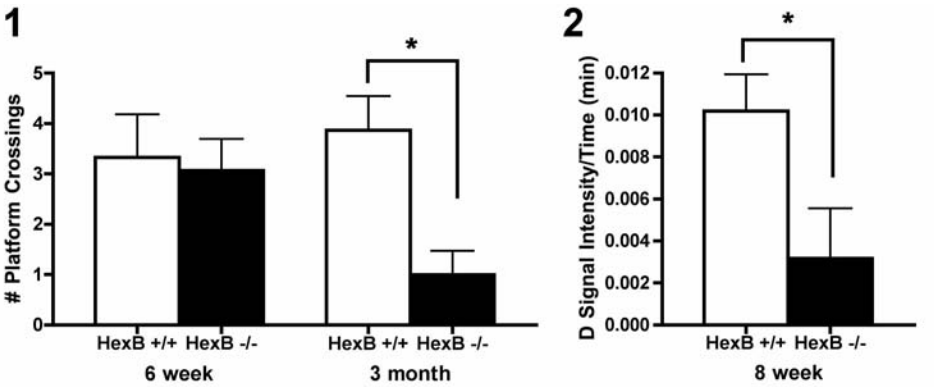

Figure **1**. Number of MWM platform crossings (y-axis) significantly (*) decrease compared to controls at 3 months of age (x-axis). N=15 for each group. P-value < 0.05.

Figure **2**. Axonal transport rate calculated as Δ in Signal Intensity (SI)/ Time (min)(y-axis) shows a significant decrease in transport at 8 weeks of age. $HexB^{+/+}$ N=5; $HexB^{-/-}$ N=4; p-value< 0.05.

[i] Kyrkanides S, Miller JH, Brouxhon SM, Olschowka JA, Federoff HJ. Mol. Brain Research 2005

[ii] Sango K, Yamanaka S, Hoffmann A, Okuda Y, Grinberg A, Westphal H, McDonald MP, Crawley JN, Sandhoff K, Suzuki K & Proia RL. Nature Genetics 1995

[iii] Smith K, Kallhoff V, Zheng H, Pautler RG. Neuroimage 2006 (Submitted)