Six Fucose-α(1–2) Sugars and α-Fucose Assigned in Human Brain using In Vivo L-COSY

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Purpose: A growing literature indicates that Fucose-α(1−2)-galactose sugars are implicated in the molecular mechanisms that underlie neuronal development, learning, and memory in the human brain. The purpose is to develop in-vivo two dimensional L-COSY to examine fucoylsated glycans the human brain in a three Tesla clinical MR scanner.

Introduction: L-Fucose is a hexose deoxy sugar with the chemical formula $C_6H_{12}O_5$. It is found on N-linked glycans on the mammalian cell surface. α-L-Fucose is usually expressed as a terminal saccharide on N- and O-linked glycoproteins and glycolipids [1]. Lean et al first used 2D MRS to assign fucosylated glycans in human colorectal cells[3]. Human cancer cell models allowed the fucose assignments to be confirmed using 2D Correlated SpectroscopY (COSY) [2] allowing assignment of five terminal fucose moieties and free α and β fucose[3]. 2D MR spectroscopy was used to study isolates of oligosaccharides to determine the structure of the blood group antigens that contain terminal fucose residues[4] showing chemical shift alter according to the type of oligosaccharide the fucose incorporated into[4].There is evidence that Fucose-α(1−2)-galactose sugars are implicated in the molecular mechanisms that underlie neuronal development, learning, and memory [5] and a pivotal role in regulating nervous system development and function[6]; and influences various neuronal processes, such as neurite outgrowth and morphology[5,7].

Methods: Data were acquired on a Siemens 60cm bore Trio (Siemens AG, Erlangen, Germany) using an 8 channel head coil or a Prisma 60cm bore (Siemens AG, Erlangen, Germany) using an 40 channel head and neck coil. **Magnetic Resonance Protocols:** T1-weighted MPRAGE volumetric sequence (TR/TE=2530/1.7 ms, 12 degree flip angle, field of view = 256x256 mm, voxel size 1x1x1mm, number of experiments = 4, acquisition time 6 minutes). Prior to the L-COSY spectroscopy data collection, routine brain MRI was performed with axial 3D-MPRAGE and reconstructed in the sagittal and coronal planes with 2 mm slice resolution for accurate localization of the voxel. The posterior cingulate gyrus (PCG), predominantly comprising grey matter was chosen as this gave the better spectra.

L- COSY was acquired in the PCG (size 3x3x3 cm^3), with the following parameters: RF carrier frequency at 2.0 ppm; TR 1.8s; water suppression using WET; spectral width of 2000 Hz; increments size of 0.8 ms in 96 t1 increments giving an indirect spectral width of 1250 Hz; 12 averages per increment; and 1024 data points. Scan time for the 2D L-COSY was 35 minutes. **Subjects:** Eighteen volunteers were recruited for this study with an age range of 22 to 55. The study was approved by the local institutional review boards and was compliant with the Health Insurance Portability and Accountability Act. All subjects provided informed consent.

Data Processing: L-COSY data were transferred to MATLAB [8] followed by row concatenation into a 2D matrix. Commercial 2D spectral processing software (Felix-2007, Accelrys, San Diego, CA, USA) was used for observer-independent spectral processing and analysis. All identifiable peaks according to Lean[3] or new assignments were reported, and peaks were measured and normalized to the creatine diagonal peak volume at 3.02 ppm.

Results: A typical two dimensional contour plot of an L-COSY data set, recorded from the posterior cingulate gyrus of a male brain, is shown in Figure 1A with assignments as described in Ramadan et al[9]. In Figure 1B a three dimensional plot of the same spectrum is shown where the intensity and relative volume of each cross peak is more clearly seen. The region containing the fucose molecules (F2: 3.95–4.50 ppm and F1: 0.90–1.70 ppm) is shown expanded in Figure 2A as a contour plot, and in Figure 2B as a three dimensional plot. The cross peaks are assigned using the nomenclature reported by Lean et al[3a], i.e. Fuc I to IV. Free α-L-Fuc, is also recorded at 4.19-1.14ppm with a second cross peak at 4.17-1.32ppm we tentatively assign to α-Fuc also. If these are indeed both α-Fuc they could arise from either being in two different environments; or as the same substrate/product but in slow exchange between two different environments. Two other cross peaks, not shown in figure 2A include Fuc II as assigned in cell models, at 4.28 - 1.14 ppm and Fuc V at 4.36 - 1.58ppm, not reported before.

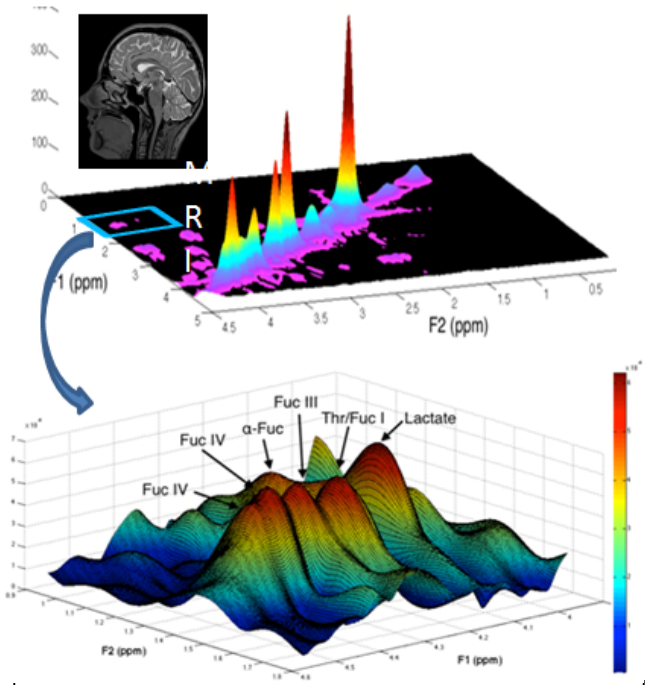

Figure: Human brain PCG, acquired at 3T using an 8 channel head coil. Region highlighted expanded below i.e. F1 0.90-1.75; F2 3.90-4.60 of the L-COSY with assignments of Fuc I to Fuc IV and α-L-Fucose.

Discussion: The improvement in MR technology is now such that a non-invasive window into neuro-glycochemistry is available using a clinical 3T scanner and the L-COSY MR spectroscopy method. We identify and assigned six fucose residues in spectra from the PCG region in the human brain. There is variation in this spectral region from person to person according to their health state and or age. These assignments were initially made in Strecher et al[10] isolated oligosaccharide-alditols carrying Lewis X, Lewis Y and A-Lewis Y determinants. They also used 2D MR spectroscopy to assign two αFuc1-2 molecules at 4.26-1.27ppm and 4.34-1.30ppm. These are consistent with Fuc II and Fuc IV assigned in the cell models and in the human brain (Table 1). Three Fucose-α(1-3) molecules were also assigned but they resonate under the water resonance in the human brain L-COSY at 4.8ppm. Assignment of Fucose-α(1-3) in the human brain requires protocol development.

Conclusions In vivo L-COSY of the human brain identifies multiple Fucose-α(1-2)-galactose species, present in relatively small amounts compared to the major neurotransmitters. Six Fucose-α(1−2)-galactose residues , and free alpha fucose, are available for inspection. Fucose-α(1−3)-galactose residues cannot yet be assigned as they resonate under the water. This new application offers a new insight into the molecular mechanisms by which fucosylated sugars contribute to neuronal processes and how they alter during development, ageing and disease.

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