Appendix 3: Protein Fucosylation in FUT1 and FUT2 Transgenic Knockout Mice

A3.1. Results

Neurite Outgrowth of Cultured FUT1 and FUT2 KO mice

As Fuα(1-2)Gal carbohydrates have been implicated in neurite outgrowth pathways, we sought to address whether FUT1 and FUT2 mice, which do not express the Fuα(1-2)Gal epitope, had defects in neuronal growth. We cultured hippocampal neurons in a substratum of poly-DL-lysine for 2 days to examine neurite lengths. Surprisingly, we saw no difference between the length of neurites between wild-type, FUT1 or FUT2 KO mice (Figure A3.1). This may suggest that the neurons respond to a Fucα(1-2)Gal

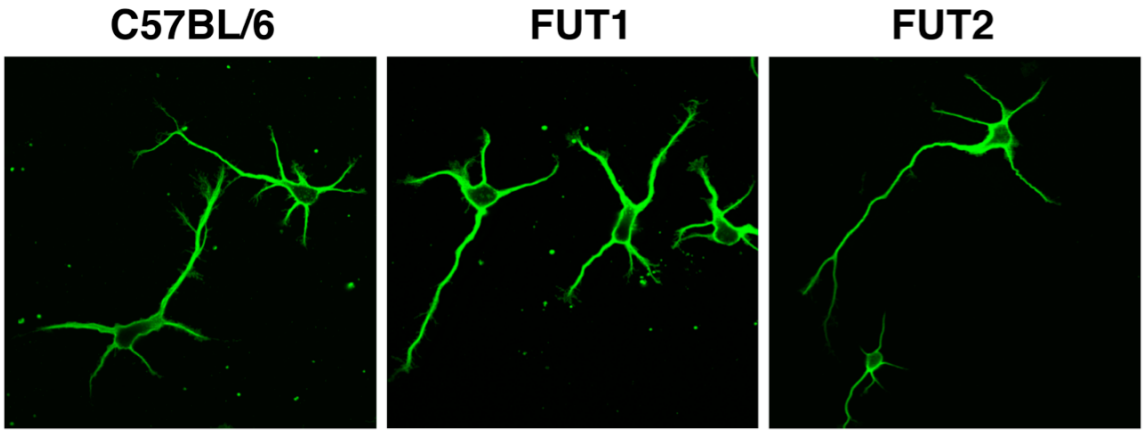

Figure A3.1. Deletion of FUT1 or FUT2 does not affect neurite outgrowth. Cultured hippocampal neurons from wild-type C7BL/6, FUT1, and FUT2 knockout mice were cultured for 2 DIV and stained with tubulin.

epitope secreted or present on other cell types such as glial cells or oligodendrocytes. In addition, there could be compensatory effects from knockdown of one gene, in which case the other fucosyltransferase can take over and synthesize Fucα(1-2)Gal glycoconjugates on the cell surface, which has never been investigated in cell culture

experiments. Furthermore, other neuronal growth pathways may compensate for loss of fucosyl expression in mediating neurite outgrowth.

Fut1 Regulates the Fucα(1-2)Gal Proteome in Murine Olfactory Bulb while FUT2 Regulates Expression of Fucα(1-2)Gal Glycoproteins in the Hippocampus

We next examined whether FUT1 or FUT2 could regulate the proteome in various adult mouse brain fractions including the cortex, cerebellum, hippocampus, striatum, and olfactory bulb. We first examined recognition by antibody A46-B/B10 in addition to the Fucα(1-2)Gal-specific lectin UEA1. Surprisingly, we did not see any difference in bands from most brain fractions labeled from antibody A46-B/10, suggesting a redundancy of Fucα(1-2)Gal expression or compensation by the other enzyme. We did observe a reduction in intensity of bands in the cerebellum of both FUT1 and FUT2 KO animals at ~35, 32, 27, and 25 kDa (Figure A3.2). These data suggest that FUT1 and FUT2 either do not regulate or play redundant functions in the synthesis of the majority of Fucα(1-2)Gal glycoproteins recognized by this antibody.

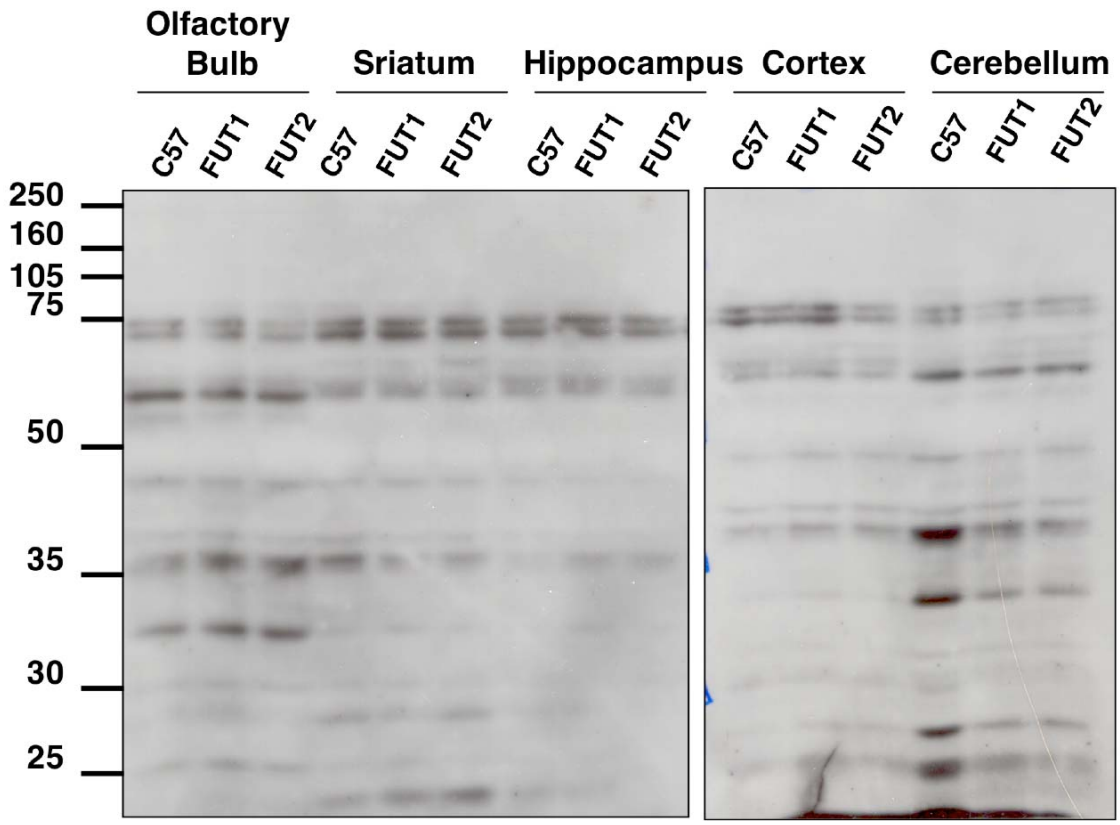

Figure A1.2. Neither FUT1 nor FUT2 regulates the fucose proteome recognized by antibody A46-B/B10. Adult mouse brain fractions from C57BL/6, FUT1, or FUT2 were homogenized, separated by SDS-PAGE, and analyzed by immunoblotting with Fucα(1-2)Gal-specific antibody A46-B/B10. Neither FUT1 nor FUT2 was found to regulated the majority of glycoproteins, except for several low molecular weight glycoproteins in the cerebellum.

In contrast, we observed a significant loss of staining of Fucα(1-2)Gal glycoproteins from the olfactory bulb of transgenic FUT1 knockout mice (Figure A1.3). In addition, UEAI lectin largely recognized a different subset of glycoproteins than antibody A46-B/B10. The majority of fucosylated glycoproteins were between 50 and over 250 kDa. Interestingly, in FUT2 KO mice, expression of Fucα(1-2)Gal glycoproteins is upregulated, suggesting potential cross-talk between FUT1 and FUT2 regulation. In the cerebellum, we observed a decrease in fucosylation of glycoproteins at ~30, 28, 25, and 24 kDa in both FUT1 and FUT2 KO animals. These proteins are similar

in molecular weights to the proteins observed from antibody A46-B/B10, suggesting that they may be the same glycoproteins. In addition, we only observed significant levels of

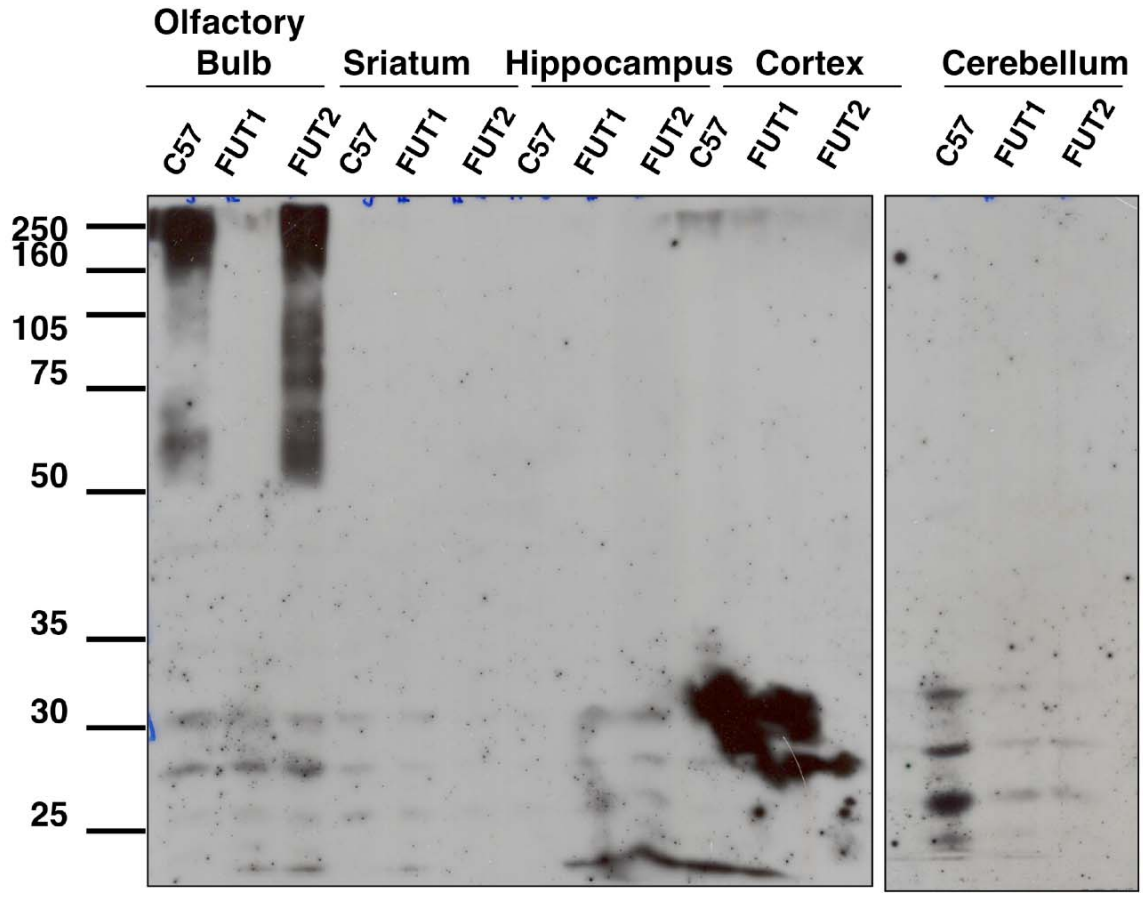

Figure A1.3. The adult mouse olfactory bulb is enriched in expression of Fucα(1-2)Gal disaccharides and the proteome is regulated by FUT1. In the cerebellum, there is reduced expression of fucose on low molecular weight glycoproteins in both FUT1 and FUT2 KO animals.

Fucα(1-2)Gal glycoproteins in the olfactory bulb of adult mouse brain, suggesting that this brain region is significantly enriched in this plasticity-relevant epitope and is discussed in Chapter 4.

We also examined darker exposures to see if we could detect Fucα(1-2)Gal glycoproteins from other brain regions in C57BL/6 and FUT2 transgenic KO animals.

While Fucα(1-2)Gal glycoproteins were highly over exposed from the olfactory bulb, we observed that the hippocampus is the next brain region with enriched expression of Fucα(1-2)Gal. Furthermore, expression of Fucα(1-2)Gal was completely ablated in FUT2 knockout mice (Figure A1.4). However, we are still in the process of repeating these experiments with FUT1 KO mice to determine the role of FUT1 in synthesis of fucosyl glycoproteins in the hippocampus.

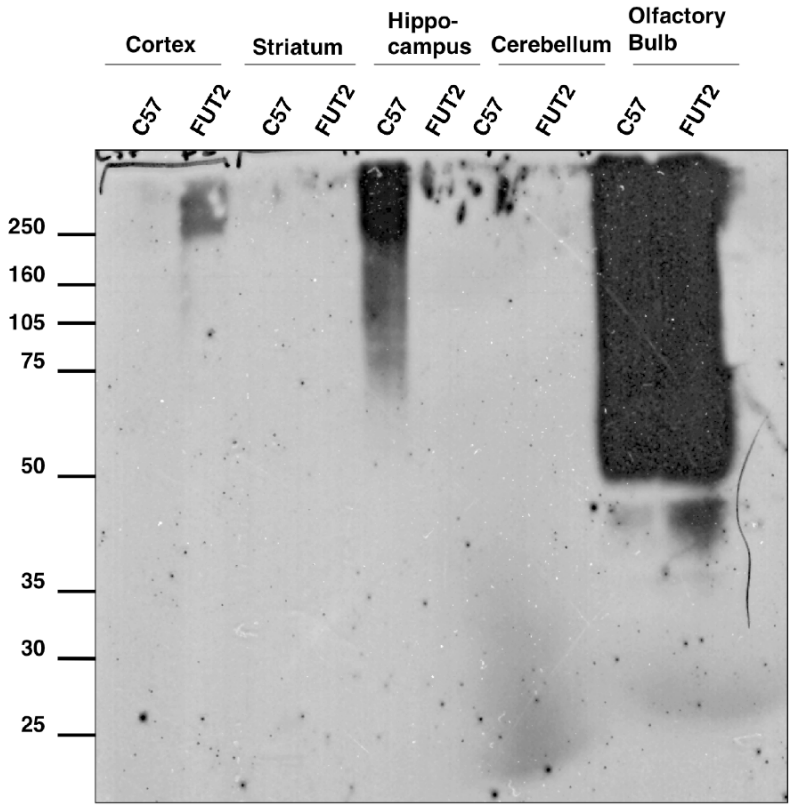

Figure A1.4. The Fucα(1-2)Gal proteome is regulated by FUT2 in the hippocampus of adult mouse brain. Blots were probed with UEAI.

We also examined expression and localization of Fucα(1-2)Gal glycoproteins in olfactory bulb and hippocampal slices of adult mouse brain. We observed staining of the

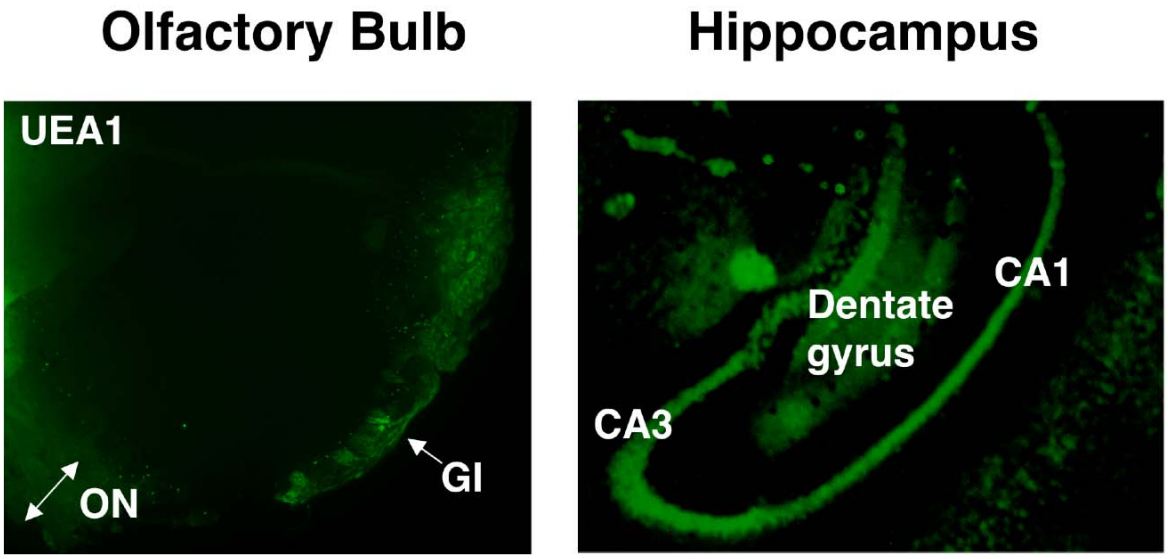

Figure A1.5. Immunohistochemistry of adult mouse brain slices from the olfactory bulb and hippocampus. Sections were labeled with UEA1 conjugated to fluorescein. In the olfactory bulb (left panel), there was strong labeling of the olfactory nerve layer (ON) and glomerular layer (Gl). In the hippocampus, Fucα(1-2)Gal glycoconjugates are present in the CA1, CA3, and dentate gyrus regions.

olfactory nerve layer and the glomerular layers in the olfactory bulb of C57BL/6 animals (Figure A1.5, left panel). In the adult hippocampus, we observe staining in the CA1, CA3, and dentate gyrus, consistent with a role for Fucα(1-2)Gal in learning and memory formation (Figure A1.5, right panel).

Discussion

FUT1 and FUT2 transgenic knockout mice have been analyzed in blastocyst adhesion and the gastrointestinal system. Here, we examined the roles of FUT1 and

FUT2 in the brain. While Fucα(1-2)Gal carbohydrates have been implicated in neurite outgrowth pathways, we did not observe any defects in neurite length of cultured hippocampal neurons. This may suggest that knockout of both Fucα(1-2)Gal-synthesizing enzymes is necessary to observe any effects on neurite outgrowth. Furthermore, the neurite outgrowth pathways mediated by Fucα(1-2)Gal may be presented on glia or secreted proteins in the extracellular matrix, in which case hippocampal neuronal culture experiments may not reveal the effects of neurite outgrowth. In addition, there may exist as yet unidentified fucosyltransferases that are responsible for the growth promoting effects of Fucα(1-2)Gal disaccharides.

The mouse olfactory bulb was found to be the brain region with the highest expression of Fucα(1-2)Gal, followed next by the hippocampus, two brain regions that retain a high degree of plasticity in the adult animal. Interestingly, FUT1 regulates the Fucα(1-2)Gal glycoproteome in both adult and neonatal mice whereas FUT2 has no effect, suggesting that FUT1 is the predominant enzyme for the synthesis of Fucα(1-2)Gal in the olfactory bulb. We also observed a loss of expression of Fucα(1-2)Gal in the hippocampus of FUT2 transgenic mice, but we were unable to determine whether FUT1 also plays a role in this brain region. Our data suggest that in some tissues, only one enzyme can be active, whereas in other tissues, FUT1 and FUT2 may compensate or have redundant functions such as in blastocyst adhesion.

Materials and Methods

See Chapter 4.