Chapter 4: Investigation of Fucosylation in the Olfactory Bulb of Wild-Type and FUT1 Transgenic Knockout Mice

Background

α(1-2)Fucosyltransferases (GDP-L-fucose:*β*-D-galactosyl-R 2-α-L-fucosyltransferase) catalyze the transfer of α-L-Fuc to the C2 position of terminal β-D-galactose residues in glycoconjugates. The Fucα(1-2)Gal epitope is important for the synthesis of the ABO blood group antigens, microbe adhesion, morphogenesis, and metastasis of cancerous cells.[1, 2] The carbohydrate structure is synthesized by two known catalytically active enzymes called FUT1 (*H* gene) and FUT2 (secretor or *Se* gene). The *SEC1* pseudogene is homologous to FUT2 but is catalytically inactive due to a frameshift mutation.[3] Substrate specificity and specific activity differs between FUT1 and FUT2 towards different oligosaccharide acceptor substrates.

FUT1 and FUT2 transgenic knockout animals have been created by homologous recombination using a targeting vector that inserts the *E. coli* β-galactosidase coding sequence in frame proximal to the transmembrane coding segment.[4] The resultant transgenic mice exhibit no gross anatomical or behavioral abnormalities. Fucα(1-2)Gal has been implicated in blastocyst adhesion required for murine implantation. However, deletion of the FUT1 and FUT2 loci appeared to be non-essential to blastocyst-uterine epithelial interactions and exhibit normal fertility. These knockout animals have also been examined in formation of glycolipids in the epithelial tissues of the gastrointestinal tract, where they act as receptors for bacteria and are implicated in the immune response.[5]

Interestingly, targeted deletion of either gene had no effect on loss of Fucα(1-2)Gal glycolipids, which suggests either compensation or redundancy of the α(1-2)fucosyltransferase genes in these tissues. Thus, FUT1 and FUT2 genes may act individually in specific tissues, retain a redundant function, or have compensatory mechanisms when the other gene is knocked out.

As previously discussed in Chapters 1 through 3, Fucα(1-2)Gal glycoproteins have been implicated in cognitive processes such as learning, memory and development. Thus, we chose to investigate the roles fucosylation mediated by FUT1 and FUT2 in the brain. Recent reports suggest that Fucα(1-2)Gal glycoconjugates are expressed in the developing and adult olfactory bulb and may be part of a "glycocode" that helps direct axonal pathfinding of olfactory sensory neurons (OSNs).[6-9] The glycocode hypothesis suggests that differential expression of glycan structures may act as chemotactic agents to help axonal targeting of OSNs to discrete areas of the olfactory bulb. This would provide a largely uncharacterized mechanism for OSN pathfinding. OSNs each express one of over 1000 different odorant receptors, whose axons connect onto topographically fixed positions in the olfactory bulb.[10-12] While the cell bodies of the OSNs are arranged in a mosaic distribution within one of four zones in the mammalian olfactory epithelium, their axons converge onto one to three glomeruli for neurons expressing a single type of odorant receptor.[11-16] The sorting of these axons appears to be controlled by the coordinated spatial and temporal expression of cell adhesion molecules such as the neural cell adhesion molecule (NCAM) and their cognate guidance receptors. However, these molecules alone are not sufficient for the highly specific topographical arrangement of OSNs and glomeruli in the olfactory bulb. Different glycan structures are also expressed

in a selective spatiotemporal arrangement, suggesting their potential involvement in axonal pathfinding of OSNs.

Previous work using lectin immunohistochemistry for Fucα(1-2)Gal, Gal, galactosamine (GalNAc), and *N*-acetylglucosamine (GlcNAc) reveals a distinct labeling of OSNs for each glycan that is spatially and temporally regulated in the vomeronasal system and accessory olfactory bulb of mice.[17, 18] In addition, Fucα(1-2)Gal and GalNAc glycoconjugates display distinct patterns of expression in OSNs of the adult olfactory bulb, consistent with the notion that a glycocode contributes to the complex topographical arrangement of OSNs in mammalian olfactory bulb.[8] A genetic knockout of FUT1[4, 19, 20] leads to impaired development of the olfactory nerve and glomerular layers of the olfactory bulb in transgenic knockout animals,[7] suggesting an important role for fucosyl-oligosaccharides in regulating proper olfactory bulb development. However, despite the intriguing possibility for the existence of a glycocode, the molecular mechanisms and proteins modified by Fucα(1-2)Gal disaccharides and other glycans were previously uncharacterized (see Chapter 3 of this thesis).

Here, we examine regulation of Fucα(1-2)Gal glycoproteins by FUT1 and FUT2 transgenic knockout animals. We demonstrate that fucosylation of these proteins is synthesized by FUT1 in the olfactory bulb. FUT1 mice exhibit developmental defects in the olfactory nerve and glomerular layers expressing the Fucα(1-2)Gal glycoproteins NCAM (neural cell adhesion molecule) and OCAM (olfactory cell adhesion molecule). Fucα(1-2)Gal glycoproteins are localized to the olfactory nerve and glomerular layers of wild-type mice, where they are expressed in all aspects of the main olfactory bulb (MOB). This staining is completely absent in FUT1 knockout (KO) mice but present in

FUT2 KO mice. In the accessory olfactory bulb (AOB), Fucα(1-2)Gal expression is localized to a distinct region that colocalizes with NCAM. The spatiotemporal expression of Fucα(1-2)Gal is consistent with the existence of a glycocode in the developing olfactory bulb. These data suggest that fucosylation of multiple proteins plays an important role in regulating olfactory bulb development and may be part of a glycocode regulating OSN targeting.

Results

Regulation of the Fucα(1-2)Gal proteome by FUT1

We first examined the expression of Fucα(1-2)Gal on glycoproteins in the olfactory bulb of wild-type C57/BL6. We identified different patterns of expression for Fucα(1-2)Gal glycoproteins from adult and the developing olfactory bulb (Figure 4.1A, lanes 1 and 4). In adult animals, we observe a smear of glycoproteins recognized by UEAI lectin between 160 and over 250 kDa, as well as prominent bands at ~ 25, 28, and 30 kDa. In the developing postnatal day 3 (P3) olfactory bulb, we detect strong of labeling Fucα(1-2)Gal on glycoproteins between 50 and over 250 kDa, as well as ~ 24, 25, 28, 30, 32, and 45 kDa. In addition, expression of the Fucα(1-2)Gal epitope is significantly upregulated in the developing P3 olfactory bulb, as we observed significantly more labeling with UEAI lectin (Figure 4.1A, lanes 1 and 4).

We also examined expression of Fucα(1-2)Gal on glycoproteins from FUT1 and FUT2 transgenic knockout mice. Deletion of the FUT1 gene led to a significant reduction in detection of Fucα(1-2)Gal glycoproteins from both adult and the developing olfactory bulb (Figure 4.1A). Surprisingly, deletion of the FUT2 gene appeared to have

little effect on protein fucosylation in the olfactory bulb, suggesting that FUT1 is the active enzyme for synthesis of Fucα(1-2)Gal glycoproteins in both adult and neonatal mouse tissues. All proteins below 50 kDa as well as proteins at 70 and ~120 kDa in both P3 and adult mice showed little to no change in protein fucosylation from either FUT1 or FUT2 KO mice. Thus, fucosylation appears to be regulated primarily by the FUT1 enzyme in the developing and mature olfactory bulb.

Since FUT1 was found to regulate the majority of Fucα(1-2)Gal expression in

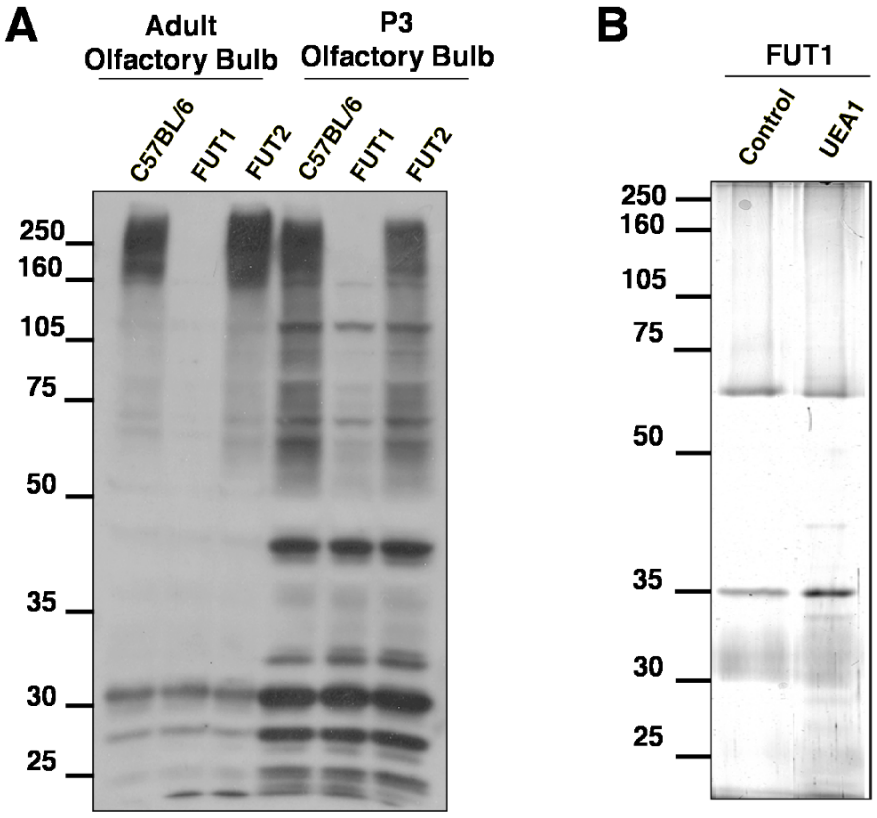

Figure 4.1. FUT1 regulates expression of Fucα(1-2)Gal in adult and neonatal mouse olfactory bulb. (A) Western blot with UEAI lectin of the olfactory bulb from C57BL/6, FUT1 and FUT2 mice. (B) Silver stain of glycoproteins isolated by UEAI lectin affinity chromatography from P3 mouse olfactory bulb. Proteins in (B) were excised, digested with trypsin, and subjected to LC-MS^2 analysis for identification.

this brain region, we sought to identify the subset of glycoproteins specifically regulated by the FUT1 enzyme. Using our lectin affinity chromatography approach (see Chapter 3), we isolated and identified proteins from FUT1 olfactory bulb lysates. Consistent with the observation that FUT1 regulates expression of the majority of Fucα(1-2)Gal glycoproteins, very few proteins were isolated from the UEAI lectin affinity chromatography in FUT1 mice (Figure 4.1B). Of the proteins that were purified from FUT1 olfactory bulb, most were under 50 KDa. This is consistent with the observation that fucosylated proteins at these molecular weights are unaffected by deletion of the FUT1 gene (Figure 4.1A). To determine protein identities, 33 bands were excised from each lane in the gel, and processed as described in Chapter 3. We implemented the same search criteria and stringent peptide cut-off of 7 peptides. Using these criteria, we identified essentially no specific proteins from FUT1 olfactory bulb (data not shown). In Chapter 3, we identified four major classes of Fucα(1-2)Gal glycoproteins from mouse olfactory bulb: the cell adhesion molecules, ion channels and solute transporters/carriers, ATP-binding proteins, and synaptic vesicle-associated proteins. Notably, none of the proteins identified from FUT1 animals was identified in the proteome from wild-type C57BL/6 mice (see Chapter 3, Table 3.1 and data not shown). This provides strong validation for identification of the Fucα(1-2)Gal proteome in Chapter 3, and is consistent with regulation of the proteome by FUT1 in mouse olfactory bulb.

Mapping of Fucα(1-2)Gal Staining in the MOB and AOB: Evidence for a Glycocode

The glycocode hypothesis suggests that glycans should be expressed in regulated spatiotemporal patterns. Thus, we mapped Fucα(1-2)Gal expression in the developing olfactory bulb and compared it to staining of adult animals. Sections were labeled with UEA1 and examined by confocal fluorescence microscopy. We observe labeling of both the glomerular layer and olfactory nerve layer (ONL) in the developing mouse olfactory bulb, the same areas labeled in adult animals. While Fucα(1-2)Gal was found to be present only on a subset of OSN axons in adult brain,[8] we observed extensive labeling of the ONL and greater numbers of glomeruli on all aspects of the developing olfactory bulb. In addition, strong UEAI labeling of the medial, lateral, and ventral faces was observed from the anterior to posterior regions of the MOB. While strong labeling of the dorsal-lateral OSNs and glomeruli was observed in the anterior region, labeling became weaker to non-existent in the posterior region (Figure 4.2). Furthermore, UEAI-positive glomeruli displayed variations in staining intensity, ranging from strong labeling especially of glomeruli in the medioventral face, to weaker or no labeling of glomeruli in the dorsal-lateral face of the olfactory bulb (Figure 4.2). Lastly, Fucα(1-2)Gal glycoconjugates were highly localized to the AOB, a structure involved in secondary detection of a particular class of chemical signals involved in regulating sexual behaviors and detecting pheromones.[21, 22] We also examined the labeling of UEAI in the olfactory bulb of FUT1 P3 mouse pups. Consistent with our observation that FUT1 regulates the murine Fucα(1-2)Gal proteome in the olfactory bulb, we observed no labeling of the

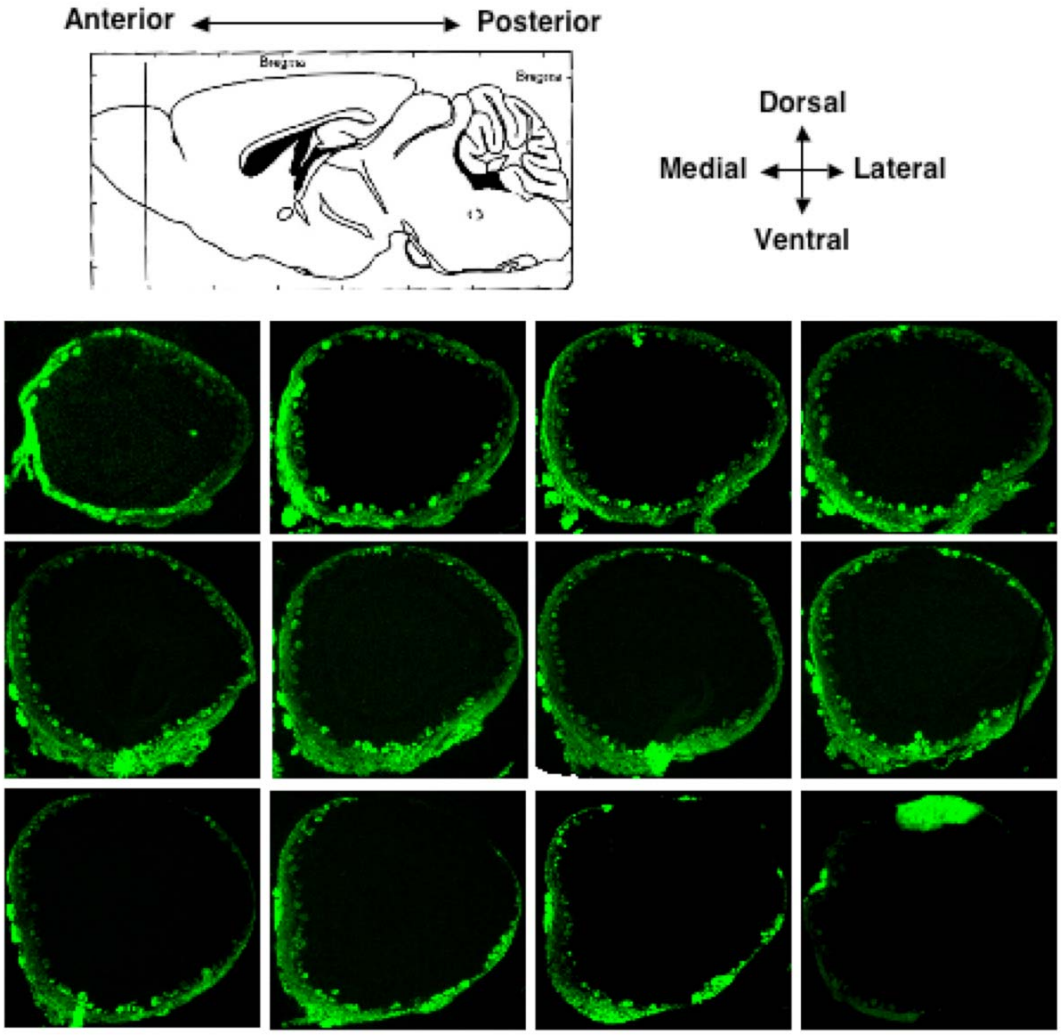

Figure 4.2. UEAI immunohistochemistry of coronal olfactory bulb sections from postnatal mice. Slices are from the anterior (upper left) to posterior (bottom right) of the olfactory bub. There is trong labeling of the OLN and the glomerular layers in all aspects of the MOB, with the strongest labeling at the medioventral face. There is also strong labeling of the AOB (bottom right panel).

glomerular or ONL in either the MOB or AOB in FUT1 transgenic KO mice (data not shown). Together, these studies demonstrate that Fucα(1-2)Gal glycoconjugates are spatiotemporally regulated, and these expression patterns are consistent with the notion of a glycocode for axonal targeting of OSNs.

Colocalization of Fucα(1-2)Gal Glycoproteins NCAM and OCAM with UEA1 in the Developing Mouse Olfactory Bulb

We next examined the colocalization of two Fucα(1-2)Gal glycoproteins identified in Chapter 3 with UEAI in the developing mouse olfactory bulb. We chose to investigate the cell adhesion molecules NCAM (neural cell adhesion molecule) and OCAM (olfactory cell adhesion molecule), which were identified as abundant fucosylated proteins from the largest class of Fucα(1-2)Gal glycoproteins (Table 3.1). NCAM and OCAM have both previously been shown to label the olfactory nerve and glomerular layers of mouse olfactory bulb. NCAM is ubiquitously expressed throughout these layers, while OCAM expression is localized to the ventral surface. Thus, we examined their colocalization with UEAI to determine the extent of fucosylation of these proteins and their localization in the developing olfactory bulb.

We first examined colocalization between NCAM and OCAM in the developing MOB. NCAM was only partially colocalized with UEAI in the glomerular and olfactory nerve layer of the MOB, consistent with the observation that many other Fucα(1-2)Gal glycoproteins are present in mammalian olfactory bulb (Figure 4.3). There were many regions where NCAM staining did not colocalize with UEAI, suggesting that NCAM may be differentially fucosylated. NCAM and UEAI overlap was most prevalent in the ventromedial aspect of the developing olfactory bulb from both anterior to posterior regions (data not shown). As expected, OCAM labeling was also partially colocalized with UEAI in the MOB. However, while most OCAM-positive glomeruli were stained by UEAI, there was little colocalization in the anterior portion of the olfactory nerve layer. (Figure 4.4A and B). In the mid-posterior region of the olfactory bulb, there was

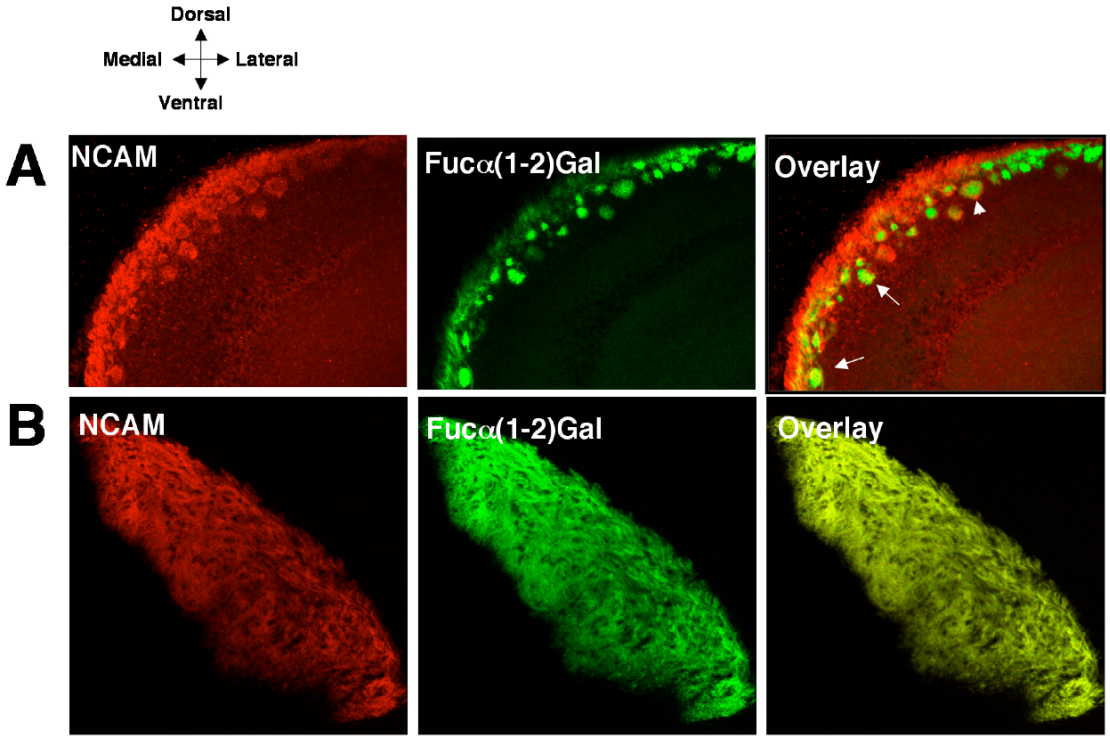

Figure 4.3. Confocal images of coronal olfactory bulb slices. (A) Immunohistochemical analysis demonstrates that NCAM (red) colocalizes with some glomeruli and a portion of the olfactory nerve layer stained by UEAI (green) in the MOB. White arrows point to glomeruli that overlay strongly with UEAI. White arrowhead points to glomerulus with NCAM staining on the outside and UEAI labeling on the inside, demonstrating the diversity in labeling of glomeruli. (B) In the AOB, NCAM is extensively colocalized with UEAI in the olfactory bulb.

significant overly between OCAM and UEAI in the olfactory nerve layer (Figure 4.4B). These data suggest that both NCAM and OCAM are likely differentially fucosylated in discrete areas of the MOB and the extent of colocalization is consistent with proteomic identification of these proteins as Fucα(1-2)Gal glycoproteins.

While both NCAM and OCAM colocalize with UEAI in the MOB, only NCAM colocalizes well with UEAI in the AOB (Figures 4.3B and 4.4C), suggesting that OSNs from both the olfactory epithelium and vomeronasal organ are labeled with fucosylated NCAM. Furthermore, lack of significant colocalization of OCAM OSNs from the

vomeronasal organ with UEAI suggests that OCAM is not a primary Fucα(1-2)Gal glycoprotein present on these neurons (Figure 4.4C). Cumulatively, these data suggest that both NCAM and OCAM are differentially fucosylated in the MOB and AOB, consistent with the notion of a glycocode involving these proteins, as fucosylation at this stage of development is spatially distinct on different Fucα(1-2)Gal glycoproteins.

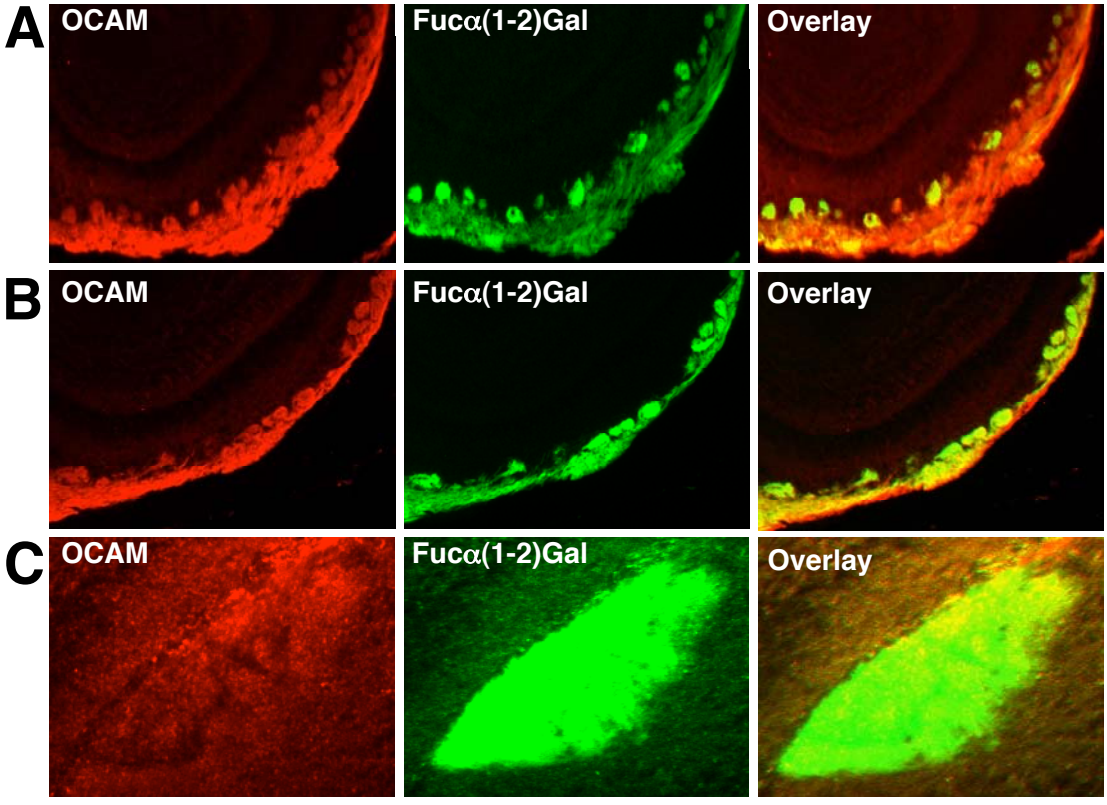

Figure 4.4. Colocalization of OCAM and UEAI in the developing mouse olfactory bulb. Fluorescence microscopy of OCAM (red) colocalizes with UEAI labeling (green) in the ONL and glomerular layers of the anterior MOB (A) and posterior MOB (B). (C) Overlay of OCAM (red) and UEAI (green) from sagittal sections of the AOB.

Development of the Olfactory Nerve Layer and Glomerular Layer is Defective in the Olfactory Bulb of FUT1 KO Mice in Areas Expressing the Fucα(1-2)Gal Glyoproteins NCAM and OCAM

Having identified the Fucα(1-2)Gal proteome and its regulation by FUT1, we next explored olfactory bulb development in areas expressing two Fucα(1-2)Gal glycoproteins, NCAM and OCAM, in FUT1-deficient mice. The olfactory nerve layer (ONL) and glomerular layers of FUT1 postnatal mice are reported to be thinner, with less large glomeruli than wild-type mice.[7] We examined whether NCAM- and OCAM-expressing OSNs display developmental defects in FUT1 transgenic knockout animals. Olfactory bulbs from wild-type (WT) and FUT1-deficient mice were fixed, cryogenically sliced in coronal sections, stained with either NCAM or OCAM antibodies, and imaged by confocal fluorescence microscopy. We observed moderate defects in the thickness of the ONL and severe defects in the number and demarcation of glomeruli for NCAM-expressing regions of the olfactory bulb (Figure 4.5A). These deficits were most pronounced in the ventromedial surface of the posterior MOB, consistent with areas containing high expression of the Fucα(1-2)Gal epitope (Figure 4.2). We did not observe any obvious defects in development of NCAM-expressing OSNs in the AOB of FUT1 KO animals (data not shown). While the ONL of NCAM-expressing areas exhibited a decrease in thickness, we did not observe any significant defects in the thickness of the ONL of OCAM-expressing OSNs (Figure 4.5B). However, there was also a significant decrease of large glomeruli expressing OCAM in both the anterior and posterior ventral MOB (Figure 4.5B). The localization and defects in NCAM- and OCAM-positive glomeruli suggest fucosylation may be important for regulating the function of these

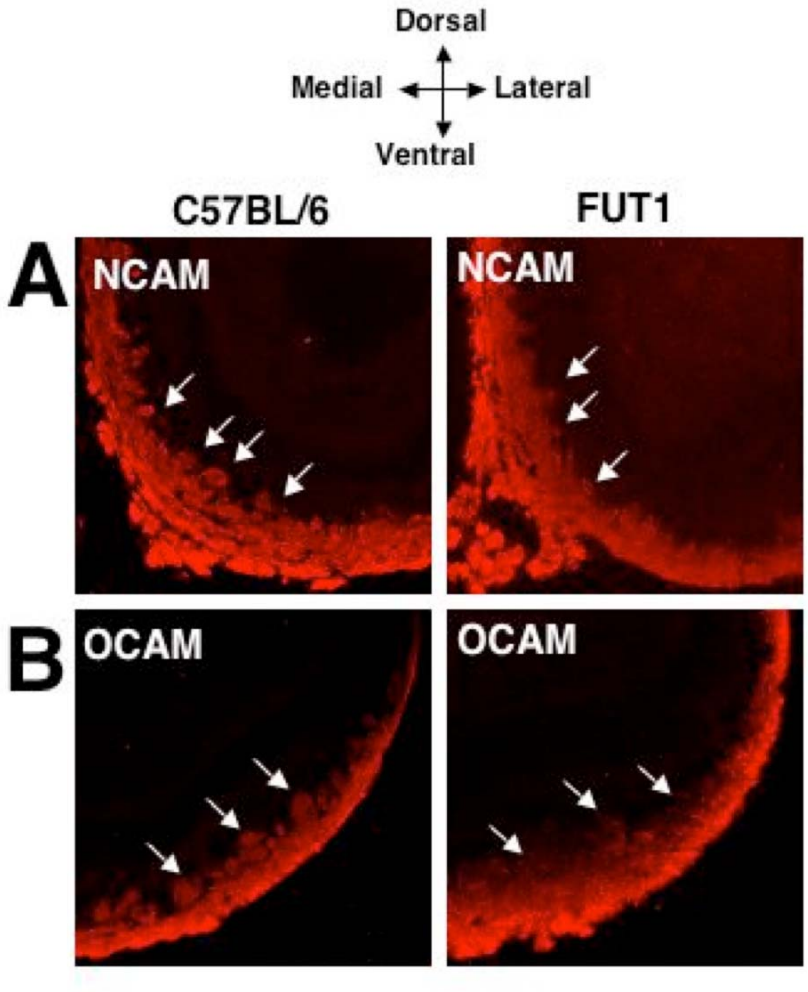

Figure 4.5. Confocal fluorescence microscopy of coronal sections of the developing WT and FUT1 KO MOB. (A) NCAM (red) expressing OSNs exhibit defects in the thickness of the ONL and in the demarcation and number of large glomeruli from FUT1 KO animals (white arrows). (B) OCAM expressing OSNs exhibit a similar thickness in the ONL. However, they also have severe defects in the presence of large glomeruli in FUT1 KO mice (white arrows).

proteins. Both proteins play functional roles in olfactory bulb development and these data suggest that fucosylation of these proteins may also be important.

Discussion

We previously identified the Fucα(1-2)Gal proteome from mouse olfactory bulb (Chapter 3) and demonstrate here that FUT1 regulates expression of the proteome in mouse olfactory bulb. These results validate identification of the proteome in Chapter 3 and suggest that FUT1 is the dominant enzyme for expression of Fucα(1-2)Gal in this tissue. The cell adhesion molecules NCAM and OCAM are important for olfactory bulb development, and their fucosylation is regulated by FUT1. We further explored fucosylation of these proteins by examining their colocalization with UEAI in the developing olfactory bulb. Both proteins were found to colocalize with Fucα(1-2)Gal to varying degrees in the MOB and AOB. NCAM expressing OSNs had a high degree of colocalization in the medioventral aspect of the olfactory bulb in both the olfactory nerve layer and a subset of glomeruli. OCAM expressing glomeruli strongly colocalized with UEAI staining in the lateroventral face of the olfactory bulb. The lack of complete localization of NCAM and OCAM with UEAI in the MOB suggests that other glycoproteins contain the Fucα(1-2)Gal moiety, consistent with our proteomics studies. In the AOB, NCAM and UEAI staining were highly colocalized, suggesting that NCAM is the predominant Fucα(1-2)Gal glycoprotein present in the AOB.

We observed a modest defect in the ONL and glomerular layers expressing NCAM and OCAM in FUT1 neonatal mice. These mice exhibit significantly less glomeruli expressing OCAM and NCAM, especially in the medioventral area of the olfactory bulb. In addition, we observed a thinner ONL for OSNs expressing NCAM. Such defects in OSNs stained by these fucosylated cell adhesion molecules suggests that the Fucα(1-2)Gal carbohydrate epitope of both NCAM and OCAM is important for proper olfactory bulb development. Previous studies have suggested that only NCAM

fucosylation is important is important in olfactory bulb development, as NCAM was the only known Fucα(1-2)Gal glycoprotein.[7] However, an actual investigation of NCAM fucosylation had been lacking. Our studies expand on what was previously hypothesized, and demonstrate that NCAM and OCAM, as well as other Fucα(1-2)Gal glycoproteins, are important for proper development of the olfactory bulb. Interestingly, NCAM-180 knockout mice are reported to display similar defects in glomerular formation as FUT1 deficient mice.[23] While the olfactory bulb of these mutants appears relatively normal, there are fewer and smaller glomeruli in NCAM-180 KO mice when compared with wild-type mice. This indicates that NCAM-180 is essential for the proper development of glomeruli in the olfactory bulb. This is consistent with a role for NCAM fucosylation in mediating proper olfactory bulb development.

The developmental abnormalities in the nerve fiber layer and glomerular formation appear to recover in adult FUT1 knockouts.[7] This may suggest that other targeting mechanisms can take over to ensure proper olfactory bulb development. While there are no obvious defects in the topology of adult murine olfactory bulb, it is still possible that some OSNs may fail to make the proper connections to appropriate glomeruli.

Our data also lends support to the existence of a glycocode for OSN pathfinding. While UEAI labeling was present throughout the ONL and glomerular layers of the MOB in the developing olfactory bulb, we observed a differential fucosylation of both NCAM and OCAM, suggesting that fucosylation may help mark OSNs to target to appropriate glomeruli. This spatiotemporal fucosylation is consistent with the glycocode hypothesis. UEA1 labeling was strongest in the medioventral area of the olfactory bulb, where the

largest defect in OSNs and glomerular formation occurred. This suggests that the Fucα(1-2)Gal epitope may be extremely important for development of this region, whereas areas of weaker UEAI staining may depend on another glycan structure for proper development. Cumulatively, our studies suggest that Fucα(1-2)Gal labeling displays a unique spatiotemporal expression consistent with a glycocode for OSN targeting.

Materials and Methods

Animals, Tissue Isolation and Homogenization.

C57BL/6 wild-type, FUT1 and FUT2 transgenic knockout animals crossed to a C57BL/6 background were a gift from Prof. Stephen Domino and maintained in accordance with proper IACUC procedures. Adult male mice ages 3-4 months and post-natal day 3 (P3) pups were anesthetized with CO_2 and dissected to remove the cerebellum, cortex, hippocampus, hypothalamus, olfactory bulb, striatum, and thalamus. For Western blotting, dissected tissues were cut into small pieces and placed immediately on ice, then lysed in boiling 1% SDS with sonication until homogeneous (5V:W). For lectin affinity chromatography, the olfactory bulbs from 30-50 P3 pups were isolated and homogenized in lectin binding buffer (100 mM Tris pH 7.5/ 150 mM NaCl/ 1mM $CaCl_2$/ 1 mM $MgCl_2$/ 0.5% NP-40/ 0.2% Na deoxycholate plus protease inhibitors) by passing through a 26G needle 5 times, then sonicated to homogeneity. Samples were clarified by centrifugation at 12,000g × 10 min. Lysates were between 6 to 10 mg/mL total protein concentration as determined by the BCA protein assay (Pierce) for lectin affinity chromatography.

Lectin Affinity Chromatography and SDS-PAGE

One mL bed volume of *Ulex europaeus* agglutinin I (UEAI) conjugated to agarose (Vector Labs) and control protein A conjugated to agarose (Vector Labs) columns were packed ~333 μL into 3 minicolumns run in parallel (BioRad). The resin was equilibrated with 10 column volumes (CV) lectin binding buffer. 3 mL of olfactory bulb lysate at 6-10 mg/mL was bound in batch at RT for 4 hours. Columns were repacked and the flowthrough was passed 3 additional times over the column. Columns were washed with 40 CV of lectin binding buffer, followed by 10 CV of lectin binding buffer lacking detergent (NP-40 and Na deoxycholate). Proteins were eluted in 10 CV of lectin binding buffer lacking detergent supplemented with 200 mM α-L-Fuc and protease inhibitors.

Protein eluates were concentrated in 10,000 MWCO centricons (Millipore) followed by 10,000 MWCO microcons (Millipore) to 100 μL. Following concentration, samples were boiled with 35 μL of 4× SDS loading dye and loaded onto 10% SDS gels for electrophoresis as described previously.[24]

Silver Staining, Peptide Extraction, and In-Gel Tryptic Digests

All silver staining reagents were prepared fresh. Gels were stained as described in Chapter 3. Gels were destained in 0.4 g $K_3Fe(CN)_6$/ 200 mL $Na_2S_2O_3 \bullet 5H_2O$ (0.2g/L) for 15 min or until no bands were visible then washed 6 × 15 min in ddH_2O overnight. Gel pieces were excised and reduced in 150 μL of 8 mM TCEP in 80 mM ammonium bicarbonate buffer, pH 7.8 / 150 μL CH_3CN. Gel pieces were reduced for 20 min at RT.

The solution was discarded and cysteines were alkylated in 150 μL of 10 mM iodoacetamide in 80 mM ammonium bicarbonate buffer, pH 7.8 / 150 μL CH_3CN. Reactions were incubated in the dark for 20 min at RT. The supernatant was discarded and gel pieces were rehydrated in 500 μL of 50 mM ammonium bicarbonate for 10 min at RT. The supernatanat was removed and gel pieces were concentrated in a speed vac for 15 min. Gel pieces were resuspended in 40 μL H_2O/ 5 μL 500 mM ammonium bicarbonate, pH 7.8/ and 5 μL of 0.2 mg/mL trypsin (promega), and left on ice for 30 min. Tubes were then incubated overnight at 37 °C. The following day, excess trypsin solution not absorbed was removed and saved in a new tube. Gel pieces were washed with 500 μL of H_2O by vortexing for 20 min. The solution was removed and combined with the tyrptic digests. Peptides were extracted at 2 × 200 μL of 5% formic acid/ 50% CH_3CN by vortexing for 20 min and combined with the tryptic and wash fractions. Samples were concentrated in a speed vac down to 20 μL for MS analysis.

Orbitrap LC-MS Analysis

Approximately 50% of gel extractions were loaded onto a 360 μm O.D. X 75 μm precolumn packed with 4 cm of 5 μm Monitor C18 particles (Column Engineering) as described previously.[25]

Western Blotting

10% SDS gels were transferred to PVDF, blocked in 3% HIO_4^- BSA,[26] and incubated with HRP-conjugated UEAI (Sigma) at 50 μg/mL in TBST for 2 h at RT or

antibody A46-B/B10 at 5 μg/mL. Membranes were washed 3 × 10 min in TBST, then developed as described previously.[26]

Immunohistochemistry

Adult mice (3-4 months) were anesthetized with sodium pentobarbital (100 mg/kg**)** and then fixed by transcardiac perfusion with PBS (pH 7.4), followed by a 4% paraformaldehyde in PBS solution. Brain tissue was removed, and immersion fixed overnight at 4 ºC in the same solution. P3 mouse pups were killed by decapitation, the brain was removed and immersion fixed overnight in 4% paraformaldehyde in PBS at 4 ºC. The following day, the solution was replaced with an ice-cold solution of 15% sucrose in PBS at 4 ºC until the brains sunk, followed by 30% sucrose in PBS. The brain tissue was mounted in OCT medium (Tissue Tek), and frozen in a dry ice/ MeOH bath. Frozen brains were stored at -80 ºC until processed for sectioning. Fixed tissues were cryogenically sliced on a Leica, CM1800 cryostat in coronal sections, 20 μm thick sections for P3 pups and 50 μm thick for adult tissues. Sections were dried at 37 ºC for 20 min, then blocked in 10% donkey serum/ 0.3% Triton X-100 in PBS for OCAM immunohistochemistry and 10% goat serum/ 0.3% Triton X-100 in PBS for NCAM immunohistochemistry for 1 h at RT. Sections were incubated with mouse anti-NCAM (Sigma) diluted 1:100 in 2% donkey serum/ 0.1% Triton X-100 in PBS, or goat anti-OCAM (R&D Systems) diluted 1:100 in 2% goat serum/ 0.1% Triton X-100 in PBS at RT for 2 h. Sections were washed three times in PBS for 10 min and then incubated with a cocktail of Alexa-568-conjugated donkey anti-goat with UEA1 conjugated to fluorescein (Sigma) at 50 μg/mL for 1 h at 37 °C.

References

1. Gorelik, E.; Xu, F.; Henion, T.; Anaraki, F.; Galili, U., Reduction of metastatic properties of BL6 melanoma cells expressing terminal fucose alpha 1-2-galactose after alpha 1,2-fucosyltransferase cDNA transfection. *Cancer Res.* **1997,** 57, (2), 332-336.
2. Bry, L.; Falk, P. G.; Midtvedt, T.; Gordon, J. I., A model of host-microbial interactions in an open mammalian ecosystem. *Science* **1996,** 273, (5280), 1380-1383.
3. Kelly, R. J.; Rouquier, S.; Giorgi, D.; Lennon, G. G.; Lowe, J. B., Sequence and expression of a candidate for the human secretor blood-group alpha(1,2)fucosyltransferase gene (Fut2) - homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. *J. Biol. Chem.* **1995,** 270, (9), 4640-4649.
4. Domino, S. E.; Zhang, L.; Gillespie, P. J.; Saunders, T. L.; Lowe, J. B., Deficiency of reproductive tract alpha(1,2)fucosylated glycans and normal fertility in mice with targeted deletions of the FUT1 or FUT2 alpha(1,2)fucosyltransferase locus. *Mol. Cell. Biol.* **2001,** 21, (24), 8336-8345.
5. Iwamori, M.; Domino, S. E., Tissue-specific loss of fucosylated glycolipids in mice with targeted deletion of alpha(1,2)fucosyltransferase genes. *Biochem. J.* **2004,** 380, 75-81.
6. Chehrehasa, F.; Key, B.; St John, J. A., The cell surface carbohydrate blood group A regulates the selective fasciculation of regenerating accessory olfactory axons. *Brain Res.* **2008,** 1203, 32-38.
7. St John, J. A.; Claxton, C.; Robinson, M. W.; Yamamoto, F.; Domino, S. E.; Key, B., Genetic manipulation of blood group carbohydrates alters development and pathfinding of primary sensory axons of the olfactory systems. *Dev. Biol.* **2006,** 298, (2), 470-484.
8. Lipscomb, B. W.; Treloar, H. B.; Klenoff, J.; Greer, C. A., Cell surface carbohydrates and glomerular targeting of olfactory sensory neuron axons in the mouse. *J. Comp. Neurol.* **2003,** 467, (1), 22-31.
9. St John, J. A.; Key, B., Expression of galectin-1 in the olfactory nerve pathway of rat. *Dev. Brain Res.* **1999,** 117, (2), 171-178.
10. Nagao, H.; Yoshihara, Y.; Mitsui, S.; Fujisawa, H.; Mori, K., Two mirror-image sensory maps with domain organization in the mouse main olfactory bulb. *Neuroreport* **2000,** 11, (13), 3023-3027.
11. Ressler, K. J.; Sullivan, S. L.; Buck, L. B., Information coding in the olfactory system - evidence for a stereotyped and highly organized epitope map in the olfactory-bulb. *Cell* **1994,** 79, (7), 1245-1255.
12. Vassar, R.; Chao, S. K.; Sitcheran, R.; Nunez, J. M.; Vosshall, L. B.; Axel, R., Topographic organization of sensory projections to the olfactory-bulb. *Cell* **1994,** 79, (6), 981-991.
13. Treloar, H. B.; Feinstein, P.; Mombaerts, P.; Greer, C. A., Specificity of glomerular targeting by olfactory sensory axons. *J. Neurosci.* **2002,** 22, (7), 2469-2477.

14. Mombaerts, P.; Wang, F.; Dulac, C.; Chao, S. K.; Nemes, A.; Mendelsohn, M.; Edmondson, J.; Axel, R., Visualizing an olfactory sensory map. *Cell* **1996,** 87, (4), 675-686.
15. Vassar, R.; Ngai, J.; Axel, R., Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* **1993,** 74, (2), 309-318.
16. Ressler, K. J.; Sullivan, S. L.; Buck, L. B., A zonal organization of odorant receptor gene-expression in the olfactory epithelium. *Cell* **1993,** 73, (3), 597-609.
17. Salazar, I.; Quinteiro, P. S., Differential development of binding sites for four lectins in the vomeronasal system of juvenile mouse: from the sensory transduction site to the first relay stage. *Brain Res.* **2003,** 979, (1-2), 15-26.
18. Salazar, I.; Quinteiro, P. S.; Lombardero, M.; Cifuentes, J. M., Histochemical identification of carbohydrate moieties in the accessory olfactory bulb of the mouse using a panel of lectins. *Chem. Senses* **2001,** 26, (6), 645-652.
19. Domino, S. E.; Zhang, L.; Lowe, J. B., Molecular cloning, genomic mapping, and expression of two secretor blood group alpha(1,2)fucosyltransferase genes differentially regulated in mouse uterine epithelium and gastrointestinal tract. *J. Biol. Chem.* **2001,** 276, (26), 23748-23756.
20. Domino, S. E.; Hiraiwa, N.; Lowe, J. B., Molecular cloning, chromosomal assignment and tissue-specific expression of a murine alpha(1,2)fucosyltransferase expressed in thymic and epididymal epithelial cells. *Biochem. J.* **1997,** 327, 105-115.
21. Bigiani, A.; Mucignat-Caretta, C.; Montani, G.; Tirindelli, R., Pheromone reception in mammals. *Rev. Physiol. Biochem. Pharmacol.* **2005**, 154, 1-35.
22. Baxi, K. N.; Dorries, K. M.; Eisthen, H. L., Is the vomeronasal system really specialized for detecting pheromones? *Trends Neurosci.* **2006,** 29, (1), 1-7.
23. Treloar, H.; Tomasiewicz, H.; Magnuson, T.; Key, B., The central pathway of primary olfactory axons is abnormal in mice lacking the N-CAM-180 isoform. *J. Neurobiol.* **1997,** 32, (7), 643-658.
24. Tai, H.-C.; Khidekel, N.; Ficarro, S. B.; Peters, E. C.; Hsieh-Wilson, L. C., Parallel identification of O-GlcNAc-modified proteins from cell lysates. *J. Am. Chem. Soc.* **2004,** 126, 10500-10501.
25. Khidekel, N.; Ficarro, S. B.; Clark, P. M.; Bryan, M. C.; Swaney, D. L.; Rexach, J. E.; Sun, Y. E.; Coon, J. J.; Peters, E. C.; Hsieh-Wilson, L. C., Probing the dynamics of O-GlcNAc glycosylation in the brain using quantitative proteomics. *Nat. Chem. Biol.* **2007,** 3, (6), 339-348.
26. Murrey, H. E.; Gama, C. I.; Kalovidouris, S. A.; Luo, W. I.; Driggers, E. M.; Porton, B.; Hsieh-Wilson, L. C., Protein fucosylation regulates synapsin la/lb expression and neuronal morphology in primary hippocampal neurons. *Proc. Natl. Acad. Sci. USA* **2006,** 103, (1), 21-26.