Title page

Differential fatty acid binding protein expression in persistent radial glia in the human and sheep subventricular zone

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Abstract

Fatty acid binding proteins (FABPs) are a family of transport proteins that facilitate intracellular transport of fatty acids. Despite abundant expression in the brain, the role that FABPs play in the process of cell proliferation and migration in the subventricular zone (SVZ) remains unclear. Our results provide a detailed characterization of FABP3, 5 and 7 expression in adult and fetal human and sheep SVZ. High FABP5 expression was specifically observed in the adult human SVZ and co-labelled with PSA-NCAM, GFAP, GFAPδ and PCNA, indicating a role for FABP5 throughout the full maturation process of astrocytes and neuroblasts. Some FABP5^{+} cells had a radial glial-like appearance and co-labelled with the radial glia markers vimentin (40E-C) and GFAP. In the fetal human brain, FABP5 was expressed by radial glia cells throughout the ventricular zone. In contrast, radial glia-like cells in sheep highly expressed FABP3. Taken together, these differences highlight the species-specific expression profile of FABPs in the SVZ. In this study we demonstrate the distribution of FABP in the adult human SVZ and fetal ventricular zone and reveal its expression on persistent radial glia that may be involved in adult neurogenesis.

Keywords: subventricular zone, radial glial cells, FABP, neurogenesis

Introduction

During brain development, neuronal precursor cells proliferate in the ventricular zone (VZ) of the developing neocortex. These neuroblasts then travel from their site of origin using radial glial fibres that serve as a scaffold to guide cellular placement [1,2]. In the adult mammalian brain neurogenesis persists within restricted areas, including the subventricular zone (SVZ) which borders the lateral ventricles, and the subgranular zone within the dentate gyrus of the hippocampus. In the human SVZ neurogenesis is more abundant than in the subgranular zone and extensive proliferation in the human SVZ remains throughout adulthood [3,4]. In addition, the SVZ retains several embryonic features of primitive germinal layers. The ependymal cells comprising layer I of the SVZ maintain direct contact with the ventricle [5] and neuronal precursors undergo long-distance migration along the rostral migratory stream (RMS) [6].

Although many embryonic radial glia disappear throughout development, a subset of cells continue to proliferate. Several types of radial glia-like cells are known to persist in the mature central nervous system at specific locations, including cerebellar Bergmann glia, retinal Muller cells, and periventricular tanycytes of the hypothalamus (reviewed in [7]). Radial glial cells are defined as bipolar elements orientated orthogonally to the growing tissue. In early development, each radial glial cell has only one apical end-foot at the ventricular surface with a characteristic long radial process reaching the pial surface of the brain. The radial fibres, particularly during the later stages of development, often form several branches that terminate with multiple end-feet [7,8]. Radial glia express a range of characteristic proteins and are commonly identified with antibodies against vimentin (40E-C), nestin (RC2), calcium-binding protein S-100b, fatty acid binding protein 7 (FABP7), the glutamate transporter GLAST, tenascin-C and glial fibrillary acidic protein (GFAP), which is considered a late stage marker [8–12]. These markers are not unique to radial glia cells and are also found in astrocytes. Furthermore, species-specific differences and the developmental stage both influence the expression of these radial glia proteins [13]. Currently the identification of radial glia cells using immunohistochemistry involves a combination of morphological features and the presence of these antigens.

In addition to their role in cell guidance, radial glial cells act as neural stem/progenitor cells during brain development [14,15]. In mice, FABP7-expressing radial glial cells give rise to most of the neurons in the brain [15]. Furthermore, analysis of FABP7 knockout mice revealed dramatic decreases in the number of astrocytes, neural stem cells and early progenitor cells in the developing brain [16]. In addition FABP3, FABP5 and FABP7 are expressed in the developing and/or adult brain in a range of species (bovine [17,18]; chicken [19]; rat [20–23]; mouse [24,25]). Functional studies have revealed a variety of roles for FABPs in brain development including the generation of neuronal and/or glial cells, differentiation, neuronal cell migration and axis patterning [22,23,25]. In particular, the distinct tissue distributions, cellular localizations, binding affinities, and often stage-specific expression patterns of these proteins imply that each individual protein serves a unique role in the cell types in which they are expressed. The implications and relevance of these factors remain poorly studied in human development, with the majority of the research focusing on rodents [20–26]. In FABP5 and FABP7 single knockout mice, proliferation of neural stem/progenitor cells is decreased by approximately 15% compared to wild type mice, and mice deficient for both genes show reduced neural stem/progenitor cell proliferation to 70% that of wild types, likely reflecting additive effects of FABP5 and FABP7 protein functioning during the proliferation phase. Survival of newborn cells is affected only in FABP7 knockout or FABP5/7 double knockout mice, but not in FABP5 knockout mice [26]. FABP5 expression is induced after peripheral nerve injury, suggesting a potential role in neuroregeneration [27]. Less is known about FABP3, which was originally identified as a heart-specific FABP [28]. FABP3 is highly expressed in neurons in the postnatal and mature brain [21].

The present study provides detailed data on the cellular localization of FABPs in both the adult SVZ and fetal VZ in human and sheep. Additionally, this study is the first to present a morphological basis for understanding the role of FABPs in human neurogenesis.

RESULTS

Expression of FABPs in the adult human SVZ

Fatty acid binding proteins were specifically expressed in the human SVZ in a variety of forms. FABP3, 5 and 7^+ puncta were located in the nucleus and cytoplasm of SVZ cells. FABP5 was abundant in the SVZ where it was located on the processes, cytoplasm, and punctate staining in the cell nuclei (Fig. 1A-F). $FABP5^+$ cells in the SVZ were often large with fibres that protruded into the caudate nucleus (CN). The fibres on these $FABP5^+$ cells reached lengths of up to 200 µm, linking the SVZ with the CN. Because these cells were morphologically similar to radial glia cells, we double labelled FABP5 with glia/radial glia markers, nestin, vimentin (40E-C) and GFAP. Specifically, we used vimentin and GFAP as markers to study the distribution of radial glia-like cells. A subset of the $FABP5^+$ cells/fibres with one to two end-feet protruding into the CN expressed vimentin (Fig. 1A-D). Overall FABP5 expression was mostly restricted to the SVZ, with the underlying CN showing little or no $FABP5^+$ cell bodies (although expression level was case dependent).

FABP7 demonstrated nuclear and punctate cytoplasmic expression and was located in the SVZ and CN. The punctate nuclear staining for the different FABPs did not co-label and were mostly localized on the border between euchromatin and heterochromatin (Fig. 1E-H). FABP3 was most abundant in the ependymal layer. In the SVZ, FABP3 was mostly localized in the nucleus (Fig. 1E, H).

Expression of FABPs in the adult sheep SVZ

By way of comparison, we investigated FABP3, 5 and 7 expression in adult sheep. Unlike rodent models, sheep have agyrencephalic brain and SVZ structure is more comparable to humans [4]. However, the expression of FABP3, 5 and 7 in adult sheep was distinctly different to that of the adult human brain (Fig. 1H-O). In the sheep brain, FABP3 was highly expressed in the ependymal layer and lamina II-IV of the SVZ. Cytoplasmic staining was observed for FABP3 throughout the SVZ. FABP3 was also expressed on fibres radiating into the CN. A subset of these fibres co-labelled with vimentin (Fig. 1I-K, yellow arrows). In the CN, cells were observed with clear $FABP3^+$ puncta in the nucleus (Fig. 1L, O).

FABP5 had a similar expression pattern in the SVZ of sheep, including nuclear puncta and labelling of cellular processes. In the CN, however, FABP5 was expressed as a punctate pattern in both the nucleus and cytoplasm. No FABP5 was seen on fibres entering the CN. No difference in fluorescent intensity between SVZ and CN was seen for FABP5 (Fig. 1L, M).

For FABP7, we observed labelling in lamina II, III and IV of the SVZ. Punctate labelling was present in the gap layer of the SVZ. Besides the expression on fibres in the SVZ, FABP7^{+} nuclear puncta were also observed in the SVZ and CN (Fig. 1L-N). The punctate nuclear labelling for the different FABPs did not co-label, and was mostly localized on the border between euchromatin and heterochromatin. Perinuclear FABP spots were frequently observed (Fig. 1M-O, yellow arrows).

FABP expression in fetal human brain tissue

As the adult human FABP5^{+} and sheep FABP3^{+} fibres connecting the SVZ to the CN resembled radial glia (Figure 1), we examined their expression patterns in fetal tissue. Human fetal tissue with an average age of 156 days gestation was used for this analysis. In this tissue the pial surface, ventricular zone (VZ), hippocampus (HP), fornix and thalamus (TH) were clearly observed (Fig. 2A, 160 day gestation). Strong expression of FABP5 was found throughout the VZ. Radial glia organisation varied depending on the region in the VZ, with the strongest FABP5 expression in the superficial fibrous layer of the cortical Stratified Transitional Field (STF). Radial fibre distribution was clearly visible, with fibres aligning closely to each other (Fig. 2C). Similar staining patterns were seen in the human fetal tissue and the adult human SVZ staining. However, the deeper layers of the STF had a less dense radial fibre distribution. In these deeper layers the radial fibres were no longer parallel but display a mesh-like distribution. In the VZ, FABP5 was expressed highly in cell bodies and on fibres. The FABP5 staining could be categorized in two typical staining patterns. (1) a nuclear and cytoplasmic staining, sometimes labelling the fibre as it leaves the soma (Fig. 2B’) and (2) radial fibre with a more intense punctate staining (Fig. 2C’-D’). The punctate staining pattern on the radial glia fibres was similar to previous reports that describe the presence of periodic thickening/nodes along the radial fibres stained with vimentin (40E-C) [29] and Golgi [30]. FABP5 expression was high in these nodes (Fig. 2C’-D’, white arrows). Co-labelling

with radial glia markers vimentin and GFAP along these fibres was evident (Fig. 3A-C). FABP7 co-labeled on both cell bodies and radial glial fibres (Fig 3D, blue and yellow arrows). Almost all PCNA$^+$ cells costained with FABP5$^+$ cell bodies (Fig. 3B, green arrows). PSA-NCAM$^+$ cell bodies costained with FABP5 (Fig. 3E). No NeuN staining was observed in the VZ. However, NeuN$^+$ cells were seen alongside FABP5$^+$ fibres in the hippocampus at this stage of development (data not shown).

FABP expression in sheep fetal brain tissue

For this study, we used sheep fetal tissue of 50-99 days gestation. As observed in adult sheep, the radial glia in the VZ of fetal sheep revealed FABP3 staining at 50, 90 and 99 days gestation. No obvious differences in FABP expression were seen between 90 and 99 days, therefore, only images of 90 days are shown. FABP3 strongly labelled the radial glial fibres and expression was restricted to lamina I of the VZ, with no expression in any other layers at 50 days (Fig. 4A). At 90 days FABP3 expression was present throughout the tissue (Fig. 4B). In the first layer of the VZ, FABP3 labelled cell bodies and radial glia fibres. FABP5 had a distinctive punctate nuclear pattern, while FABP7$^+$ cells were present throughout the VZ. However, a clear gradient was observed, with the highest intensity present in layer I of the VZ. In this layer, FABP7$^+$ cells co-labelled with FABP3 (Fig. 4C). A punctate staining of FABP7 and FABP5 (but less pronounced), not associated with cell bodies, was also seen in layer II of the VZ. These puncta co-labelled with the FABP3$^+$ radial glia throughout the z-stack (see yellow arrow, insert Fig. 4D). Strong co-labelling with the radial glia marker vimentin was observed (Fig. 4E, blue arrows). At this stage of development, the radial glial fibres were very pronounced, relatively wide in relation to the cell bodies, and were similar to the mesh-like structure observed in human fetal VZ (Fig. 3E'). FABP3$^+$ fibres also co-labelled with GFAP (data not shown for 50 day).

At 90 days gestation, FABP3 was still expressed on radial glial fibres (Fig. 5, blue arrows), but compared to 50 days gestation the intensity of the fibres was lower, and the fibres were thinner and more spread out in the parenchyma. The punctate pattern on the radial glia fibres observed at 50 days gestation for FABP7, and to a lesser degree for FABP5, was no longer visible after 90 days (Fig. 5). A clear co-labelling of FABP3$^+$ cells and fibres with

vimentin and GFAP was still observed (Fig. 5A, C). PCNA staining was seen throughout the brain tissue. Some FABP3$^+$ cells were PCNA$^+$ (Fig. 5B, yellow arrows), but the PCNA staining in these cells was punctate and less intense than other neighbouring PCNA$^+$ cells. FABP3 stained the whole nucleus, whereas FABP5 and FABP7 had a clear punctate nuclear staining pattern (Fig. 5D-E). FABP3, 5 and 7 were found in the same cells with varying intensity. Cells with high FABP5 staining also had high FABP7 staining (Fig. 5D-E, orange arrows).

Neurochemical characterization of FABP5$^+$ cells in the SVZ and CN of adult human brain

Because of the high expression of FABP5 in the SVZ of the adult human brain, we double labelled FABP5 with various cell specific markers, revealing co-labelling with PCNA, PSA-NCAM, GFAPδ and GFAP. Not all FABP5$^+$ cells were PCNA$^+$, but most FABP5$^+$ cells co-labelled with GFAPδ (Fig. 6A-D). Co-labeling with two $GABA_A$ receptor subunits was performed as they were expressed highly in the SVZ and labelled fibres in a previous SVZ study [31]. FABP5$^+$ cells expressed the $GABA_A$ receptor subunit α_3. In addition, FABP5$^+$ cells showed processes that protruded into the CN that were costained with the GABA subunit γ_2 (Fig. 6E-F).

Compared with the SVZ, we observed a lower intensity of FABP5 expression in the CN and less FABP5$^+$ cells. In some cases, no FABP5 labelling was observed in the CN. The cells in the CN that were FABP5$^+$ did not co-label with GFAP or NeuN. Some of the FABP5$^+$ cells were PSA-NCAM$^+$ (Fig. 6G-H).

FABP5 expression was concentrated in the ventral rostral migratory stream (RMS) and was clearly distinguishable from the connecting CN where no FABP5 was present. The expression level was similar to that observed in the SVZ. FABP5$^+$ processes from the RMS extended into the CN to make contact with nearby blood vessels. FABP7 was expressed in the CN cells, but not in the RMS (Fig. 6I, yellow arrows). In parts of the RMS, we found FABP5$^+$ cells clearly aligning along the RMS with some PSA-NCAM$^+$ cells lining the FABP5$^+$ fibres (Fig. 6J-K). This staining pattern was similar to that observed in fetal tissue (Fig. 6J, yellow arrows)

FABP7 expression in SVZ and CN in adult human brain

Depending on the case, the SVZ sometimes showed a large amount of FABP7 puncta in the gap layer that were not associated with any cell bodies (Fig. 7A). Unlike FABP5, no accumulation was observed in the SVZ. Throughout the CN, FABP7$^+$ cells were uniformly distributed, however we did observe a layer of cells with high nuclear staining for FABP7 immediately beneath the SVZ (100-300 µm band below SVZ; Fig. 7B). Punctate FABP7 staining became less intense with increasing distance from the SVZ. FABP7$^+$ cells co-labelled with PCNA (yellow arrows) and PSA-NCAM. FABP7 expression in the SVZ varied, and cells with high FABP7 expression (blue arrow) did not label for PCNA (Fig. 7C-D). In the CN, we found that cells with high nuclear and cytoplasmic FABP7 expression were almost exclusive to NeuN$^+$ cells (Fig. 7B, E). Only a few faint FABP7$^+$ puncta were observed in GFAP$^+$ cells in the CN (Fig. 7F, pink arrows).

DISCUSSION

FABPs are known to play central roles transporting fatty acids both as intracellular carriers and to various regions within the cell, where they can modulate gene expression by binding to transcription factors [32–34]. However, most studies are limited to rodents, so the relevance to humans is unclear. In this study, we compared FABP3, 5 and 7 expression in the embryonic and adult human and sheep brain. We found cytoplasmic and nuclear staining for FABP3, 5 and 7 and FABP5 staining was also detected within the end-feet of FABP$^+$ processes touching blood vessels. The major finding of this study demonstrates the presence of persistent FABP$^+$ radial glia in adult human and sheep SVZ that extend into the CN with the sheep SVZ expression showing strong similarities with radial glial expression in adult canary brain [9]. FABP expression on radial glia was species specific. In adult humans radial glial cells express FABP5, whereas in adult sheep FABP3 was predominantly expressed by radial glial cells. This was confirmed consistently in fetal tissue by double labelling with radial glia markers such as vimentin (40E-C) and GFAP. High FABP5 expression was observed in the human SVZ and co-labelled with PSA-NCAM, GFAP, GFAPδ and PCNA, indicating a role for FABP5 throughout the maturation process. Interestingly different $GABA_A$ receptor subunits also co-localised with FABP5 in the human SVZ. Radial glia and astrocytes have been shown

to express $GABA_A$ receptors in rodents [35]. A special type of GFAP-expressing cells with radial morphology persists in the subgranular zone of the hippocampus [36]. These radial glia-like cells display a passive current profile and functional $GABA_A$ receptors [37]. Fatty acids are known to influence $GABA_A$ receptor binding [38], while GABA is important in stem cell proliferation. Thus, fatty acids may play a role in regulating the effects of GABA in the SVZ.

In the present study, there were species-specific differences in FABP expression on radial glia, which could be caused by a different preferential lipid usage. Alternatively, FABPs show a distinct preference for certain fatty acids, but there is partial overlap in their expression profile. Thus, despite the ligand specificity, FABPs likely perform complementary functions as suggested in single and double knockout experiments [26]. FABP7 shows higher specificity to long polyunsaturated fatty (PUFA) acids such as eicosapentaenoic acid (EPA; 20:5), docosahexaenoic acid (DHA; 22:6), and n-6 PUFA, arachidonic acid (24:4), but low binding affinity to saturated fatty acids like lauric acid (12:0), palmitic acid (16:0), and stearic acid (18:0). By contrast, FABP3 has higher affinity to shorter PUFAs, such as oleic acid (18:1) and linoleic acid (18:2), than for long PUFAs, such as EPA and DHA. FABP5 binds to long PUFAs and eicosanoids such as 5 (S)- and 15 (S)-hydroperoxyeicosatetraenoic acid (HPETE)[39,40]. The various functions of FABPs and their ligands are complex but may explain the differences in FABP3, 5 and 7 expression observed between rodents, sheep and humans.

Previous rodent studies have shown that FABP7 is strongly expressed in radial glia and immature astrocytes in prenatal and perinatal periods, but its markedly attenuated in astrocytes of the adult brain. FABP5 is distinctly expressed in both neurons and glia in the prenatal and early postnatal brain, while FABP3 is expressed in neurons in the mature brain[21,41]. We observed a similar overlap in expression of the various FABPs throughout the VZ and SVZ. FABP3, 5 and 7 were all expressed in the SVZ, but varied in expression level and location. In the sheep VZ, FABP3 expression on radial glial fibres decreased and the fibres were thinner when comparing between 50 and 90 days gestation. Radial glial fibres in fetal human brain strongly expressed FABP5 in the VZ, and this high expression remained

localized to the adult SVZ, the area with the greatest level of plasticity/neurogenesis in the adult human brain [3]. Double labelling with PSA-NCAM, GFAP, GFAPδ and PCNA in the SVZ showed that FABP5 was present throughout a range of maturation steps in stem cell astrocytes (expressing GFAPδ). These cells divide to give rise to transit-amplifying GFAPδ and PSA-NCAM$^+$ type C cells, which in turn, generate PSA-NCAM positive neuroblasts [42,43]. This suggests that FABP5$^+$ cells are involved in more than just neuronal migration and potentially play a role in neurogenesis.

No FABP7 expression was observed in the human RMS, even though cells with high FABP7 expression were found in the adjacent caudate nucleus on neurons. This contrasts with previous reports in rodents showing strong expression of FABP7 in radial glia and immature astrocytes in prenatal and perinatal brains, but not on neurons [21,41]. FABP5 expression was high in the adult human RMS, and in the SVZ and RMS in mice [44]. As the RMS is important for neuronal migration from the SVZ to the olfactory bulb, this further implicates FABP5 in adult neurogenesis, differentiation and neuronal migration.

The role of radial glia during brain development, where radial glial cells not only perform an important role in cell guidance but also as neural stem/ progenitor cells, is well described [14,15]. The FABP5$^+$ radial glial cells in the adult SVZ share these characteristics. A combination of morphological features and various markers is frequently used to identify radial glia [8,13]. Of these markers, vimentin (40E-C) and GFAP labelled the radial glia and fibres in human and sheep sections. The radial glial specificity of FABP5 and FABP3 was further confirmed in fetal tissue, which exhibited a similar characteristic periodic thickening/nodes, as previously observed in canary [9], ferret and human [45]. An abundance of radial glia was observed in the VZ in the fetal brains, and even though FABP7 is frequently used as a radial glia marker in mice [8,25], rats [46] and zebrafish [47], we found that radial glia-like cells in the human fetal VZ and SVZ instead highly expressed FABP5. By contrast in sheep, radial fibres expressed only FABP3. In both species, these FABP$^+$ fibres expressed radial glial markers and showed the typical morphology of radial glial cells in a neurogenic active region. This data strongly support that presence of persistent radial glial cells in the adult SVZ, similar to the cerebellar Bergmann glia, retinal Muller cells, and

tanycytes in the hypothalamus [7]. All these specialized cells share basic morphological, immunological and biochemical features [48]. The function of the radial glia however depends on the context and functional requirements of the neurogenic niche, which differ between species. The close interaction of the radial glial cells in the SVZ with blood vessels and cerebral spinal fluid expose these cells to an array of embryonic signals [49]. The existence of residual FABP^{+} radial glia in the adult SVZ and RMS suggests a potential role in adult neurogenesis. This could include providing structural support during axon growth as observed in the corpus callosum [50], regulating the maturation process through GABA signal or might be restricted to cell guidance into the nearby caudate nucleus or in the RMS.

CONCLUSION

Our results provide a detailed characterization of FABP expression in the human and sheep SVZ. Based on morphological features and neurochemical markers, we have demonstrated the presence of persistent radial glial cell in the fetal and adult SVZ, with species specific differences in patterns of FABP expression (FABP5 vs FABP3) between the human and sheep brain during development and in adulthood.

MATERIAL &METHODS

Human brain tissue

The human adult brain tissue for this study was obtained from the New Zealand Neurological Foundation Douglas Human Brain Bank (Centre for Brain Research, University of Auckland). The University of Auckland Human Participants Ethics Committee approved the protocols used in these studies (2014/011654) and all tissue was obtained with full informed consent of the families. All methods in this study were performed in accordance with the relevant guidelines and regulations. All adult brain tissue was neurologically normal, with an average age of 56.8 years (range 41-67 years), with no history of neurological disease and no

evidence of neuropathology. The cases were chosen because they were similar in age, relatively young by human brain study standards, and had a similar cause of death. No significant histological abnormalities were found. The cases had a *post-mortem* interval between 8 and 24 h after death (average *post-mortem* interval 5.1 h) and included 1 female and 5 males (Table 1).

For the immunohistochemical studies, the human brains were processed as previously described [51]. In brief, the human brains were fixed by perfusion through the basilar and internal carotid arteries, first with phosphate-buffered saline (PBS) with 1% sodium nitrite, followed by 15% formalin in 0.1 M phosphate buffer, pH 7.4. After perfusion, tissue blocks were carefully dissected and kept in the same fixative for 24 h. The tissue blocks were cryoprotected in 20% sucrose in 0.1 M phosphate buffer with 0.1% Na-azide for 2-3 days, and then in 30% sucrose in 0.1 M phosphate buffer with 0.1% Na-azide for a further 2-3 days. The blocks were sectioned on a freezing microtome at 50 µm and the sections were stored at 4°C in PBS with 0.1% sodium azide [51].

Human fetal tissue containing the VZ was selected from the databases of the Department of Neuropathology of the Academic Medical Center (AMC), University of Amsterdam, The Netherlands. Informed consent was obtained for the use of brain tissue for research purposes. Tissue was obtained and used in a manner compliant with the Declaration of Helsinki and the AMC Research Code provided by the AMC Medical Ethics Committee (W11_073). Four cases (2 females, 2 males) with an average age of 156 days gestation were used (range 149-166 days). We performed a careful histological and immunohistochemical analysis and evaluation of clinical data. We only included, as control cases, specimens displaying a normal hippocampal and cortical structure for the corresponding age and without any significant brain pathology.

Animal brain tissue

Non-treated Romney/Suffolk fetal sheep were instrumented using sterile techniques at 50–99 days of gestation (term=147 days). All procedures were approved by the Animal Ethics Committee of the University of Auckland, New Zealand (AEC R1359). The experimental

procedures used for experiments have been previously published [52]. Sheep brains were fixed in 15% formalin. The tissue blocks were cryoprotected, sectioned and stored identically to the human free floating tissue.

Immunofluorescent procedures

1.1.1. Free floating 50 µm thick sections

Selected sections were stained whilst free-floating in tissue culture wells using standard immunohistochemical procedures [53,54]. All sections were washed and permeabilized overnight at 4°C in PBS-triton (0.2% Triton-X) before being processed for immunohistochemistry. All sections for antigen retrieval were transferred to six-well tissue culture plates and washed in 0.1 M sodium citrate buffer, pH 4.5 (3 x 10 min). Subsequently the sections were transferred to 10 ml of fresh sodium citrate buffer solution, microwaved without lid in a 650 W microwave oven for 40 s and allowed to cool (45 min) before washing (3 x 10 min) in PBS-triton. The sections were incubated with the required primary antibodies (Table 2) diluted in 1% normal goat or donkey (when Sheep-FABP3 was used) serum at 4°C on a moving platform for 3 days. Cells were subsequently washed in PBS (3 x 10 min) and incubated overnight at ambient temperature with species-specific fluorescent secondary antibodies (goat secondary, 1:400; donkey secondary, 1:100, Invitrogen). The secondary antibodies were washed off (3 x 10 min) and incubated with Hoechst 33342 (Invitrogen) at 1:20,000 for 20 min at RT. Sections were mounted with PBS on slides and cover slipped with ProLong gold antifade reagent (Invitrogen). Control sections were routinely processed to determine non-specific staining using the same immunohistochemical procedures detailed above except that the primary or secondary antibody was omitted from the procedure. Fluoromyelin green (Invitrogen) was used to delineate SVZ/CN border and applied per manufacturer's instructions.

1.1.2. Paraffin embedded 7 µm-thick sections

Immunohistochemistry was performed on paraffin embedded 15% formalin-fixed samples. Sections were cut on a microtome at 7-µm thickness; heated for 1 h at 60°C on a heating block, dewaxed in xylene (2 x 30min) and rehydrated through an alcohol series. Antigen

retrieval was performed by incubating the slides in a pressure cooker in Tris-EDTA buffer pH 9.0 and left to cool down in the cooker for 1.5 h. Paraffin sections were permeabilized in PBS containing 0.1% triton X-100 at 4°C for 10 min. Sections were blocked in 10% normal goat or donkey serum in PBS for 1 h at RT. Subsequently, the sections were incubated with primary antibodies overnight at 4°C (Table 2). Sections were then incubated with the corresponding goat/donkey secondary Alexa Fluor (488, 594, 647) conjugated secondary antibody (Invitrogen) at 1:400/1:200 for 3 h at RT. Finally, sections were incubated with Hoechst 33342 (Invitrogen) at 1:20,000 for 5 min at RT, and then cover slipped with ProLong gold antifade reagent (Invitrogen). All washes were done with PBS (3 x 5 min). Control sections where the primary antibody was omitted showed no immunoreactivity. The control experiments showed that the secondary antibodies did not cross-react with each other. We were unable to get FABP3 staining to work on paraffin sections. All confocal recordings were done using an FV1000 confocal microscope (Olympus) with a 40x oil immersion lens (NA 1.00). Fluorescent and brightfield recordings were performed with a MetaSystems VSlide slide scanner with a 20x dry lens (NA 0.9).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Author contributions

BVD designed the study, performed experiments and analyzed the data. JD provided sheep tissue and expertise. HW assisted in GABA receptor experiments and provided anatomy knowledge, EA provided fetal tissue. RF and MC run the human brain bank and directed the work. BVD, JD, HW, EA, RF, MC wrote the manuscript.

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Tables

Table 1 Brain tissue used in this study

	Age years	sex	PM delay h	weight g	cause of death
H215L	67	F	23.5	1232	Ischaemic heart disease
H135	62	M	16	1560	Ischaemic heart disease
H189	41	M	16	1412	Asphyxia
H231R	65	M	8	1527	Ischaemic heart disease
H211R	41	M	9.5	1513	Ischaemic heart disease
H195	65	M	18	1390	ischemic heart disease
Average ± SEM	56.8 ± 5.05		15.2 ± 2.32	1439 ± 49.6	

Table 2 antibodies used in this study. These primary antibodies have been extensively validated on human brain sections in our group, through the Clinical Proteomic Technologies for Cancer Initiative (CPTC) and in other labs[31,54–58]. Most antibodies are included in the Journal of Comparative Neurology (JCN) antibody database. *GP-GABA$_A$R α_3 and GP-GABA$_A$R γ_2 antibodies were a gift from H Möhler and JM Fritschy University of Zurich Switzerland. The following mouse (M), rabbit (R), guinea pig (GP), sheep (Sh) antibodies were used. ⊕ Antibody was not used on paraffin sections

antigen	Free floating dilution	Paraffin dilution	Catalog number	company	validation
M-radial glial marker (IgM)	1:200	1:200	40E-C	DSHB	[29]
Sh-FABP3 (IgG)	1:200	1:200	AF1678	R&D systems	[59]
R-FABP5 (IgG)	1:1500	1:1500	ab84028	Abcam	[60]
M-FABP5 (IgG)	1:300	1:300	CPTC-FABP5-1S	DSHB	CPTC
R-FABP7 (IgG)	1:200	1:200	ab27171	Abcam	[JCN]
GP-GABA$_A$R α_3 (IgG)	1:5,000	⊕	GP-GABA$_A$R α_3	*gift	[56][JCN]
GP-GABA$_A$R γ_2 (IgG)	1:5,000	⊕	GP-GABA$_A$R γ_2	*gift	[56][JCN]
M-GFAP (IgG)	1:20,000	1:20,000	G3893	Sigma	[JCN]
R-GFAP (IgG)	1:15,000	1:1000	Z0334	Dako	[JCN]
R-GFAPδ (IgG)	1:1,000	⊕	ab28926	Abcam	[JCN]
M-NeuN (IgG)	1:300	1:100	MAB377	Millipore	[JCN]
R-NeuN (IgG)	1:300	1:100	ABN78	Millipore	[JCN]
M-PCNA (IgG)	1:500	1:300	sc-56	Santa Cruz	[JCN]
R-PCNA (IgG)	1:500	1:300	sc7907	Santa Cruz	[61]
M-PSA-NCAM (IgM)	1:600	1:400	MAB5324	Millipore	[62,63][JCN]
M-PSA-NCAM (IgM)	1:600	1:400	5A5	DSHB	[62]

Figure 1 Confocal Images of FABP expression in the adult human subventricular zone (SVZ) in adult human and sheep. **(a)** FABP5 is highly expressed in the human SVZ as elongated fibres and in scattered cells lateral to the SVZ but with lower expression (cell body: yellow and fibres: blue arrows). Insert from box drawn in A shows FABP5$^+$ radiating cell (yellow arrow) with fibres (blue arrows) penetrating the caudate nucleus (CN). **(b-d)** Radial glial cell in the SVZ with processes expressing both radial glia marker vimentin (40E-C) and FABP5 (blue arrows). The radial glia (yellow arrow) has one basal end-foot at the ventricular surface (orange arrow) with a characteristic (1-2) long radial fibres(s) (blue arrow) reaching the pial surface of the brain. The radial fibres, particularly during the later stages of development, often form several branches that terminate with multiple endfeet. **(e)** FABP3, 5 and 7 expression in adult human SVZ. **(f-h)** High magnification of cells co-expressing FABP3 (cyan), FABP5 (red), FABP7 (green) in the cytoplasm and nucleus of cells found in the human SVZ. (i) FABP3 is highly expressed in sheep SVZ. **(j-l)** Co-labelling of elongated fibres vimentin (40E-C) and FABP3 (yellow arrows). **(m)** FABP3, 5 and 7 expression in adult sheep SVZ. **(n-p)** Triple labelling of FABP3, 5 and 7 in the sheep SVZ and underlying CN showing non-overlapping distributions of labelling. Scale bars represent 10µm.

Figure 2 160 day-old human fetal brain tissue **(a)** overview image of FABP5 and GFAP regional expression. Ventricular zone (VZ), thalamus (TH), hippocampus (HP) **(b-e)** Magnification of ventricular zone (VZ) with radial glial expression labelled with GFAP throughout the VZ. Four regions showing differential orientation of radial fibres. White lines in bottom of each panel depict radial fibre distribution throughout the VZ. **(b)** Strong FABP5 labelling at the pial surface, but not associated with any fibres or cell bodies. **(c)** Punctate FABP5 labelling on the radial fibres and occasional mature looking astrocytes (pink arrows) in the lower portion of the cortical plate. **(d-e)** Radial fibres are no longer parallel but display a mesh-like distribution. **(b'-e')** Higher magnification of each zone in ventricular zone of GFAP-labelled ventricular zone/subventricular zone (VZ/SVZ) at midgestation. **(b')** Some FABP5$^+$ cell bodies (yellow arrows) present alongside radial glia fibres (blue arrows). **(c'-d')** Punctate FABP5 labelling (white arrows) on the radial fibres (blue arrows). Mature looking astrocytes (pink arrows). **(e')** FABP5$^+$ cell bodies (yellow arrows) with punctate FABP5 labelling (white

arrows) present alongside radial glia fibres (blue arrows). Non-annotated scale bars represents 10µm.

Figure 3 FABP5 expression in the ventricular zone of fetal human tissue. **(a-e)** FABP5$^+$ expression on radial glia cells (yellow arrows) and fibres (blue arrows) with various markers in 160 days old fetal human brain. The presence of periodic thickening/nodes along the radial fibres for FABP5 is frequently seen (blue arrows); **(a)** Vimentin (40E-C) co-labelling on radial fibres (blue arrows). Vimentin (40E-C) co-labels strongly with FABP5$^+$ radial glia fibres where periodic thickening/nodes is seen (white arrows); **(b)** PCNA co-labelling with FABP5$^+$ cell bodies (yellow arrows); **(c)** FABP5 labelling on the radial glial cells (yellow arrows) and fibres (blue fibres). Mature looking astrocytes (pink arrows) are found throughout in the lower portion of the cortical plate; **(d)** FABP7 expression in cells and radial fibres co-labels with FABP5$^+$ cell bodies and fibres; **E** FABP5$^+$ cells co-label with PSA-NCAM (white arrows) and are oriented perpendicular to the FABP5$^+$ radial glia fibres (blue arrows). Scale bars represents 10µm.

Figure 4 FABP3 expression in fetal sheep **(a)** FABP3 double labeling with radial glia marker vimentin (40E-C) after 50 days gestation. Expression of FABP3 on radial glial fibres is observed over 600µm into the ventricular zone (VZ) from the pial surface **(b)** FABP3 double labeling with vimentin (40E-C) after 90 days gestation. **(c)** FABP expression in fetal sheep VZ after 50 days gestation FABP3 (red), FABP5 (green), FABP7 (magenta). FABP3$^+$ expression is abundant on thick radial glial fibres. **(d)** High magnification of box in (c). FABP3 sometimes co-labels with both FABP5 and FABP7 (yellow arrows). Insert shows zoom of single confocal plane. **(e)** FABP3$^+$ radial cell glia (yellow arrow) with glial fibres (blue arrows) extending into the VZ. Glial fibres co-label with radial glia marker vimentin (40E-C). Abbreviations: white matter (WM), caudate nucleus (CN). Non-annotated scale bars represents 10µm.

Figure 5 confocal recordings of FABP expression in the ventricular zone of 90 days old fetal sheep tissue. **(a-e)** Co-labeling of FABP3$^+$ radial glia cells and fibres with various makers. **(a)** Vimentin (40E-C) co-labels with FABP3$^+$ radial glial fibres (blue arrows); **(b)** FABP3$^+$ cells were

often PCNA positive (yellow arrows); **(c)** GFAP co-labels with FABP3$^+$ radial glial fibres (blue arrows); **(d)** FABP7; **(e)** FABP5, cell bodies with high FABP5 had high FABP7 staining and were also FABP3$^+$ (orange arrows). Scale bars represents 10µm.

Figure 6 FABP5 expression in the adults human SVZ and rostral migratory stream (RMS). **(a-h)** FABP5$^+$ cells (blue arrows) located within the SVZ costaining with various markers (yellow arrows). **(a)** PCNA; **(b)** PSA-NCAM; **(c)** GFAPδ; **(d)** GFAP; **(e)** $GABA_A$ α_3. **(f)** Double labelling with $GABA_A$ γ_2 of a SVZ cell radiating into the CN. **(g)** FABP5$^+$ cells located in the CN did not co-label with GFAP. **(h)** Some FABP5$^+$ cells in the CN cells did co-label with PSA-NCAM. Expression levels of FABP5 in CN are much lower than in SVZ. **(i)** FABP5 is highly expressed in the RMS (RMS borders depicted with yellow lines) and FABP7 expression (yellow arrows) is restricted to the caudate nucleus (CN). **(j)** FABP5 co-labelling with PSA-NCAM expression in a confocal projection of the RMS. PSA-NCAM expression on cells lining FABP5$^+$ fibres (yellow arrows). Yellow dotted line delineates RMS. **(k)** Overlay in red depicts cells observed throughout confocal stack. Yellow dotted line delineates RMS. Scale bars represent 10µm.

Figure 7 FABP7 expression in the human SVZ and caudate nucleus (CN). **(a-b)** Overview image of FABP7 expression in SVZ and underlying CN. Yellow line indicates border SVZ/CN as delineated with the fluoromyelin stain. Second yellow line in (b) indicates approximate border ventral from SVZ border where very bright FABP7 (white arrows) expression is observed. **(c)** Punctate FABP7$^+$ cells express PCNA (blue arrows). There is no co-labeling with PCNA when FABP7 is present throughout the whole nucleus. FABP7 expression ranged from a few defined punctate FABP7$^+$ spots (blue arrows) to an overall punctate labelling (yellow arrow). **(d)** FABP7$^+$ cells express PSA-NCAM in the SVZ. **(e)** Most FABP7$^+$ cells express NeuN in the CN. **(f)** GFAP$^+$ cells (blue arrow) express a few FABP7$^+$ spots (pink arrow) in the CN, but most FABP7$^+$ cells do not express GFAP. FABP7 was rare in GFAP$^+$ cells in SVZ. Scale bars represents 10µm.