Published in final edited form as:

Neuroreport. 2013 December 18; 24(18): 1035–1040. doi:10.1097/WNR.0000000000000056.

Profiling metabolite changes in the neuronal differentiation of human striatal neural stem cells using ¹H-Magnetic Resonance Spectroscopy

Yuen-Li Chung¹, Gehan El Akabawy^{2,3}, Po-Wah So⁴, Bhavana S. Solanky^{2,*}, Martin O. Leach¹, and Michel Modo^{2,5}

¹The Institute of Cancer Research and Royal Marsden Hospital, Cancer Research UK and EPSRC Cancer Imaging Centre, Sutton, Surrey, SM2 5PT, UK

²Kings College London, Institute of Psychiatry, Department of Neuroscience, London, SE5 9NU, UK

³University of Menoufia, Department of Anatomy and Embryology, Faculty of Medicine, Menoufia, Egypt

⁴Kings College London, Institute of Psychiatry, Department of Neuroimaging, London, SE5 9NU, UK

⁵University of Pittsburgh, Department of Radiology, McGowan Institute for Regenerative Medicine, Pittsburgh PA5203, USA

Abstract

Objective—Neural stem cells (NSCs) are playing an increasing clinical role for stroke. However at present, it is not yet possible to non-invasively monitor their differentiation once implanted into the brain.

Methods—We here describe the use of high-resolution ¹H-magnetic resonance spectroscopy (MRS) to define a metabolite profile of undifferentiated human striatal NSCs from the STROC05 cell line and their differentiation after 3 weeks of treatment with purmorphamine.

Results—The undifferentiated conditions were characterized by ~95% of cells expressing nestin and ~77% being Ki67+, indicating that these were still proliferating. Phosphocholine +glycerophosphocholine (PC+GPC) was increased in these cells, as well as myo-Inositol (mI). PC +GPC and mI were dramatically reduced upon differentiation, potentially serving as markers of the NSC state. Upon differentiation (~45% neurons, ~30% astrocytes, ~13% oligodendrocytes),

Address for Correspondence: Mike Modo, University of Pittsburgh, McGowan Institute for Regenerative Medicine & Department of Radiology, 3025 East Carson St, Pittsburgh, PA 15203 USA, +1 (412) 624 9462, modomm@upmc.edu.

*Current Address: NMR Research Unit, Department of Neuroinflammation, Institute of Neurology, University College London, Queens Square, London, WC1N 3BG, UK

Authors' Contribution: YLC: Contributed to design of study, Acquired NMR spectra, Processed and Analyzed NMR data, drafted manuscript, interpretation of data; GEA: Grew and differentiated cells, Dual Phase Extraction; Contributed to the Manuscript; PWS: Contributed to the design of the study, set-up dual phase extraction, interpretation of the results, drafting of the manuscript; BS: acquiring pilot data; MOL: Contributed to design of the study, interpretation of the results, review of results and manuscript, financial support; MM: Conceived of the study, financial support, interpretation of the results, drafted the manuscript, final approval of the manuscript.

the concentration of many metabolites decreased in absolute value. The decreasing trend of the N-acetyl-aspartate (NAA) level was observed in differentiated cells when compared to NSCs. An increase in plasmalogen (enriched in myelin sheets) could potentially serve as a marker of oligodendrocytes.

Conclusion—These metabolite characteristics of undifferentiated and differentiated NSCs provide a basis for exploring their possible use as markers of differentiation after cell transplantation.

Keywords

Neural Stem Cell; ¹H-Magnetic Resonance Spectroscopy; Purmorphamine; DARPP-32; Neuronal Differentiation; Metabolites; Striatum; Ganglionic Eminence; human

Introduction

Neural stem cells (NSCs) are gradually emerging as a therapeutic tool with a developing clinical translation. However, the in vivo monitoring of transplanted cells remains a major challenge [1]. Tracking of cells by cellular MRI that relies on tagging the cells prior to injection with MRI contrast agents is feasible and has found specific applications in a clinical setting [2]. Although this approach can indicate the location of transplanted cells by using the MRI contrast agent as a surrogate marker of cell location, it is not possible to determine the cells differentiation status.

Neural stem cells differentiate into neurons, astrocytes and oligodendrocytes. However, after implantation a significant proportion of cells also do not express mature phenotypic markers, despite having ceased to proliferate. One potential approach to address this currently unmet monitoring need is to implement ¹H-magnetic resonance spectroscopy (MRS). ¹H-high-resolution MRS can determine in vitro a particular metabolic profile of cell extracts [3] and afford a distinction, for instance, between neurons and glia, as well as differentiating them from brain tumor cells [4]. Distinguishing NSCs from brain tumor cells is also potentially an important aspect of monitoring the safety of cell transplantation. Manganas et al. [5] have suggested a specific metabolite marker to identify exogenous or endogenous neural stem cells (e.g. a peak at 1.28 ppm), which would be desirable, although alternative explanations of this marker have emerged [6-8].

To monitor the phenotypic differentiation of NSCs, it is essential to establish the metabolite profile present in these cells while in proliferative, yet undifferentiated conditions, as well as in their post-mitotic differentiated state. Jansen et al.[9], for instance, have profiled changes in mouse embryonic stem (ES) cells during neuralization to NSCs. ES cells were characterized by an overall low level of metabolites with non-detectable glycerophosphocholine (GPC) levels, but a high content of phosphocholine (PC). NSCs were found to exhibit a profile akin to glial cells with higher PC, GPC and myo-inositol (mI). As the metabolite profile is dependent on the developmental stage, as well as its regional source from the brain, it is hence important to clearly define the cellular characteristics of the investigated cells. To our knowledge, this is the first report on the

metabolic characterizations of undifferentiated and differentiated human neural stem cells (hNSCs).

Methods

Striatal human neural stem cell (hNSC) line

The cmyc-ER^{TAM} conditionally immortalized striatal hNSC line (STROC05, ReNeuron), was previously described by Johansson et al. [10]. In brief, STROC05 cells were isolated from the whole ganglionic eminence of a 12 weeks' old human fetal brain, expanded on laminin-coated culture dishes, and transfected with the retroviral vector pLNCX-2 (Clontech) encoding the cmyc-ER^{TAM} gene. Transfected cell colonies were isolated following neomycin selection before being expanded into a clonal cell line [11]. To maintain proliferation through the conditional immortalization gene, 4-hydroxy-tamoxifen (4-OHT; 100 nM/ml; Sigma-Aldrich) was added to all undifferentiated media.

Expansion of undifferentiated STROC05 cells

Cells were expanded in culture flasks (Falcon) coated with mouse laminin at a concentration of 1:100 (mouse, 10 µg/ml; Trevigen) at a seeding density of 2.5×10^6 cells/flask. Every 2 days, cells were passaged at 90% confluency and medium was completely replaced. To stimulate proliferation, basic fibroblast growth factor (bFGF; 10 ng/ml) and epidermal growth factor (EGF; 20 ng/ml) (Peprotech) were added to the media (Suppl. Table 1).

Long-term differentiation of hNSCs

STROC05 cells differentiate spontaneously into appropriate striatal phenotypes over 3 weeks of differentiation. However, neuronal yield and differentiation into the lateral ganglionic eminence (LGE)-derived GABAergic output neurons is low. To increase neuronal yield and specifically produce more DARPP-32 cells, purmorphamine was added here to the differentiation condition [12]. As described in El Akabawy et al. [12], long-term cultures were exposed to purmorphamine for 3 weeks. To ensure a long-term attachment, cells were seeded at a density of 2.5×10^6 cell/flask onto laminin and poly-L-lysine (PLL, 100 µg/ml, Sigma). Cells were grown to 90% confluency before initiation of the differentiation protocol. For the first week of differentiation, media was identical to that of undifferentiated cells (Suppl. Table 2) with the exception of bFGF, EGF and tamoxifen omitted from the media.

Dual-Phase Extraction

From each culture vessel, 1 ml of the supernatant was stored at -80°C. Cells were harvested by incubation with Trypzean EDTA (Sigma) for less than five minutes at 37°C, followed by addition of soybean trypsin inhibitor to inactivate the enzymatic activity. After harvesting, cells were centrifuged for 5 minutes at 1500 rpm, and the cell pellet was re-suspended in 1 ml ice cold phosphate buffered saline (PBS). Cells were counted and their viability was assessed using the trypan blue exclusion test. 10^7 cells of each sample were then centrifuged for 5 minutes at 1500 rpm, and the cell pellets were re-suspended in 2ml ice-cold methanol. Then, 2ml of ice-cold chloroform were added to each tube and vortexed for 30 seconds, followed by addition of 2 ml of ice-cold de-ionised water into each tube, and vortexed again

for another 30 seconds. Samples were centrifuged (4°C) at approx. 4600 rpm for 20 minutes for phase separation. The water-methanol and lipid phases, as well as the protein layer, were carefully separated and kept at -80°C. Prior to storage the water-methanol layers were pre-treated with Chelex 100 (1 mg/ml) to remove divalent ions.

High-Resolution ¹H-Magnetic Resonance Spectroscopy

The methanol-water fraction (containing water soluble metabolites) was freeze-dried and reconstituted in 580 µl deuterated water (D₂O, Sigma). 20 µl of 0.75% sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP) in D₂O (Sigma) was added to the samples for chemical shift calibration and quantification. 500 µl of the reconstituted extracts were then placed in 5 mm NMR tubes (Apollo Scientific). Lipid extracts were dried and then reconstituted in 450 µl deuterated chloroform (Sigma) and 150 µl of 0.1% tetramethylsilane in deuterated chloroform (Sigma) was added to the samples for chemical shift calibration and quantification. Media from the cultured cells were also analyzed by ¹H-MRS. 500 µl of the media sample and 50 µl of D₂O were placed in the NMR tube. 50 µl of 0.75% TSP in D₂O was added to the samples for chemical shift calibration and quantification. Spectral assignments were based on literature values [13,14]. Metabolite uptake and excretion levels in undifferentiated and differentiated cells were evaluated relative to its own respective starting media.

¹H-MRS measurements were performed on a 500 MHz spectrometer (Bruker). Pulse angle of 45°, repetition time of 2 seconds, spectral width of 7500Hz, 128 averages, temperature of 298K, acquisition time approximately 5 minutes. The water resonance was suppressed by a gated irradiation centered on the water frequency. Spectral processing was carried out using the Topspin-2 software package.

Statistics

One-tailed Mann-Whitney tests were used to compare data from undifferentiated and differentiated cells. Statistical significance was set at $p < 0.05$. In vitro cell culture studies consisted of 3 biological replicates, each consisting of 3 technical replicates. ¹H-MRS results consisted of 4 biological replicates with viability measurements, as well as passage number recorded for each.

Results

Neuronal Differentiation of human striatal neural stem cells

Human neural stem cells (hNSCs) from the STROC05 cell line were differentiated for 3 weeks by addition of purmorphamine. In comparison to undifferentiated cells, markers of NSCs, such as nestin, and proliferation, such as Ki67, were significantly downregulated (Suppl. Figure 1A, Suppl. Table 3). Concomitantly markers of differentiated cells of the caudate putamen were significantly upregulated. Specifically, 45.7% of cells were of a neuronal phenotype (β-III-tubulin+ cells), 29.7% expressed GFAP, a marker of astrocytes, and 12.9% were oligodendrocytes (GalC+ cells). Calbindin+ neurons (9.11%) were the most common neuronal phenotype followed by DARPP-32+ GABAergic output neurons (6.4%) and calretinin+ neurons (6.29%). However, within these differentiated cells, no cells

expressed choline-acetyl transferase (ChAT), as a marker of cholinergic interneurons, and only a very small number expressed tyrosine hydrolyase (TH) (<1%), as a marker of dopaminergic cells. The viability of these cells after harvesting was excellent for undifferentiated cells (95%), but decreased slightly for differentiated cells (87%) (Suppl. Figure 1B). Differentiated cells were at a slightly later passage number (p28) than undifferentiated cells (p23) (Supplementary Figure 1C). These in vitro conditions therefore afforded the investigation of the metabolites that changed during the differentiation of striatal hNSCs.

Water-soluble metabolites in undifferentiated and differentiated hNSCs

For some metabolites, such as combined phosphocholine and glycerophosphocholine (PC +GPC) and myo-inositol (mI), very prominent peaks are evident and changes in these metabolites lead to striking differences in concentration profile between undifferentiated and differentiated cells (Suppl Fig 2). Significant decreases in threonine, alanine, glutamate, PC +GPC, mI, uridine triphosphate + uridine diphosphate (UTP+UDP) and adenosine-triphosphate + adenosine diphosphate (ATP+ADP) were found in differentiated STROC05 cells when compared with undifferentiated cells (Table 1). The concentration of mI was reduced almost 50 fold in differentiated cells, whereas most other metabolites were reduced between 2-5 fold. Although the concentration of NAA decreased by 50% during differentiation, this did not reach statistical significance. These differences must be considered together with changes or differences in secreted factors in the culturing media. Resonances from 4-(2-hydroxyethyl)-1- piperazine-ethane sulfonic acid (HEPES) were only readily observed in extracts of differentiated cells, resulting from cell uptake from the media, as HEPES was only present in the media of the differentiated cells.

Uptake and excretion of metabolites in the culture media

As metabolite changes can be a consequence of different culturing conditions, it is important to establish how the uptake and excretion of metabolites is affected by the cell culture media (Table 2). Significant increases (1.1 to 3.5 fold) in the uptake of leucine, iso-leucine, valine, glutamine, tyrosine, phenylalanine and glucose were found in differentiated compared to undifferentiated STR cells, which is consistent with these metabolites (except for glutamine) being higher in the differentiated media (1.3 to 2 fold). In contrast, a decrease in alanine excretion was also observed in differentiated cells.

Lipid metabolites in undifferentiated and differentiated hNSCs

The spectra from the lipid phase of an undifferentiated and a differentiated STROC05 cell extract show subtle but significant changes in levels of a variety of lipids (Suppl. Figure 3). Specifically, cholesterol and cholesterol ester, the methyl resonance of the fatty acids, the -CH=CH- resonance of the fatty acids, phosphatidylcholine and plasmalogen were significantly increased in differentiated STROC05 cells (Table 3). A cholesterol ester signal (CS) at 1.58 ppm, as well as unidentified resonances at 2.99, 3.84, 6.44, 6.96 and 7.35 to 8.10 ppm were only observed in differentiated cells. These lipids are specific to differentiated cells, as these resonances are not present in the lipid extracts of media from differentiated or undifferentiated STROC05 cells.

Discussion

¹H-Magnetic resonance spectroscopy (MRS) enables the detection of metabolites in undifferentiated and differentiated hNSC extracts [15]. Significant decreases in water-soluble metabolites were evident, with mI indicating an almost 50 fold decrease in concentration, whereas most other metabolites (e.g. alanine, glutamate) underwent a 2-5 fold decrease. Many metabolites in the aqueous phase decreased, whereas lipids increased upon differentiation. Specific metabolite changes, reflecting different biological and biochemical processes (e.g. proliferation), therefore characterize the undifferentiated from the differentiated state of hNSCs.

Profiling the neural stem cell state

Neural stem cells are a fairly homogenous population of cells with all cells being in a similar proliferative state and therefore generate proteins and cell organelles for cell division, hence constantly increasing their biochemical composition. Indeed, metabolite concentrations in undifferentiated cells were overall much higher than in differentiated cells. PC+GPC, for instance, was significantly higher in undifferentiated NSCs, reflecting a higher turnover rate of the cellular membrane, consistent with the higher score of the proliferative marker Ki67. There was a significant decrease in UTP+UDP and ATP+ADP with unchanged creatine in differentiated cells. This lower energetic demand of differentiated cells is consistent with its non-proliferative state. Higher levels of PC+GPC and ATP could therefore indicate if transplanted cells are still proliferating or if they have ceased cell division.

Importantly, NSCs are restricted to become brain cells, but are unspecified in terms of becoming a mature brain cell, i.e. a neuron, astrocyte or oligodendrocyte. They therefore share commonalities in their metabolite profile with other proliferative cells, such as embryonic stem (ES) cells or tumor cells. Choline metabolism, leading to the elevated levels of choline-containing metabolites, is known to be a key component or result of the transformation of tumor cells and is upregulated upon immortalization [16]. STROC05 cells, which are conditionally immortalized, do show elevated PC+GPC in their undifferentiated state. Interestingly, Jansen et al. [9] reported the absence of GPC and mI in ES cells and their presence in NSCs, indicating that changes in these two metabolites might indeed be related to the NSCs state. mI is easily identified in undifferentiated NSCs and putatively might serve to distinguish NSCs from ES and differentiated brain cells. Still, mI is an abundant metabolite in the adult brain and thought to act as an osmolyte, while being located in glial cells and to a lesser extent in neurons [17]. This metabolite profile therefore is useful to generally describe the NSC state, but is unlikely to specifically define NSCs per se [18].

Further, caution must be employed, as we have to use a different growth media during the last two weeks of cell differentiation to ensure the differentiated cells' survival and attachment. Differences in media composition between undifferentiated and differentiated cells could influence metabolism, as well as the presence or absence of specific amino acids. Still, the magnitude of changes observed here, such as a 50 fold decrease in mI, cannot account for the small differences in concentration of these components in both media.

Cell type-specific metabolites

The differentiation of NSCs into mature brain cells produces a heterogeneous cell population consisting of neurons, astrocytes and oligodendrocytes. Each cell type has specific biochemical features that are associated with different metabolites [19]. Most commonly in vivo, the presence and the level of NAA expression are considered indicators of neurons [20]. However, despite a content of 45.7% of neuronal cells, absolute NAA levels here were not significantly different in differentiated cells when compared with undifferentiated cells. Although primary fetal rat neurons contain NAA in vitro when analyzed using MR spectroscopy [3], there is also robust evidence indicating that at least immature oligodendrocytes can also contain NAA [21].

Whilst the maturation of human neurons is a lengthy process, this is considered less so for astrocytes and oligodendrocytes. We here achieved a yield of 29.7% astrocytes and 12.9% oligodendrocytes, amounting to a substantial portion of our cell population. Nevertheless, mI concentration typically associated with astrocytes [22], decreased with differentiation. Glutamine, the main product of astrocytic metabolism [23], was also decreased in differentiated compared to undifferentiated cells. Absolute levels of glycine were unchanged after 3 weeks of differentiation, whereas taurine was neither detected in undifferentiated, nor in differentiated, cells. Taurine is synthesized by astrocytes and accumulates in neurons [24]. It will be important to establish how these levels compare with primary astrocytes derived from an adult brain. In contrast, plasmalogen, a lipid which is enriched in myelin sheets [25], is increased 3-fold, potentially being a marker for oligodendrocytes [26].

In vivo detection of NSCs and neuronal differentiation

In vitro studies afford the investigation of specific cell types under defined conditions. This information is useful to determine which metabolites could be used in vivo to monitor the differentiation of implanted human NSCs. However, in vivo MR spectroscopy in humans or animals is typically performed at lower field strengths and is subject to susceptibility effects associated with tissue structure, leading to broader peaks and higher levels of noise in spectra. Therefore only clearly identifiable peaks, such as mI, will be useful markers to distinguish undifferentiated and differentiated cells. The localized injection of NSCs to repair damage in neurological conditions, such as stroke, supplies large quantities (>400,000) of NSCs into striatal or cortical tissue constituting a large proportion of an in vivo voxel. ¹H-MRS would therefore potentially allow clinicians to determine how many cells were delivered (based on changes in metabolite levels in mI), but would also provide a means to determine if most of the cells stopped proliferating.

Conclusion

¹H-Magnetic resonance spectroscopy readily identifies metabolites, such as PC+GPC, associated with the proliferation of neural stem cells. A combination of PC+GPC and mI might be useful markers of the NSC state, but comparisons with cells at a similar developmental stage are required to better define if these changes are specific to NSCs and if this profile is consistent across NSCs derived from different brain regions. These changes in

metabolites may be useful to monitor the differentiation of NSCs after intracerebral implantation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by an MRC (G0802552) and a EU Framework VII grant (201842-ENCITE). MM was the recipient of a RCUK fellowship. GEA was the recipient of a PhD studentship from the Egyptian Government (MM45/07). YLC and MOL acknowledge the support received from the CR-UK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334, NHS funding to the NIHR Biomedical Research Centre.

References

1. Sztriha LK, O'Gorman RL, Modo M, Barker GJ, Williams SC, Kalra L. Monitoring brain repair in stroke using advanced magnetic resonance imaging. *Stroke*. 2012; 43:3124–3131. [PubMed: 23010674]
2. Modo M, Hoehn M, Bulte JW. Cellular MR imaging. *Mol Imaging*. 2005; 4:143–164. [PubMed: 16194447]
3. Bhakoo KK, Williams IT, Williams SR, Gadian DG, Noble MD. Proton nuclear magnetic resonance spectroscopy of primary cells derived from nervous tissue. *Journal of neurochemistry*. 1996; 66:1254–1263. [PubMed: 8769892]
4. Florian CL, Preece NE, Bhakoo KK, Williams SR, Noble M. Characteristic metabolic profiles revealed by ¹H NMR spectroscopy for three types of human brain and nervous system tumours. *NMR Biomed*. 1995; 8:253–264. [PubMed: 8732181]
5. Manganas LN, Zhang X, Li Y, Hazel RD, Smith SD, Wagshul ME, et al. Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. *Science*. 2007; 318:980–985. [PubMed: 17991865]
6. Jansen JF, Gearhart JD, Bulte JW. Comment on ‘Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain’. *Science*. 2008; 321:640. author reply 640. [PubMed: 18669846]
7. Friedman SD. Comment on “Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain”. *Science*. 2008; 321:640. author reply 640. [PubMed: 18669845]
8. Hoch JC, Maciejewski MW, Gryk MR. Comment on “magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain”. *Science*. 2008; 321:640. author reply 640. [PubMed: 18669844]
9. Jansen JF, Shablott MJ, van Zijl PC, Lehtimäki KK, Bulte JW, Gearhart JD, et al. Stem cell profiling by nuclear magnetic resonance spectroscopy. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2006; 56:666–670.
10. Johansson S, Price J, Modo M. Effect of inflammatory cytokines on major histocompatibility complex expression and differentiation of human neural stem/progenitor cells. *Stem Cells*. 2008; 26:2444–2454. [PubMed: 18635871]
11. Pollock K, Stroemer P, Patel S, Stevanato L, Hope A, Miljan E, et al. A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. *Exp Neurol*. 2006; 199:143–155. [PubMed: 16464451]
12. El-Akabawy G, Medina LM, Jeffries A, Price J, Modo M. Purmorphamine increases DARPP-32 differentiation in human striatal neural stem cells through the Hedgehog pathway. *Stem cells and development*. 2011; 20:1873–1887. [PubMed: 21345011]
13. Sitter B, Sonnewald U, Spraul M, Fjosne HE, Gribbestad IS. High-resolution magic angle spinning MRS of breast cancer tissue. *NMR Biomed*. 2002; 15:327–337. [PubMed: 12203224]

14. Sze DY, Jardetzky O. Characterization of lipid composition in stimulated human lymphocytes by ¹H-NMR. *Biochim Biophys Acta*. 1990; 1054:198–206. [PubMed: 2400782]
15. Ramm Sander P, Hau P, Koch S, Schutze K, Bogdahn U, Kalbitzer HR, et al. Stem cell metabolic and spectroscopic profiling. *Trends in biotechnology*. 2013
16. Bhakoo KK, Williams SR, Florian CL, Land H, Noble MD. Immortalization and transformation are associated with specific alterations in choline metabolism. *Cancer Res*. 1996; 56:4630–4635. [PubMed: 8840976]
17. Haris M, Cai K, Singh A, Hariharan H, Reddy R. In vivo mapping of brain myo-inositol. *Neuroimage*. 2011; 54:2079–2085. [PubMed: 20951217]
18. Loewenbruck KF, Fuchs B, Hermann A, Brandt M, Werner A, Kirsch M, et al. Proton MR spectroscopy of neural stem cells: does the proton-NMR peak at 1.28 ppm function as a biomarker for cell type or state? *Rejuvenation Res*. 2011; 14:371–381. [PubMed: 21548757]
19. Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1993; 13:981–989. [PubMed: 8441018]
20. Wang Y, Li SJ. Differentiation of metabolic concentrations between gray matter and white matter of human brain by in vivo ¹H magnetic resonance spectroscopy. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 1998; 39:28–33.
21. Bhakoo KK, Pearce D. In vitro expression of N-acetyl aspartate by oligodendrocytes: implications for proton magnetic resonance spectroscopy signal in vivo. *J Neurochem*. 2000; 74:254–262. [PubMed: 10617127]
22. Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci*. 1993; 15:289–298. [PubMed: 7805581]
23. Brand A, Richter-Landsberg C, Leibfritz D. Metabolism of acetate in rat brain neurons, astrocytes and cocultures: metabolic interactions between neurons and glia cells, monitored by NMR spectroscopy. *Cell Mol Biol (Noisy-le-grand)*. 1997; 43:645–657. [PubMed: 9298588]
24. Merle M, Pianet I, Canioni P, Labouesse J. Comparative ³¹P and ¹H NMR studies on rat astrocytes and C6 glioma cells in culture. *Biochimie*. 1992; 74:919–930. [PubMed: 1334701]
25. Nagan N, Zoeller RA. Plasmalogens: biosynthesis and functions. *Prog Lipid Res*. 2001; 40:199–229. [PubMed: 11275267]
26. Wilhelm MJ, Ong HH, Wehrli SL, Li C, Tsai PH, Hackney DB, et al. Direct magnetic resonance detection of myelin and prospects for quantitative imaging of myelin density. *Proc Natl Acad Sci U S A*. 2012; 109:9605–9610. [PubMed: 22628562]

Abbreviations

4-OHT	4-hydroxy-tamoxifen
ATP+ADP	adenosine-triphosphate + adenosine diphosphate
bFGF	basic fibroblast growth factor
ChAT	choline acetyltransferase
Cr	creatine
CS	cholesterol sulfate
D₂O	deuterated water
DARPP32	Dopamine- and cAMP-regulated neuronal phosphoprotein
EDTA	Ethylenediaminetetraacetic acid
ECF	extracellular fluid

EGF	epidermal growth factor
ES	embryonic stem cell
GABA	γ -aminobutyric acid
GalC	Galactocerebroside C
GFAP	Glial Fibrillary Acid Protein
Glx	glutamine+glutamate
GPC	glycerophosphocholine
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hNSC	human neural stem cell
LGE	lateral ganglionic eminence
mI	myo-inositol
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAA	N-acetylaspartate
n.c	not computed
n.d.	not detected
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
PC	Phosphocholine
PCr	phosphocreatine
PLL	poly-L-lysine
ppm	parts per million
TH	tyrosine hydroxylase
TMS	tetramethylsilane
TSP	sodium 3-trimethylsilyl-2,2,3,3-tetradeuteriopropionate
UTP+UDP	uridine triphosphate + uridine diphosphate

Table 1

Water-soluble metabolites

Metabolites detected in extracts of undifferentiated (n=4) and differentiated (n=4) STROC05 cells are expressed as Mean $\pm$ SEM. Metabolite concentration in fmol/cell. Abbreviations: N-acetyl-aspartate = NAA; phosphocholine + glycerophosphocholine = PC+GPC; uridine triphosphate + uridine diphosphate = UTP+UDP; adenosine-triphosphate + adenosine diphosphate = ATP+ADP; nicotinamide adenine dinucleotide = NAD(H).

Peak	Metabolite	Undifferentiated	Differentiated	Fold Change	p value
2	Threonine	1.82 $\pm$ 0.30	0.56 $\pm$ 0.11	$\downarrow$ 3.25	0.02
3	Lactate	9.55 $\pm$ 3.84	4.06 $\pm$ 0.80		0.29
4	Alanine	4.78 $\pm$ 1.03	0.93 $\pm$ 0.11	$\downarrow$ 5.14	0.03
5	Acetate	2.59 $\pm$ 0.27	2.75 $\pm$ 0.19		0.68
6	NAA	3.65 $\pm$ 0.72	1.85 $\pm$ 0.18		0.09
7	Glutamate	14.25 $\pm$ 2.35	5.96 $\pm$ 0.52	$\downarrow$ 2.39	0.03
8	Succinate	0.32 $\pm$ 0.06	0.22 $\pm$ 0.03		0.21
9	Glutamine	1.80 $\pm$ 0.51	2.74 $\pm$ 0.46		0.26
10	PC+GPC	12.83 $\pm$ 1.33	4.42 $\pm$ 0.90	$\downarrow$ 2.78	0.003
11	Glycine	0.17 $\pm$ 0.06	0.15 $\pm$ 0.04		0.81
12	Creatine	0.20 $\pm$ 0.06	0.13 $\pm$ 0.01		0.33
13	Myo-inositol	9.25 $\pm$ 0.86	0.19 $\pm$ 0.04	$\downarrow$ 48.68	<0.001
14	UTP+UDP	4.09 $\pm$ 0.41	2.08 $\pm$ 0.07	$\downarrow$ 1.97	0.01
15	ATP+ADP	3.91 $\pm$ 0.29	2.32 $\pm$ 0.08	$\downarrow$ 1.69	0.01
16	NAD(H)	0.66 $\pm$ 0.06	0.54 $\pm$ 0.06		0.21

Supernatant of cell culture media of undifferentiated (n=4) and differentiated (n=4) STROC05 cells was analyzed for changes in metabolite uptakes and excretions. Data are expressed as Mean $\pm$ SEM. Metabolite concentration in $\mu\text{mol}/\text{million}$ cells.

Table 2
Metabolite uptake and excretion in the culture media

Cell	Metabolite	Undifferentiated	Differentiated	Change	p value
Uptake	Leucine	0.48 $\pm$ 0.04	0.99 $\pm$ 0.03	$\uparrow$ 2.06	0.001
	Iso-leucine	0.46 $\pm$ 0.04	0.87 $\pm$ 0.09	$\uparrow$ 1.89	0.001
	Valine	0.24 $\pm$ 0.05	0.40 $\pm$ 0.04	$\uparrow$ 1.66	0.02
	Glutamine	2.93 $\pm$ 0.05	3.32 $\pm$ 0.28	$\uparrow$ 1.13	0.05
	Histidine	0.08 $\pm$ 0.02	0.08 $\pm$ 0.03		0.47
	Tyrosine	0.10 $\pm$ 0.01	0.35 $\pm$ 0.07	$\uparrow$ 3.5	0.008
	Phenylalanine	0.13 $\pm$ 0.01	0.29 $\pm$ 0.01	$\uparrow$ 2.23	0.03
Excretion	Glucose	6.91 $\pm$ 0.60	8.81 $\pm$ 0.39	$\uparrow$ 1.27	0.03
	Lactate	31.44 $\pm$ 3.54	16.88 $\pm$ 0.82		0.23
	Alanine	0.58 $\pm$ 0.10	0.35 $\pm$ 0.07	$\downarrow$ 1.65	0.04

Lipid metabolites

Table 3

Metabolites detected in extracts of undifferentiated (n=4) and differentiated (n=4) STROC05 cells are expressed as Mean ± SEM. Metabolites are expressed as Lipid Metabolite/CDCl₃ ratio per million cells. (not detected = n.d.; not computed = n.c.).

Peak	Lipid Metabolite/CDCl ₃	Undifferentiated	Differentiated	Change	p value
2	Cholesterol-C18H3	0.002 ± 0.001	0.008 ± 0.001	↑4.00	0.001
3	Cholesterol ester	0.130 ± 0.02	0.160 ± 0.02		0.45
4	CH3-FA	0.130 ± 0.01	0.180 ± 0.02	↑1.38	0.01
5	Cholesterol-C19H3	0.009 ± 0.001	0.012 ± 0.001	↑1.33	0.05
6	(CH2) <i>n</i> -FA	0.840 ± 0.03	1.040 ± 0.09		0.07
7	Cholesterol ester (1.58ppm)	n.d.	0.110 ± 0.01	↑	n.c.
8	CH2-CH2-CH2	0.460 ± 0.02	0.410 ± 0.02		0.16
9	CH2-CH2-CH=	0.094 ± 0.008	0.115 ± 0.011		0.18
10	2OC-CH2-CH2	0.063 ± 0.005	0.078 ± 0.006		0.10
11	=CH-CH2-CH=	0.024 ± 0.001	0.036 ± 0.002	↑1.50	0.004
12	Phosphatidylethanolamine	0.005 ± 0.001	0.007 ± 0.001		0.07
13	Phosphatidylcholine	0.057 ± 0.003	0.083 ± 0.013	↑1.45	0.01
14	Triacylglyceride	0.004 ± 0.001	0.005 ± 0.001		0.26
15	CH2=CH2	0.053 ± 0.009	0.072 ± 0.010	↑1.35	0.03
16	Plasmalogen	0.001 ± 0.000	0.003 ± 0.000	↑3.00	0.001