Supporting Online Material

Materials and Methods

Cell Culture. Preparation of neurospheres: Neurosphere cultures were prepared essentially as described previously *(13)*. Embryonic day 12 (E12) brains of C57Bl/6 mice were isolated and digested in 2 mg/mL collagenase type-2 for 2 hrs at 37^0C. Cells were filtered through a 40 μm filter three times and plated at a density of 50,000 cells/mL on plates coated with 2-hydroxyethyl methacrylate. Cells were grown in Neurocult Basal Media (NBM) with 10% Proliferation supplement. Growth factors (EGF, FGF-2, 20ng/mL) were added every two days *(13)*. Neurospheres were collected after 14 days and trypsinized to single cells. After washing in phosphate-buffered saline (PBS, pH 7.25), they were resuspended in the PBS and analyzed at different concentrations (0.1-10 X 10^6 cells per sample) using ^{1}H-NMR. For differentiation experiments, neurospheres were plated onto polyornithine/laminin coated coverslips, and maintained in the NBM with 10% Differentiation supplement *(13)*. Preparation of hair follicle-derived spheres: The entire skin from the dorsum of a postnatal day 30 (P30) C57Bl/6 mouse was excised. The skin was digested in trypsin for 1 hr at 30^0C and then 2 mg/mL collagenase type-2 for 2 hrs at 37^0C. Cells were filtered three times using a 40 μm filter and then plated at 30,000 cells/mL on plates coated with 2-hydroxyethyl methacrylate. Cells were maintained in the NBM containing 10% Proliferation supplement for 2 weeks. Growth factors EGF and FGF-2 were added every two days *(14).* The spheres were collected after 14 days and prepared for spectroscopy as above. Primary cultures of astrocytes: Astrocytes were derived from the P2 cortices of C57Bl6 mice, and digested in 2 mg/mL collagenase for 2 hrs at 37^0C *(29)*. Cells were filtered three times using 40 μm filter and plated at 500,000 cells per 10 cm tissue culture dish coated with poly-D-lysine. Cells were maintained in Earle's MEM containing 10% horse serum and 0.6% glucose. The media was changed every two days. After reaching confluency (2 weeks), the cells were detached with trypsin/EDTA, washed three times, resuspended in the PBS, and analyzed at different concentrations (1-10 X 10^6 cells per sample) by ^{1}H-NMR. Primary cultures of neurons: Rat primary hippocampal neurons were purchased from QBM Cell Science. Neurons were plated on poly-D-lysine and laminin coated dishes and cultured in Neurobasal medium at a density of 200,000 cells/mL. The media was changed every 2 days. Two weeks after plating, neurons were collected, washed three times, and resuspended in the PBS for analysis by ^{1}H-NMR. Primary cultures of oligodendrocytes: Primary oligodendrocytes were derived from the P2 cortices of C57Bl/6 mice using a shaking method, as described *(30)*. Cultures were maintained in poly-D-lysine-coated 75cm^2 flasks in plating media (Dulbecco's modified Eagle's medium (DMEM), 20% fetal bovine serum, 1% penicillin-streptomycin) which was changed every 2 days. After 10 days, the flasks were shaken for 1 hr at 200rpm to remove adherent microglia/macrophages, washed with the same medium, and then shaken overnight at 200rpm to separate oligodendrocytes from the astrocyte layer. The suspension was plated onto uncoated Petri dishes and incubated for 1 hr at 37°C to further remove residual microglia and astrocytes that adhere to the dishes. The oligodendrocytes were collected through a 15μm sieve and plated onto poly-ornithine coated culture plates. Purified oligodendrocytes were cultured for 7–9 days in DMEM containing 0.1% bovine serum albumin, 50μg/mL apo-transferrin, 50μg/mL insulin, 30nM sodium selenite, 10nM D-biotin, 10nM hydrocortisone, 200μM L-cystine, 10ng/mL PDGF, and 10ng/mL basic FGF. After 2 weeks in culture, oligodendrocytes were collected and prepared for the ^{1}H-NMR as above. Preparation of oligodendrocyte progenitor cells (OPC): Dissociated neonatal rat forebrains were cultured in DMEM with 10% fetal calf serum on poly-D-lysine coated flasks. After 2 weeks, the flasks were placed on a rotary shaker at 200rpm for 1 hr to remove the majority of loosely adherent microglia. A subsequent prolonged

shake for 16 hrs was used to dislodge OPC from the astrocyte monolayer into the supernatant. OPC were maintained in the Sato's media and processed immediately *(31)*. Other cell lines: The Macrophage cell line (J774) (courtesy of Rebecca Rowehl, Stony Brook University) was maintained in DMEM supplemented with 10% FBS at 37^0C. The T cell line (Jurkat) (courtesy of Dr. Martha Furie, Stony Brook University) was maintained in RPMI supplemented with 10% FBS at 37^0C. Microglia were isolated from cultures of mixed cortical cells as described previously *(32)*. Briefly, cortices from the neonatal C57Bl/6 mice were trypsinized and triturated, and the resulting single-cell suspension was plated into poly-L-Lysine coated 75 cm^2 tissue-culture flasks. The medium (DMEM, 10% FBS, and 40 mg/L gentamycin) was changed every 3 days. After 10-14 days in culture, the mixed cortical cells establish a confluent layer with bright rounded microglial cells visible on top of the layer. These microglia were removed by 15mM lidocaine treatment with gentle shaking. After centrifugation, the pellet was resuspended in the medium and plated onto poly-L-Lysine coated glass coverslips. Isolated embryonic stem cells (ESC, courtesy of Dr. Alea Mills, Cold Spring Harbor Laboratory) were seeded in a 35mm tissue culture dish containing a confluent layer of mouse embryonic fibroblasts. ESC were grown at 37^0C in DMEM containing 15% FCS, 1% L-glutamine, 1% Non-essential amino acids, 1% Penicillin/Streptomycin, 0.2% mercaptoethanol and 0.0001% leukemia inhibiting factor. ESC were passaged every 2-3 days.

Single cell suspensions isolated from mouse whole brains. Whole brains, isolated from E12 and adult P30 C57Bl/6 mice, were digested in 2mg/ml collagenase type-2 for 2 hrs at 37^0C. Cells were filtered through a 40μm filter three times and washed in PBS prior to ^{1}H-NMR analysis.

Biochemical experiments. Neurospheres were treated with cerulenin (5 μg/mL) for 24 hrs at 37^0C. They were then trypsinized, washed, and resuspended in PBS for ^{1}H-NMR. To test if the 1.28ppm biomarker metabolite is in the lipid fraction, neurospheres were solubilized in chloroform:methanol (2:1), sonicated, and analyzed by ^{1}H-NMR. ^{1}H-NMR was also done for palmitic acid (SFA), oleic acid (MUFA), and arachidonic acid (PUFA) as controls, used at 100μg/mL.

Gas Chromatography (GC). GC was performed by Scientific Research Consortium, Inc. (SRC, St Paul, MN). Lipids from NPC and astrocytes were extracted with chloroform:methanol 2:1, the sample was mixed and centrifuged, the aqueous layer was decanted, and the chloroform layer filtered to remove the protein. The solution was then dried with a stream of nitrogen and re-dissolved in chloroform. The lipid extracts were then applied to silica gel thin-layer plates and developed in petroleum ether/diethyl ether/acetic acid, 80:20:1. Lipid classes were visualized 0.1% 2,7-dichlorofluorescein solution under UV light, and then scraped into glass tubes with teflon screw caps. The free fatty acid class was trans-esterified with 12% BF3 in methanol at 75°C. The resulting methyl esters were extracted with water and petroleum ether, dried under nitrogen, re-dissolved in heptane and analyzed by GC. GC analysis was carried out with a gas chromatograph equipped with a 50m x 0.25mm capillary column and linked to an integrator. The column was temperature programmed from 180-220°C at 2°C/min with an initial time of 10min and a final time of 30min. Helium carrier gas and a split ratio of 100:1 was used. Identification of fatty acid peaks was made by comparison with authenticated standards.

NPC transplantation. Five million NPC (1 X 10^6 cells/μL) were grown *in vitro* as neurospheres and trypsinized to single cells before transplantation. NPC were injected transcranially into the left hemisphere of an adult rat cortex (stereotaxic coordinates X:Y:Z=1:1:3 mm from the bregma). The same volume of PBS (5 μL) was injected into the contralateral hemisphere as a sham control. Rats were imaged within 4 hrs after injection, as described below. All animal care was in accordance with institutional guidelines.

Electroconvulsive shock (ECS). ECS experiments were performed using a Ugo Basile (57800) ECS unit. Bilateral ECS was administered via moistened pads on ear clips using a pulse generator in male adult C57bL/6 mice (frequency 50Hz, shock duration 0.5msec, pulse width 0.5msec, and current 50mA) and in male adult Sprague Dawley rats (frequency 100Hz, shock duration 0.5msec, pulse width 0.5msec, and current 50mA) *(33).* ECS was performed at the same time each day for five consecutive days. On the fifth day, animals were injected with 150mk/kg BrdU. Sham-control animals were exposed to the same procedure, but did not receive the shock. Twenty four hours later, mice where either sacrificed and their hippocampi were dissected and prepared for ^{1}H-NMR, or they were perfused and brains prepared for immunostaining with the anti-BrdU antibody. Rats were imaged by mMRI spectroscopy as outlined below. Following mMRI, they were perfused, brains were fixed, sectioned, and immunostained with the anti-BrdU antibody.

NMR spectroscopy. One-dimensional ^{1}H-NMR spectra of the aqueous suspensions of cells (0.55mL, pH 7.25) containing 10% D_2O as a field frequency lock were measured using a 700 NMR spectrometer. Spectral analysis was conducted using XWinNMR, version 3.5. In all the experiments, the temperature was maintained at 35°C and pH at 7.25. The spectra were acquired with a Free Induction Decay (FID, 32,768 points in a spectral width of 8389.3 Hz, readout time of 1.95 seconds, repetition time of 2 seconds and 128 averages). To minimize the large water peak, the water signal was pre-saturated with a low power radiofrequency (RF) pulse. Before Fourier transform, FIDs were line broadened to 1.0 Hz with an exponential weighting function. ^{1}H-NMR spectra were phase and baseline corrected for distortions and referenced to tetramethylsilane (TMS) chemical shift (0.00ppm).

MicroMRI acquisition. Proton mMRI spectroscopy was performed on a 9.4T Biospec Avance 94/20as scanner. Adult Sprague-Dawley rats were anesthetized with ketamine/xylazine mixture and allowed to breathe spontaneously. Anesthesia was maintained with 1-1.5% isoflurane in a 1:1 O_2/air mixture delivered via a T-piece. The rat head was immobilized in a custom-build head holder and positioned on a 3.0cm RF surface coil. Initial hydration was administered by an intraperitoneal injection of Lactated Ringer (4mL/kg/hr). Fluids were continuously administered through the intraperitoneal catheter. To reduce salivation and maintain optimal conditions for spontaneous ventilation, glycopyrulate (0.1mg/kg) was given prior to positioning within the mMRI scanner. The rat ears were covered with cotton/gauze custom-build ear fittings to protect against noise from the mMRI gradients during scanning. A high resolution axial T2 image was used to specify the spectroscopy voxel of interest (VOI, 2.5mm^3), located within the hippocampus for imaging of endogenous NPC and within the cortex for imaging of transplanted NPC. Spectra were collected using point resolved spectroscopy (PRESS), TE/TR = 8ms/2,000ms, and 2,048 data points extending over a spectral width of 16.01ppm (6410 Hz), yielding a spectral resolution of 1.57 Hz/point (imaging time 54min). The suppression bandwidth was a 400 Hz CHESS (chemical-shift selective) pulse. First and second order shims were accomplished using the Fastmap sequence followed by automatic local shim adjustment. The spectrometer frequency and receiver gain were adjusted to achieve 50–60% digitizer filling. Pulse angles were further adjusted to reduce the digitizer filling to between 30–45%. All animal care was in accordance with institutional guidelines.

Human brain MRI. Adult human brain ^{1}H-MRS was obtained using a 3T MRI scanner, with the following parameters: TE/TR=30ms/2,000ms, voxel size 30x12x12mm^3 oriented along the hippocampus and 16x16x16mm^3 within the gray matter in the cortex, spectral width 2,000 Hz, 1,024 points, 128 averages, and total image time 4min 55sec. Five healthy volunteer subjects of both sexes were tested at baseline and 90 days after. In addition, three pre-adolescent (8-10 year

old) and three adolescent (14-16 year old) were imaged using the same parameters. Spectra of cortical and hippocampal regions were obtained and analyzed with custom-made SVD-based signal processing. Informed consent was obtained from all volunteers.

SVD-based signal processing. The signal processing method is implemented interactively and is based on a parametric approach. It is assumed that the data can be represented by a Lorentzian model, which is a superposition of decayed complex sinusoids. Each sinusoid is identified by four parameters, amplitude, initial phase, frequency, and damping factor of which the frequency and the damping factor are nonlinear parameters. This approach is adequate primarily because of the low signal-to-noise ratio (SNR) in the data and the need for high frequency resolution. The various resonances are due to the different metabolites in the sample, and their intensities are proportional to the number of nuclei that resonate at the corresponding frequencies. The method proceeds as follows.

First, a Fast Fourier transform (FFT) of the raw data is computed with the objective of finding the strongest peak in the spectrum of the data. This peak corresponds to water, and the frequency where it is located is used as a reference and is centered at 0 Hz (or at the sampling frequency). Its value in terms of part per million (ppm) is assigned as 4.7 ppm.

In the next step the water is removed from the data. We apply the HSVD method (singular value decomposition of the acquired signal arranged in a Hankel matrix) by first computing the signal poles that correspond to water and from them the signal frequencies and damping factors *(23).* The poles corresponding to water are identified by their location in the z-plane. Then we estimate the amplitudes of the signal components by the least squares method, and proceed with constructing the water signal from the estimated parameters. Subsequently, the water signal is subtracted from the raw data. The spectrum of the signal after water removal is checked for presence of remaining water. If additional water removal is needed, the process is repeated on the residual data. After water removal, we multiply the obtained data with a decaying exponential function with the purpose of increasing the overall SNR. The function is of the form $e^{-\pi c n t_s}$, where $n = 0, 1, 2, \ldots, N-1$ with N being the length of the data set, t_s is the machine's sampling interval, and c is a user defined constant (for *in vitro*, human, and rat data we used $c = 0.5, 0.7,$ and 3, respectively).

Next, we proceed with a second frequency alignment. We first apply FFT to the resulting time series data from the previous step. In the obtained spectrum, one can clearly see either the lactate doublets for the *in vitro* samples, or the NAA peak for the *in vivo* samples. The frequency alignment is performed by centering the lactate doublets to 1.33ppm, or by adjusting the position of the NAA peak to 2.02ppm.

We continue with the method by filtering the frequency band of interest. To that end, we apply the ER (extraction and reduction) filter from *(24).* The filtering amounts to applying FFT to the water-removed data followed by selecting the frequency bandwidth of interest. Then, one shifts the selected spectrum to the baseband and applies the inverse FFT. In the time domain, these operations correspond to convolution of the water-removed signal with the impulse response of an ideal bandpass filter, modulation of the filtered signal, and decimation with a factor equal to the ratio of the original bandwidth and the bandwidth of interest.

The filtered data are then again processed using the HSVD method by first forming the Hankel matrix and computing the SVD of the matrix and the modes of the signal. From the obtained modes, we find the corresponding amplitudes. With the obtained parameters, we construct the signals that we want to remove from the filtered data (these signals are the ones close to the NPC signal in the frequency domain). This procedure is in general conducted iteratively. In the first iteration, the stronger signal components are estimated and removed, and

in the following iterations, the weaker ones. Typically, however, the removal of the signals after the first iteration is sufficient. Once the filtering is completed, there is an additional fine tuning of the frequency, which is followed by detection of the NPC peak. If there is a mode in the final set of data within the window of 1.28 ± 0.025 ppm, we declare that there are NPC in the sample, and from the amplitude of the mode, we can estimate their quantity.

Statistics. Statistical analysis was performed using Statistica. In experiments comparing two groups, Student t-test was used. In experiments comparing three or more groups, the normal distribution of the data histograms and the homogeneity of variances (Levene's test) were determined before Analysis of Variance (ANOVA). ANOVA was followed by post-hoc analysis with Tukey-HSD test (High Significance Difference) for pair-wise multiple comparisons or with the Dunnet test when comparing experimental with a control group. In linear regression analysis, the correlation coefficient R^2 is indicated. All quantification was done with the SVD-based method. Bar graphs represent mean +/- SEM; *, $p<0.05$; **, $p<0.01$; and ***, $p<0.001$. Detailed statistics data for each figure are listed in Supporting Text.

Supporting Text

Fig. 1. The 1.28ppm biomarker identifies Neural Progenitor Cells. (B) The 1.28ppm mean signal amplitudes are: NPC: 1,870 ± 214; N: 4.10 ± 0.32; O: 338.0 ± 82.9; and A: 7.10 ± 0.41. Statistics: ANOVA p=0.000007, Dunnet test: NPC vs N p=0.000016, NPC vs O p=0.00004, and NPC vs A p=0.000016. NAA mean signal amplitudes are: NPC: 6.9 ± 3.5; N: 49.4 ± 5.2; O: 6.20 ± 0.66; and A: 11.2 ± 2.8. Statistics: ANOVA p=0.000047, Dunnet test: N vs NPC p=0.000064, N vs O p=0.000059 and N vs A p=0.00013. Cho mean signal amplitudes are: NPC: 74.1 ± 7.1; N: 169.1 ± 18.9; O: 98.7 ± 7.8; and A: 274.0 ± 8.6. Statistics: ANOVA p=0.000007, Dunnet test: A vs NPC p=0.000014, A vs N p=0.00052, and A vs O p=0.000022. **(C)** The 1.28ppm mean signal amplitudes are: NPC: 15,700 ± 1,130; ESC: 1,370 ± 177; SSC: 6,470 ± 1,530; OPC: 5,530 ± 823; MΦ: 237 ± 96; TC: 100 ± 23; and MG: 657 ± 146. Statistics: ANOVA p<0.000001, Dunnet test: NPC vs ESC p=0.000009, NPC vs SPC p=0.000014, NPC vs OPC p=0.00001, NPC vs MΦ p=0.000009, NPC vs TC p=0.000009, and NPC vs MG p=0.000009. **(D)** The 1.28ppm mean signal amplitudes are: 0.25 X 10^6 NPC = 5,050 ± 435; 0.5 X 10^6 NPC = 8,810 ± 669; and 1 X 10^6 NPC = 16,500 ± 721.

Fig. 2. Analysis of the specificity and molecular composition of the NPC biomarker using ^{1}H-NMR. (A) The 1.28ppm mean signal amplitudes are: D0: 12,400 ± 324; D1: 13,600 ± 392; and D5: 3,890 ± 158. Statistics: ANOVA p=0.000001, Tukey test: D0 vs D1 p=0.084, D0 vs D5 p=0.00023 and D1 vs D5 p=0.00023. The NAA mean signal amplitudes are: D0: 1,220 ± 219; D1: 2,040 ± 37; D5: 2,100 ± 37. Statistics: ANOVA p=0.0046, Tukey test: D0 vs D1 p=0.0093, D0 vs D5 p=0.0066 and D1 vs D5 p=0.94**.** The Cho mean signal amplitudes are: D0: 74 ± 7; and D7: 351 ± 12.7. Statistics: t-test, p<0.000001. **(B)** The 1.28ppm mean signal amplitudes are: E12: 3,080 ± 244; P30: 450 ± 43, t-test p<0.000001. The NAA mean signal amplitudes are: E12: 4,220 ± 853; P30: 16,600 ± 1,470; t-test p=0.002. The mI mean signal amplitudes are: E12: 300 ± 7; P30: 430 ± 31; t-test p=0.013. **(C)** The 1.28ppm mean signal amplitudes are: CTX: 276 ± 76; and HIPP: 1,390 ± 205; t-test p=0.007. **(E)** The 1.28ppm mean signal amplitudes are: control (CTRL): 12,600 ± 665; CRL: 5,570 ± 751, t-test p=0.00078. The Cho mean signal amplitude are: CTRL: 310 ± 8; CRL: 443 ± 6, t-test p=0.00077.

Fig. 3. Identification of NPC in the rat brain *in vivo*, using microMRI spectroscopy. (A) The 1.28ppm mean signal amplitudes are: CTX: 2,790 ± 966; and HIPP: 32,100 ± 8,480; t-test p=0.026. 1.28ppm:Cr ratios are: CTX: 0.00814 ± 0.00140; and HIPP: 0.100 ± 0.038; t-test

p=0.076. **(B)** The 1.28ppm mean signal amplitudes are: ST: 12,000 ± 1,210; and NT: 417,000 ± 123,000; t-test p=0.029. 1.28ppm:Cr ratios are: ST: 0.037 ± 0.037; and NT: 0.817 ± 0.220; t-test p=0.025. **(C)** The 1.28ppm:Cr ratios are: ECS-: 0.0312 ± 0.0070; and ECS+: 0.0647 ± 0.0113; t-test p=0.046. **(D)** The number of BrdU positive cells: ECS-: 975 ± 93; and ECS+: 1,670 ± 166; t-test p=0.011.

Fig. 4. Identification of NPC in the human hippocampus *in vivo*, using ^{1}H-MRI spectroscopy. **(A)** The 1.28ppm mean signal amplitudes are: CTX: $0.116 \times 10^{-6} \pm 0.126 \times 10^{-6}$; left hippocampus (LH): $2.47 \times 10^{-6} \pm 0.78 \times 10^{-6}$; and right hippocampus (RH): $1.88 \times 10^{-6} \pm 0.89 \times 10^{-6}$. Statistics: ANOVA p=0.004, Dunnet test: CTX vs LH p=0.0028 and CTX vs RH p=0.021. The 1.28ppm:Cr ratios are: CTX: $0.217 \times 10^{-2} \pm 0.230 \times 10^{-2}$; LH: $3.12 \times 10^{-2} \pm 0.86 \times 10^{-2}$; and RH: $2.42 \times 10^{-2} \pm 0.74 \times 10^{-2}$. Statistics: ANOVA p=0.023, Dunnet test: CTX vs LH p=0.016 and CTX vs RH p=0.069. **(B)** The 1.28ppm signal amplitudes are: Day 1: $2.47 \times 10^{-6} \pm 0.78 \times 10^{-6}$, t-test p=0.747; Day 90: $3.13 \times 10^{-6} \pm 1.05 \times 10^{-6}$. **(C)** The 1.28ppm mean signal amplitudes are: pre-adolescent: $50.0 \times 10^{-6} \pm 4 \times 10^{-6}$, adolescent: $26 \times 10^{-6} \pm 3 \times 10^{-6}$ and adult: $4 \times 10^{-6} \pm 14 \times 10^{-6}$. Statistics: ANOVA p<0.000001, Dunnet test: pre-adolescent vs adolescent p= 0.004, pre-adolescent vs adult p<0.000001 and adolescent vs adult p<0.000001.