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Systems Biology Approach to Imaging of Neural Stem Cells

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Abstract

Over the past decade, the advances in human brain magnetic resonance imaging (MRI) have significantly improved our ability to gain insightful information about the structure and function of the brain. One of the MRI imaging modalities that still awaits more comprehensive data mining is magnetic resonance spectroscopy (MRS). MRS provides information on the functional status of the brain tissue and can detect metabolic abnormalities that precede structural changes. The chemical specificity of proton MRS (^1H -MRS) allows detection of several biomarkers that are specific for neurons (*N*-acetyl aspartate, NAA) and astrocytes (myoinositol (mI) and choline (Cho)), the two most abundant cell types present in the brain tissue. However, apart from a dozen metabolites, current methodologies utilized for MRS analysis do not allow further biomarker discoveries. Herein, we introduce a bioinformatics approach to MRS data processing and discuss possible discoveries that such approach may provide. Specifically, we describe the methodology for neural stem/progenitor cell (NPC) detection in vitro and in vivo, utilizing metabolomic profiling and singular value decomposition analyses.

Keywords

NMR; MRS; metabolomics; neuroprogenitors; hippocampus; multivariate statistics; singular value decomposition

1. Introduction

The ability to identify human neural stem/progenitor cells (NPCs) by brain imaging may have profound implications for diagnostic, prognostic, and therapeutic purposes. The study of human NPC in vivo has been hindered by the absence of well-defined markers that can distinguish them from other neural cell types. Recently, this obstacle has been approached from a systems biology point of view; namely metabolomics (1).

Metabolomics investigates a global metabolic pool of cells, tissues, and fluids. While metabonomics aims to measure the global, dynamic metabolic response of living systems to biological stimuli or genetic manipulation, metabolomics represents an analytical description of complex biological samples, aiming to characterize and quantify all small molecules in such a sample (2). These small molecules represent a “metabolome,” a metabolic state as regulated by net interactions between the genes and the environment (2). Two major modes of data acquisition in metabolomics are nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) (3). Despite the relative lower sensitivity and specificity, NMR has several advantages over MS, in particular that it could be translated to in vivo studies utilizing the NMR correlate, magnetic resonance spectroscopy (MRS). However, due to inherent limitations of both NMR and MRS instrumentation and spectral analysis, specific care needs to be taken to ensure that data acquisition is as optimal as it can be. Precise data acquisition and processing are most critical when differences

between classes approach the limitations of instrument precision. Studies where the key metabolites are present in high concentrations may not require great sensitivity, whereas studies of less abundant metabolites will require optimizing sensitivity and signal-to-noise ratios (SNR). Thus, new NMR and MRS methodologies geared toward a specific application of interest have to provide standardized experimental and mathematical approaches to enable reliable and reproducible data generation across different users.

In this chapter, we focus on NMR and MRS data acquisition and analysis aimed to identify a variety of metabolic biomarkers in neural cell types in vitro and brain tissue in vivo. Specifically, we describe our methodology for detection of metabolites enriched in NPC in both in vitro and in vivo conditions. We describe spectral preprocessing, statistical multivariate analysis for potential biomarker discovery, and analytical methods used for identification and quantification of individual metabolites. We also note the specific empirical lessons we have learned from our studies. We propose that our methodology and lessons we learned potentially provide the foundation of general approaches, amenable for further modifications based on specific experimental hypothesis, and thus not only for detection of NPC and metabolomic profiling of the human brain.

2. Material

2.1. Reagents

1. Phosphate buffered saline (PBS).
2. PBS + 10% antibiotic (Invitrogen Cat. #15140-122).
3. Epithelial growth factor (EGF). Prepare a stock solution of 20 µg/mL of rhEGF (Sigma Cat. #E9644, powder at 200 g/vial) by adding 0.1 mL of sterile 10 mM acetic acid (Mallinckrodt Cat. #V193-05) with 0.1% BSA (Sigma Cat. #A1470) to initially dissolve the rhEGF powder and then add 9.9 mL of NeuroCult basal medium with 10% proliferation supplements. Store stock solution as 0.5 mL aliquots at −20°C. Add 1 µL of 20 µg/mL rhEGF to every 1 mL of NeuroCult basal medium with 10% proliferation supplements to give a final concentration of 20 ng/mL of rhEGF. Mix well.
4. NeuroCult proliferation supplements (StemCell Technologies Cat. #05701) should be thawed for 1 h at 37°C before addition to the NeuroCult basal medium (StemCell Technologies Cat. #05700). A white precipitate may form during storage at −20°C which will disappear upon thawing at 37°C. Add the entire volume of NeuroCult proliferation supplements (50 mL) to one bottle (450 mL) of NeuroCult basal medium. Avoid repeated exposure of medium to room temperature and light. If the entire volume is not needed immediately, aliquot into appropriate volumes to be used within 1 week.
5. NeuroCult proliferation medium (StemCell Technologies Cat. #05700) with EGF (20 ng/mL).
6. NeuroCult chemical dissociation kit (StemCell Technologies Cat. #05707).
7. Trypan blue.
8. D₂O, >99.9% atom% D.
9. 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS, Cambridge isotope laboratories) for calibration of NMR spectra.

2.2. Equipments

1. For metabolomic experiments of cultured cells: NMR spectrometer (500–900 MHz, Bruker or Varian). NMR fingerprinting can be done at any operating frequencies, but the higher, the better. Use a 5-mm diameter ^1H -detection NMR probe and NMR processing software (Topspin 2.1 (Bruker); HiRes (<http://mrs.cpmc.columbia.edu/hires.html>)).
2. For metabolomic experiments on animal brain in vivo: 9.4 T mMRI scanner (Biospec Avance 94/20 as, Bruker Biospin MRI, Inc.) with operating system Paravision 5.0 and newer versions. Higher magnetic strength may also be used; however, MRS acquisition might be technically more difficult.
3. For metabolomic experiments on human brain in vivo: 3 T MRI scanner (Phillips, Siemens, GE).
4. Software: Excel for data normalization; SIMCA-P, version 11 (Umetrics AB) for multivariate statistical analysis.

3. Methods

3.1. NPC Preparation In Vitro

1. NPCs are derived from the mouse embryos at gestational day E12–13. It is very important to dissect the embryos at this stage and not later, as properties of NPC change with age. Thus, to derive NPC at E12–13 consistently, one needs to establish a timed-pregnancy colony. NPC can be derived from both wild type and transgenic animals in which NPC-specific promoter nestin drives the expression of fluorescent proteins, as the expression of these proteins does not affect the metabolomic profile of cultured NPCs.
2. Mother is euthanized in the CO_2 chamber.
3. Abdomen is washed with 70% ethanol (EtOH), opened, and the embryos are removed. They are transferred to a 10-cm dish with cold PBS, pH 7.25 and 10% antibiotics, placed on ice. This part can be done on the lab bench, particularly if using heterozygote colony of transgenic mice. Thus, embryos that express fluorescent proteins may be selected under the microscope.
4. The selected embryos are transferred to the tissue culture room. The embryos are taken out of the sac and rinsed twice with cold PBS, pH 7.25 with 10% antibiotics.
5. The remaining of the procedure is done in a laminar flow hood under sterile conditions. Embryonic brains are dissected out and transferred to a 35-mm plate containing cold PBS. Using tweezers, the tissue is triturated to obtain a fine suspension (make special care not to introduce air bubbles into the tissue suspension). The suspension is filtered through a 40- μm strainer (BD Cat. # 352340) directly into a 50-mL tube.
6. The cells are centrifuged at 1,000 rpm (or $200\times g$) for 5 min. Supernatant is removed and the pellet resuspended smoothly (no bubbles) in NeuroCult proliferation medium with EGF (20 ng/mL). Count cell numbers (a 1/5 or 1/10 dilution could be necessary) with Trypan Blue and hemacytometer. Plate 5×10^6 cells per 75 cm^2 flask and maintain the cells in the tissue culture incubator at 37°C and 5% CO_2 .
7. After 2 days in culture, the cell aggregates are visible. Collect the medium containing floating neurospheres and centrifuge them at 1,000 rpm for 5 min.

Discard the medium and cell debris and proceed with the NeuroCult chemical dissociation kit (StemCell Technologies Cat. #05707) to get single cell suspension. If necessary, use a cell strainer to discard non-disaggregated neurospheres.

8. Count cell numbers (a 1/5 or 1/10 dilution could be necessary) with Trypan Blue and hemacytometer. Again, plate 5×10^6 cells per 75-cm² flask and maintain them at 37°C/5% CO₂ incubator.
9. Keep neurospheres at 100–200 µm in diameter using the NeuroCult chemical dissociation kit twice per week. As the neurosphere size increases, the possibility of hypoxia in the neurosphere core also increases. This may jeopardize the “healthiness” of the neurosphere as cells in the core may undergo cell death. Thus, regular dissociation and replating are necessary.
10. After two passages/dissociations (approx. 7 days of culture), proceed with experiments. Cultured neurospheres obtained and maintained the way described here may be used for up to 10 passages and optimally by 6–7 passages. Thus, we routinely prepare fresh batch of neurosphere cultures once a month.

3.2. NMR Data Acquisition

1. Dissociate the neurospheres to single cell level. The cell quantity required to obtain a reliable and reproducible metabolomic profile using the NMR depends on the magnet strength. For our studies, we used 10×10^5 to 10^6 NPC to obtain the NPC-associated signal utilizing 700 MHz NMR (4). Furthermore, all experiments are done in triplicate within the same culture (i.e., on the same day) to ensure reproducibility, with $N = 3$ different cultures to ensure specificity (*see Note 1*). Control solutions are run with each experiment (*see Note 2*).
2. Centrifuge the sample at 1,000g for 1–2 min at 4°C to remove the culture media. Use PBS to resuspend the pellet. Add 0.45 ml PBS and 0.05 ml D₂O with 0.3 mM DSS as concentration standard and chemical shift reference (calibrated to 0.00 ppm). The sample may be recovered after the spectra collection and stored at –80°C for several weeks to allow further measurements by NMR or other analytical techniques.
3. Set acquisition temperature for NMR between 26 and 36°C depending on the type of analysis. Load sample into probe and leave sufficient time to equilibrate. Pay special attention to use powder-free gloves when handling NMR tubes to avoid fatty and other deposits from bare hands onto the glass (*see Note 3*).
4. Tune and match the probe. Set the RF carrier frequency offset value to the H₂O resonance and determine the water saturation power. Transfer these settings to the experiments to be submitted. Select 1D NOESY-presat pulse sequence (on a Bruker instrument, this is called *noesypr1d*). Record an NMR spectrum. The time required to obtain the consistent spectra depends on the magnet strength and can vary from 3 to 30 min. Figure 21.1 shows NMR of the NPC acquired at 800 MHz NMR (Bruker) with specific emphasis on the 1.28 ppm peak enriched in NPC (4) (*see Note 4*).

¹The same NMR acquisition methodology can be applied for metabolome analysis of other cell types, such as primary cultures of neurons, astrocytes, oligodendrocytes, other stem cells, or any other isolated cell population of interest (4).

²To ensure the specificity of the findings, all control solutions should be analyzed in parallel. These include media, conditioned media (also useful for footprinting of excreted metabolites), and any compounds or kits used to dissociate and wash the cells.

³The described method utilizes live cells and not cell extracts. Thus, special care needs to be taken to ensure the homogeneous suspension of cells in the NMR probe.

3.3. microMRI Data Acquisition

1. Sprague-Dawley adult rats are anesthetized with isoflurane and allowed to breathe spontaneously. Anesthesia is maintained with 1–2% isoflurane in a 1:1 O₂/air mixture delivered via a T-piece.
2. To reduce salivation and maintain optimal conditions for spontaneous ventilation, glycopyrrulate (0.1 mg/kg) is given prior to positioning within the mMRI scanner. Rodent head is immobilized by a custom-build head holder and positioned on a 1 cm RF surface coil. Initial hydration is given by an intraperitoneal injection of lactated ringer (4 cc/kg/h). Fluids are continuously administered through the intraperitoneal catheter. Heart rate, body temperature, and respiratory rate are continuously monitored. The rodent ears are covered with cotton/gauze custom-build ear fittings to protect against noise from the MRM gradients during scanning.
3. The MR acrylic holder with the anesthetized and monitored rodent and RF coil is positioned in the center of the 9.4 T magnet. Physiological monitoring is continued from afar via a computerized system (PC-SAM, SA Inc.) which also allows ¹H-MRS data acquisition using cardiac and respiratory scan synchronizations.
4. T2-weighted fast spin echo MR images (TR/TE 2,000/20 ms) are collected with in-plane spatial resolution of 100–200 μm and consecutive 500 μm slices. Single ¹H-MRS voxel-of-interest (VOI) is centered over the region of interest. For investigations of NPC, the VOI is placed within the hippocampus. Voxel size is approximately 2.5 × 2.5 × 2.5 mm³, tailored to the size of animal's brain.
5. Data are acquired using a double spin echo sequence (Point RESolved Spectroscopy, PRESS, 8,192 acquisitions, 4 kHz bandwidth, TR/TE=2,000/20 ms, NEX=1,024 water suppressed spectra, total acquisition time = 60 min). The T2 decay of the unsuppressed water signal from the PRESS experiment is measured at 10 different echo times to determine the metabolite concentrations corrected for the partial volume of CSF (5). This approach allows minimization of inter- and intra-subject variability of the ¹H-MRS data sets as it allows measuring of the water content in vivo with a precision of 1.5% (5) (*see Note 5*).

3.4. MRI Data Acquisition

1. The MRI imaging of human subjects is done at 3 T MRI scanner (Phillips Achieva). The hippocampal voxel is planned using a 3D T1-weighted, inversion-prepared, spoiled gradient echo image, acquired axially and reformatted in the sagittal and oblique coronal planes to visualize the hippocampus (sequence parameters: TE/TR/TI = 5/8/400, matrix = 256 × 256, resolution 1 mm isotropic, SENSE factor 1.6, 160 slices).
2. Hippocampal voxel is placed along the full length of hippocampus, as determined on axial, sagittal, and coronal sections. The VOI size is tailored to the individual brain and is approximately 27.5 × 10 × 10 mm³ for the adult brain.

⁴Since the cells are resuspended in PBS and not media, they are deprived from all the nutrients and most likely switch to an anaerobic metabolism, leading to the presence of the lactate doublet (1.33 ppm) observed in most of the in vitro spectra.

⁵Metabolomic analysis of the MRS data is challenging and high-quality data are critical for the success of this work. Some of the issues which may be encountered in MRS are (1) poor shimming, in particular of the hippocampal spectra (the irregular structure and adjacent CSF provide a challenge for shimming), (2) suboptimal water suppression, which introduces baseline deviations and affects accurate metabolomic peak area estimates, (3) measurement of short T2 metabolites, which may be important for improving the metabolomic profile, (4) insufficient signal-to-noise ratio (SNR) for detection of low concentration metabolites. SNR can always be improved with increased scan time or by testing alternate coil options, including highly parallel coils (>64 channels) which are beginning to show marked SNR improvements for brain scanning.

3. Pre-scan calibrations include an in-house developed, second-order iterative shimming procedure to obtain minimal line-width and maximal separation of metabolic components and an adiabatic, hyperbolic secant CHESS water suppression pulse utilizing an in-house developed optimized search algorithm for obtaining complete water suppression and minimal baseline deviation. Minimal line-width should be between 10 and 15 Hz.
4. The ^1H -MRS is preformed using a PRESS sequence with the following acquisition parameters: TE/TR = 30/2,000 ms, spectral width 2 kHz, 1,024 points, NEX 128, and a total scan time of approximately 5 min (*see* Note 5).

3.5. Spectral Data Processing for Metabolomics Analysis

1. Preprocessing converts the raw spectra into an appropriate form for the statistical analysis. In general, preprocessing includes data import and organization by high-resolution MRS (HiRes). In HiRes, NMR spectra acquired from Bruker spectrometers are imported automatically or by manually entering parameters. Series of spectra are imported in a single step. All the spectra and associated information are organized in a project and listed in a table that can be easily sorted by each column.
2. Spectral data processing procedures can be applied either to all the spectra or to the selected individual spectra. Free induction decays (FIDs) are zero-filled or truncated to desired, specified length. Binning is assigned, and spectra are divided into small regions or bins. The averaged intensity of each bin is used to generate a list of variables that represent the original spectrum. Spectra are compared based on the same bins or variables. Constant phase and linear phase corrections are performed. The disadvantages of binning are loss of spectral resolution, generation of possible artifacts caused by signals on the border of bin boundaries, and compensation of intensities within individual bins. Temporal filtering (Gaussian 5) is performed and then the baseline is corrected to remove the baseline distortion and obtain the correct signal intensities. The next step in data processing is water suppression, resonance adjustment (ppm), and calibration or peak alignment (0.00 ppm of DSS).
3. The spectra are then exported from HiRes in the text format into Excel, where they are normalized. Normalization is essential to reduce the systemic variations in different samples and bring them to a more comparable scale. Spectra can be normalized to the total spectral integral or to the specific reference metabolite, such as creatine – a single peak around 2.98–3.08 ppm. We prefer to use the former method, as it is more general, particularly when analyzing *in vitro* data and spectra from different species where exact metabolite identities are not known for all the peaks. Furthermore, creatine amplitude may differ in disease conditions and thus may skew the data interpretation. The normalized file is saved as delimited unicode text file for multivariate statistical analysis.
4. Multivariate statistical analysis of metabolite fingerprints is performed by SIMCA 11 P. The statistical methods used to analyze sets of metabolite NMR/MRS fingerprints fall into two main categories: unsupervised methods such as principal component analysis (PCA) and supervised methods such as partial least squares-discriminant analysis (PLS-DA). PCA is an unsupervised analysis which highlights the differences in a data set using a smaller number of factors, called principal components (PC), which contain the most variance through the data set (6). The collection of all NMR or ^1H -MRS spectra can be thought of as a matrix with a size of $N \times K$ dimensions, where N is the number of observations (individual patient

spectra) and K is the number of variables, often integrals over small segments of the spectra, which can be narrowed down to the individual digital points of the spectra (6). The first principal component (PC) is the vector that accounts for the most variation in the data, and each observation is projected onto this line. The second PC is represented by another vector in K dimensional space, orthogonal to the first PC, and represents the second largest variation in the data set. Many PCs may be obtained from a single data set; however, the variation quickly tapers off within a few consequent PCs. The PCA data are presented as a score plot, which reveals clustering in the observations based on similarities in the spectra. Additionally, a loadings plot is obtained from the PCA, which assists in the interpretation of the score plot by highlighting the spectral regions responsible for clustering (7). In $^1\text{H-MRS}$ data, it is expected that actual peaks, not noise, are the highest contributing factors.

5. Forms of scaling. The results of both PC and PLS modeling are scale dependent. Selecting the scaling of the variables is therefore an important step in fitting projection models such as PC or PLS. The data are scaled to reduce the dominance of large spectral features. If one has no prior information about the importance of the variables, autoscaling all variables to *unit variance* (UV) is recommended. This is equivalent to making all of the variable axes have the same length, which is, giving all of the variables equal importance. In UV, the variable j is centered and scaled to “unit variance,” i.e., the base weight is computed as $1/\text{sd}_j$, where sd_j is the standard deviation of variable j computed around the mean. If one has prior information about the importance of the variables, it may be desirable to scale some variables up or down by modifying their unit variance weights. A modifier can be selected (default = 1.0) that changes the scaling of a variable relative to its base weight. Block scaling can also be specified. The SIMCA default for all selected variables is a UV base weight and a block scaling factor of 1 (no block scaling) and thus needs to be changed if one desires to apply modified scaling. Another form of scaling is *Pareto scaling*. There, the variable j is centered and scaled to Pareto variance, i.e., the base weight is computed as $1/\sqrt{\text{sd}_j}$, where sd_j is the standard deviation of variable j computed around the mean. Pareto scaling is in between no scaling and UV scaling and gives the variable a variance equal to its standard deviation instead of unit variance. The scaling weight of the variable may also be frozen (will not be recomputed when observations in the workset change or the variable metric is modified after the freezing) (*see* Note 6).
6. The default workset, at the project start, is the whole data set with variables defined as X s and Y s as specified at import and scaled to UV. The associated model (unfitted) is listed in the active area. To fit a model with excluded variables, transformations, or different scaling, it is necessary to first modify the workset. An unfitted model is generated by SIMCA-P when the workset is specified (select a starting workset new or as model). It uses the whole original primary data set with X s and Y s as defined at import. The next step is to include or exclude observations or group them into classes for classification. X/Y variables are defined, transformation is performed, and scaling is selected. A modifier can be selected (default = 1.0) that changes the scaling of a variable relative to its base weight. Block scaling can also be specified. Trimming/Winsorizing the workset does not affect the data set as a whole but just that particular workset. The model level options are specified and then the model is fitted.

⁶All scaling methods should be tested on the data before selecting the best method. We recommend using UV scaling for in vitro data and Pareto scaling for in vivo data.

7. The scores plot is generated by PCA or PLS-DA. PC of the given table (or parts of it) gives vectors of scores, with values t_i , which summarize all the variables entering the analysis. It is customary to calculate two or three score vectors, up to 10, and then plot them against each other (tt-plots). This provides the best summary of the process behavior. In scores plot we can observe trends, unusual behaviors (such as outliers), and other information of interest.
8. In addition, the PC loadings plot is generated, demonstrating the variables responsible for data clustering in the scores plot. The PCA also provides values of the so-called loading vectors, showing how the variables are combined to form the scores. A plot of the loading vectors indicates which of the variables are important and correspond to the directions in the scores plot (*see* Note 7) (8).

3.6. Spectral Data Processing Using Singular Value Decomposition-Based Algorithm

The SVD-based program may be utilized for detection and quantification of molecules that are present in low quantities in the obtained spectra. The main assumption of our SVD-based algorithm is that the individual metabolites resonating in the magnetic field have Lorentzian shape. This assumption can be challenged if the magnetic field is not homogeneous during the acquisition. Thus, the robustness of the SVD-based algorithms has to be tested if such inhomogeneity is highly suspected.

Our SVD-based software has been developed in MATLAB and primarily used for detection and quantification of 1.28 ppm peak, associated with NPC, in the spectra acquired in vivo (4). The input to the program is a time domain free induction decay (FID) signal represented by complex data.

1. Remove the water in the raw data using an SVD-based method. Apply a fixed model of order 10 and remove the peaks located in the water region.
2. From the obtained data, estimate the noise variance σ^2 . Perform line broadening by multiplying the signal with an exponentially decaying sequence.
3. Apply fast Fourier transform (FFT) to the data and calibrate the spectrum using the DSS peak (0.00 ppm) for in vitro spectra or *N*-acetyl aspartate (NAA, 2.02 ppm) peak for in vivo spectra.
4. If necessary, using SVD, remove the nuisance metabolic peaks in the vicinity of the peak of interest.
5. Select the final quantification region based on the location of the peak of interest. Filter the data with the predefined bandwidth which is around 0.5 ppm. Process the filtered data with models of orders $K = 2, 3, \dots, K_{\max}$. A signal is considered to be detected with a given model if the SVD frequency estimates of the signal are in a small bandwidth centered at the frequency of the biomarker. Soft constraints are imposed so that the estimated damping factor of the detected signal must be between 0 and 15 Hz. The estimated signal power is the median of the estimated powers from the different models.
6. The SNR of the detected signal is calculated as a function of the estimated signal amplitude a , estimated signal damping factor d , signal length N , and the estimated data noise variance σ^2 and according to

⁷Multivariate statistical analysis indicates metabolomic differences in main neural cell types and points to certain metabolites enriched in NPC which distinguishes them from the rest of the cells found in the healthy brain (1). However, this methodology may be applied to any spectral analysis regardless of the mode of acquisition and thus for both in vitro and in vivo data acquisition.

$$\text{SNR} = 10 \log \frac{|a|^2}{\sigma^2} + 10 \log \frac{1 - e^{-2dN}}{N(1 - e^{-2d})},$$

where the SNR is expressed in dB. Based on the SNR value, the reliability of the quantification is determined. For example, for human brain ¹H-MRS data, 80% reliability in signal quantification (such as 1.28 ppm peak enriched in NPC) is obtained when the minimal SNR required is −20 dB. An example of the human MRS of the hippocampal voxel and the SVD-based processing of the spectral range of interest is shown in Fig. 21.2 (*see* Note 8).

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⁸The presented methodologies for metabolomics analysis and quantification of the selected peaks using SVD-based algorithms are always subjected to problems associated with biological variation and closely tied analytical validation. Thus, optimization of the acquisition and processing methods is essential to provide informative and reproducible data.

Fig. 21.1.

Spectral profile of live cultured neural progenitor cells (NPC; 100,000 cells) obtained using 800 MHz NMR and 10 min acquisition. *Arrow* indicates the spectral peak of a metabolite enriched in NPC, resonating at 1.28 ppm. Inset shows enlarged spectral area between 0.8 and 1.4 ppm. Lactate doublet (1.33 ppm) is visible, as well as a CH₃-associated peak (0.88 ppm).

Fig. 21.2.

¹H-MRS of the human brain hippocampus in vivo, obtained using a 3 T MRI scanner (Phillips, Achieva) and 5 min acquisition. **a** Anatomical scans are used to localize the hippocampal voxel of interest. FFT spectra are shown. **b** Singular value decomposition (SVD)-based spectral processing of the spectra obtained in (**a**). Modeling of the individual peaks within the region of interest (1.0–1.5 ppm) outlines the 1.28 ppm metabolite (peak of interest).