SPME Probes: A Novel Chemical Biopsy Tool for Spatially Resolved Profiling of Human Brain Tissue in vivo

Barbara Bojko (✉ bbojko@cm.umk.pl)

Nicolaus Copernicus University

Joanna Bogusiewicz

Nicolaus Copernicus University

Katarzyna Burlikowska

Nicolaus Copernicus University

Kamil Łuczykowski

Nicolaus Copernicus University

Karol Jaroch

Nicolaus Copernicus University

Marcin Birski

10th Military Research Hospital and Polyclinic

Jacek Furtak

10th Military Research Hospital and Polyclinic

Marek Harat

10th Military Research Hospital and Polyclinic

Janusz Pawliszyn

University of Waterloo

Research Article

Keywords: brain, SPME, chemical biopsy, neurochemistry

Posted Date: July 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-668958/v1>

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Abstract

It is extremely challenging to perform chemical analyses of the brain, particularly in humans, due to the restricted access to this organ. Imaging techniques are the primary approach used in clinical practice, but they only provide limited information about brain chemistry. Solid-phase microextraction (SPME) has been presented recently as a chemical biopsy tool for the study of animal brains. The current work demonstrates for the first time the use of SPME for the spatially resolved sampling of the human brain *in vivo*. Specially designed multi-probe sampling device was used to simultaneously extract metabolites from the white and grey matter of patients undergoing brain tumor biopsies. Samples were collected by inserting the probes along the planned trajectory of the biopsy needle prior to the procedure, which was followed by metabolomic and lipidomic analyses. The results revealed that studied brain structures were predominantly composed of lipids, while the concentration and diversity of detected metabolites was higher in white than in grey matter. Although the small number of participants in this research precluded conclusions of a biological nature, the results highlight the advantages of the proposed SPME approach, as well as disadvantages that should be addressed in future studies.

Introduction

Neuroimaging methods enable the analysis of anatomical structures and functional changes in the brain. These techniques are based on wide range of physicochemical phenomena, such as electrical activity (e.g., encephalography), X-ray measurements (e.g., computed tomography), and magnetic field and computer-generated radio waves (e.g., magnetic resonance). Although the further development of these methods (e.g., the injection of radiocontrast or radiotracers) has enabled greater temporal and spatial resolution and analysis of functional changes in the brain, more invasive techniques are required for detailed explorations of the chemical composition of brain tissue ^{1,2}. Histopathology is the gold standard for diagnosing and analyzing diseases in clinical analysis, especially in neuro-oncology, followed by genetic and biochemical testing ³. However, the main disadvantage of “out of organism” examination is the fact that enzyme activity does not automatically stop when the sample is removed ⁴. To overcome this problem, some *in vivo* approaches, such as microdialysis (MD) and methods based on electrochemical detection, have been applied in neuroscience applications ⁵.

The development of mass spectrometry (MS) has yielded new sampling techniques that can be applied for *in vivo* brain analysis. One such technique is rapid evaporative ionisation mass spectrometry (REIMS), which measures analytes present in “smoke” generated during the electrosurgical cutting of tissue. Another MS-based technique is probe electrospray ionization (PESI), which entails depositing a drop of water mixed with ethanol on the tissue surface. Analytes are extracted via this solution, and subsequently transferred to the electrospray ionization source of the mass spectrometer with a metal needle, which enables the ionization of the analytes when connected to high voltage ^{6,7}.

In recent years, solid-phase microextraction (SPME) has been successfully applied in *in vivo* studies ⁸, including brain analyses of macaques ⁹ and rats ^{10–13}. SPME is based on the interaction between

compounds in a sample and an extractive sorbent, with extraction taking place until equilibrium is reached between the respective analyte concentrations in the sample and sorbent. Under certain conditions (i.e., when the volume of the sample is much higher than the volume of the extraction phase) the amount of analyte extracted is independent of the sample volume ¹⁴. Therefore, SPME can be used successfully in *in vivo* studies where the volume of the sampled tissue or body fluid is not known or difficult to measure. The sampling procedure involves inserting a thin fiber coated with a biocompatible sorbent into the tissue for a predetermined period of time. Following sampling, the fiber is removed and subjected to a desorption step; after this desorption step has been completed, the extracted substances can be analysed. *In vivo* SPME is commonly referred to as a “chemical biopsy,” as there is no need to remove tissue or draw blood in order to analyze small metabolites ^{9,10,12,13,15}.

The present study aims to assess SPME's suitability for the spatially resolved metabolic characterization of living human brain tissue using a single sampling device and a short extraction time. Ultimately, the proposed strategy is intended to be applied for the simultaneous extraction of biomarkers from neoplasma and non-neoplastic tissue, as well as from injured and non-injured tissue. To simplify the experiment and uniform sampling procedure in this proof of concept study, a profiling of white and grey matter was selected.

Materials And Methods

Chemicals

External calibrant Pierce LTQ Velos ESI Positive Ion Calibration Solution and Pierce LTQ Velos ESI Negative Ion Calibration was purchased from Thermo Scientific, while all other chemicals were purchased from Sigma Aldrich (Poznan, Poland). This research used LC-MS-grade isopropanol, methanol, water, acetonitrile, and mobile phase additives (formic acid, acetic acid, and ammonium acetate), and HPLC-grade chloroform. The probes were prepared using N,N-dimethylformamide ACS reagent and polyacrylonitrile.

Materials

Three types of SPME fibers were used in the experiments: fibers coated with mix-mode ((MM) mixture of strong cation-exchange (SCX) and octyl (C8) particles) and octadecyl (C18) sorbents, both of which were kindly provided by Supelco, Merck; and hydrophilic-lipophylic balanced (HLB) fibers, which were manufactured in-house according to the protocol described in ¹⁶. HLB particles were kindly provided by Waters (Wilmslow, U.K.).

Human biological material

Sampling was conducted at the 10th Military Research Hospital and Polyclinic in Bydgoszcz during brain biopsy procedures on patients with brain lesions localized at least 2 cm below the brain surface. Chemical biopsies (SPME samplings) were performed simultaneously on white and grey matter not

affected by neoplastic changes using a specially designed device (Fig. 1). An acquisition time of 4 minutes was employed for sampling. Samples were obtained from eight patients whose detailed characterization is presented in Table S.A.1. Written consent was obtained from each patient prior to undergoing the biopsy procedure.

In order to avoid additional brain damage, the SPME fibers were inserted along the planned trajectory of the biopsy needle. Sampling was performed by the neurosurgeon conducting the brain tumor biopsy procedure.

The study was approved by the Bioethical Committee of Nicolaus Copernicus University at Collegium Medicum in Bydgoszcz (KB 142/2017). The study protocol was performed in accordance with the relevant guidelines. The written informed consent and consent for publication was obtained from human participants.

Solid-phase microextraction

General protocol for SPME

Fibers with a coating length of 3 mm were used for the experiments. To increase the coverage, four fibers were immobilized together at a set position, with polyacrylonitrile (PAN) being used as a biocompatible glue. Two longer fibers were introduced into the white matter of the brain, while the other two (1 cm shorter) were inserted into the grey matter. The construction of the device is illustrated in Fig. 1. Depending on the analysis, two types of sorbent were used: MM for metabolomics, and C18 for lipidomics.

Metabolomic analysis

Prior to sampling, the MM-fiber-based devices were preconditioned overnight in methanol:water solution. After preconditioning, the fibers were transferred to individual glass vials, where they were sterilized in ethylene oxide in accordance with hospital protocols. The prepared devices were then used to conduct sampling during the biopsy procedure (Fig. 2). The sampling procedure (extraction of analytes) had a duration of 4 minutes. After extraction, the SPME device was removed and washed for 3 sec. via static immersion in water. Following washing, the device was dismantled, and the fibers were separated, securely placed in labeled vials, and stored at -30°C until liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis. Immediately prior to instrumental analysis, the metabolites were desorbed from the fiber using an acetonitrile:water (80:20 v/v) solution for 1 hour with agitation at 1200 rpm. To increase recovery rates, two fibers used to sample the same structure were desorbed together in one vial with 150 µl of desorption solvent.

Lipidomic analysis

Before being used, the C18-fiber-based devices were immersed in a chloroform:methanol (2:1 v/v) solution for 45 min with agitation at 1200 rpm, and then preconditioned overnight in methanol:water (1:1 v/v) solution. Following preconditioning, the fibers were sterilized using the same protocol as was used

for the MM devices. Similarly, the experiments using the C18-fiber-based devices also utilized the same sampling and storage procedures used in the experiments for the MM-based devices. Directly before instrumental analysis, the analytes were desorbed from the fibers via submersion in 150µl of an isopropanol:methanol (1:1 v/v) solution for 1 hour, with agitation at 1200 rpm. As in the metabolomics analyses, the two fibers used to sample each structure (i.e., two fibers for grey matter, and two for white matter) were desorbed together in one vial in order to increase recovery. Silanized glassware was used for the lipidomic analyses.

Selection of SPME coating for lipidomics

The effectiveness of the sorbents used in the lipidomic studies was assessed using brain tissue from a C57BL/6 mouse. According to European Union regulations, permission from the Local Ethical Commission is not required for the *post mortem* use of animal tissue or organs for scientific purposes. The mouse was euthanized via cervical dislocation, and its brain was immediately collected and stored at -80°C until analysis. Prior to sampling, the brain was thawed at room temperature, and then SPME probes were then inserted into the hypothalamus for 15 min. The six fibers used for sampling were preconditioned overnight in a solution consisting of methanol:water (1:1 v/v) (three C18 and three HLB). After sampling, the fibers were desorbed under the same conditions used in the lipidomic analyses (described above).

Liquid chromatography-high resolution mass spectrometry (LC-HRMS)

Liquid chromatography coupled with high-resolution mass spectrometry (Q Exactive Focus) was performed. Data acquisition was performed using dedicated Thermo Scientific software, namely, Xcalibur 4.2 and Free Style 1.4.

The mass spectrometer was externally calibrated, resulting in mass accuracy of < 2 ppm.

Samples were randomized within the sequence, and instrument performance was monitored by running pooled quality control (QC) samples composed of 10 µl of each sample every 4–5 injections. Instrumental blanks (desorption solvent) and extraction blanks (negative control) were also injected every 4–5 samples.

The metabolomic analyses utilized reversed-phase liquid chromatography (RPLC) and hydrophilic-interaction liquid chromatography (HILIC) in positive and negative ion mode. The protocol used in these analyses has been detailed in ^{17,18}.

The structure of the detected compounds was confirmed by matching their fragmentation patterns to spectra libraries at a mass accuracy of < 3ppm. To this end, full MS/dd-MS2 discovery mode was used. The fragmentation parameters were described in Supplementary Material A.

Reversed-phase liquid chromatography (RPLC) and hydrophilic interaction chromatography (HILIC) in positive ion mode were conducted for the lipidomic analyses. The protocol used for these analyses is detailed in ¹⁷ and with some modification in ¹⁸.

As with the metabolomics analyses, the structure of the detected compounds was confirmed by matching their fragmentation patterns to spectra libraries at a mass accuracy of < 3ppm. Full MS/dd-MS2 discovery mode was used for this purpose. The fragmentation parameters were described in Supplementary Material A.

Data processing and statistical analysis

Compound Discoverer 2.1. (Thermo Fisher Scientific, San Jose, California, USA) software was used for metabolomics data processing and statistical analysis.

The data for the lipidomic studies was processed using LipdSearch 4.1.30 (Thermo Fisher Scientific, San Jose, California, USA).

Multivariate statistical analysis was performed using the online Metaboanalyst software package ¹⁹. Missing values were estimated and replaced by small numbers (i.e., half of the minimum positive values in the study data). Logarithmic transformation and autoscaling were also applied. Finally, the following multivariate approaches were utilized: principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and volcano-plot.

Basic statistical analysis was performed using Statistica 13.3 PL (StatSoft, Inc., Tulsa, Oklahoma, USA) software, while homogeneity was assessed via Levene's test and normality was checked using the Shapiro-Wilk test. The Student's t-test was used to evaluate the statistical significance of the differences between the studied groups, and the Mann-Whitney U test was employed when hypotheses about the normality and homogeneity of variance were not fulfilled. A p value of 0.05 was set as a threshold of statistical significance, and an F-test was used to compare the standard deviations.

Results

Chemical biopsy device

The four-fiber SPME device was successfully used to perform chemical biopsies in the examined patients (Figs. 1, 2). The SPME device, which is smaller than the biopsy needle, was inserted prior to the standard biopsy procedure along the trajectory planned for the biopsy needle in order to prevent any excessive tissue damage. The entire SPME procedure took less than 5 minutes. It is also worth noting that no mechanical damage (e.g., crumbling of the sorbent, fiber fractures, decomposition) to the device was observed.

Metabolomics

The first untargeted analysis was performed using the metabolomics protocol. A total of 75449 features were detected after running all four LC-HRMS modes. After filtering out the background noise and the unstable features, a total of 2071 compounds were identified in the analyzed samples, which constituted 541 hits within the ChemSpider database (Figs. SA1-SA4). A comparison of the number of compounds detected in the grey and white matter samples revealed no significant differences, with an average grey-to-white compound ratio of 0.931. Greater variations in analyte coverage were usually detected inter-individually for the given matter rather than between the two types of brain matter in subsequent individuals (Fig. SA5).

The metabolomic workflow allows a wide range of the analytes extracted from the samples to be analyzed. The accurate masses were putatively identified prior to the analysis of the metabolic pathways (Fig. 3). However, due to the lack of separation on PCA (Fig. SA6), the supervised chemometric analysis was not applied. The largest number of pathways was found to be associated with amino acid metabolism, but only one hit (compound) was found for each of these pathways. The best match status was observed for the metabolism of sphingolipids, nitrogen compounds, glycerophospholipids, phenylalanine, and fatty acids (Fig. 3). Further fragmentation of detected analytes revealed that the majority of confirmed compounds were lipids (Tables SA2 and SA3).

Lipidomics

Selection of SPME coating for lipidomics

The first step in the lipidomic analysis process was to determine the optimal sorbent type, as this would ensure maximum effectiveness. To this end, fibers coated with C18 and HLB particles were used to obtain samples from the mouse brain specimens. The fibers were then subjected to HILIC-HRMS analysis, which detected 164 lipids, and RPLC-HRMS analysis, which detected 463 (Table SB1 and SB2, respectively). The average area under the peak for almost all of the detected compounds was larger when sampling was performed using the C18 fibers (100% of compounds detected in HILIC-HRMS, and 86.0% of the compounds detected in RPLC-HRMS) (Table SB1 and SB2). Only 3.7% of the peak areas for the compounds detected via HILIC-HRMS, and 10.8% of the compounds detected via RPLC-HRMS analysis were statistically different between the two types of sorbent. Additionally, an F-test was conducted in order to compare the two coatings' coefficients of variation (CV) for the extracted compounds. The results revealed similar CVs for the majority of detected metabolites; however, sampling with the C18 fibers yielded statistically significant higher CVs for 4.3% of the features in HILIC-HRMS and 8.8% of the features in RPLC-HRMS. When the HLB fibers were used, CVs were only higher for 0.9% of the detected tentative analytes in reversed-phase chromatography (Table SB1 and SB2). Ultimately, the C18-coated fibers were selected for use in the human subject experiments, as they provided larger average areas under the peaks.

Human brain analysis

A wide range of tentative lipid species (phospholipids, glycerolipids, and sphingolipids) were detected in positive ion mode for HILIC-HRMS and RPLC-HRMS chromatography (189 and 245 compounds, respectively). RPLC-HRMS analysis mainly enabled the detection of triglycerides (TG) (Table SA4), while HILIC-HRMS chromatography facilitated the analysis of more hydrophilic species, mainly phospholipids (Table SA5). Multivariate analysis conducted with the online Metaboanalyst software package revealed weak separation between the white and grey matter (Figs. 4A, 4C). As with the metabolomics results, PCA did not show any separation between the two types of matter. In addition, the PCA findings showed that the data points representing grey matter were closer to each other than the ones for white matter.

Next, statistical analysis was conducted on the compounds with confirmed structures. This analysis revealed that the white matter contained higher levels of phospholipids and hexoyl ceramides (HexCer) compared to the grey matter (Table 1). In more quantitative terms, 8% of phosphatidylocholines (PC) and 13% of phosphatidyletanolamines (PE) were at least two times more abundant in white matter (Table SA5). For other lipids, the area under the peak was on average about 30% bigger in the white-matter samples compared to the grey-matter samples. Interestingly, the amount of detected triglycerides in the grey matter was over three times higher than in the white matter (Table 2). Furthermore, the coefficients of variation for TGs were also very high, at 138% in the white-matter samples, and 143% in the grey-matter samples (Table 2). More in-depth analysis of individual lipid species can be found in Table SA4. As can be seen, 75.9% of TGs were present in grey matter at levels twice as high as in white matter, with ratios higher than 5.0 for 31.8% of these compounds. Similarly, 63.6% of the phospholipids analyzed with RPLC-HRMS were present in white matter at concentrations at least twice as high as in grey matter. HexCer was also more abundant, occurring about 2.6 times as often in white matter compared to grey matter.

Table 1
The comparison of the average summary peak area for lipid classes in HILIC-HRMS

Lipid Class	QC		WHITE		GREY		WHITE/GREY
	mean	CV [%]	mean	CV [%]	mean	CV [%]	
HexCer	24 351 215	14%	38 479 183	83%	18 059 876	45%	2.1
LPC	40 822 619	11%	73 482 043	40%	61 125 592	45%	1.2
PC	413 307 542	7%	598 952 413	37%	372 526 825	22%	1.6
PE	461 953 238	7%	603 696 780	28%	437 538 384	19%	1.4
HexCer – Hexosyl ceramide; LPC – Lysophosphatidylcholines; PC – Phosphatidylcholines; PE – Phosphatidyletanolamines; QC – Quality Control; CV – coefficient of variation							

Table 2
The comparison of the average summary peak area for lipid classes in RPLC-HRMS

Lipid Class	QC		WHITE		GREY		WHITE/GREY
	mean	CV [%]	mean	CV [%]	mean	CV [%]	
HexCer	51 852 594	1%	88 023 649	103%	34 491 630	54%	2.6
PE	96 472 642	2%	168 284 801	56%	74 717 935	37%	2.3
TG	1 419 676 537	1%	981 999 982	138%	3 626 716 190	143%	0.3
HexCer – Hexosyl ceramide; PE – Phosphatidylethanolamines; TG – Triglycerides; QC – Quality Control; CV – coefficient of variation							

Discussion

The introduction of new mass-spectrometry-based techniques to neuroscience has created a wide array of new opportunities within this discipline^{20,21}. Nonetheless, many challenges related to areas such as safety, biocompatibility, and practicability must still be overcome. The present article explores chemical biopsy as a new tool that can be used in neurochemistry applications. Previous *ex vivo* and *in vivo* investigations of animal brains have demonstrated that some of this technology's unique features may be able to fill the gaps present in existing methods^{10,11}. In addition, prior findings have shown that chemical biopsy is safe for use in laboratory animal studies, and enables the non-depletive analysis of labile endogenous substances, as well as metabolism quenching due to its ability to restrict the access of enzymes to absorbed substances^{10,13,15,22}.

This paper documents the first application of chemical biopsy for the analysis of human brain tissue *in vivo*, which was achieved by introducing a few adjustments to previously reported protocols. Firstly, it was necessary to sterilize the SPME fibers before inserting them into the human participants' brains. To this end, ethylene oxide was used in this study, but other research has shown that steam sterilization can be used as well²³.

Another modification to previous SPME protocols made in this research was the compromise that was struck between analytical needs and practicality in a clinical setting. Specifically, the selected sampling time must be long enough to ensure adequate sensitivity and data quality, but short enough to be acceptable for the neurosurgeon and the patient. The length of the extractive phase coating on the probe must also be sufficient to attain reasonable recovery of the metabolites, but not too long to fit within the selected location. A literature review revealed that a sampling time of 15–30 min is usually used in metabolomics studies²⁴. However, such an extraction time would be impractical for the current study; since the extractions were performed on patients prior to the actual biopsy procedure while they were still

conscious, the extraction time used in this research could be longer than a few minutes in duration. Therefore, 4 min was chosen as an optimum extraction time. Huq et al.'s foundational work on measuring free drug concentrations in solid tissue demonstrated that equilibrium time is established more quickly in complex matrices than in simple solutions like phosphate buffered saline (PBS) or agarose gel. This phenomenon is due to the fact that analytes are bound by tissue components (e.g., proteins), which in turn act as a local reservoir for the molecules to be extracted ²⁵. As a result, the substances distributed in the cellular and intercellular spaces are able to be rapidly transported to the sorbent. Based on this finding, it is easy to conclude that even a short extraction time (e.g., 5 min) might be sufficient to achieve satisfactory recovery when extraction is performed from a complex matrix ²⁵.

Unlike SPME, gold standard method in neuroscience – MD requires a relatively long sampling time, as it takes over 30 min to collect a sufficient amount of dialysate to enable the determination of target compounds. Moreover some experiments require even longer sampling times in order to obtain satisfactory recovery ²⁶. Furthermore, a special cannula must be installed in the patient's skull 3–4 days prior to the experiment in order to allow the MD probe to be introduced to the brain; thus, dates of MD experiments must be planned in advance. Attempts to modify this technology led to the development of push-pull probes (PPP), which enable faster analysis, but lower sensitivity and accuracy ²⁶. Compared to MD and SPME, PPPs and electrochemical methods guarantee better temporal resolution, although push-pull probes are still prone to capillary clogging ^{26,27}. Additionally, while electrochemical methods are able to return results in a few seconds, they only enable the analysis of selected substances, which may considerably reduce the scope of research ^{26,28}.

As previously mentioned, extraction time and coating length must be customized in order to perform extractions from brain structures using SPME. The grey matter is a thin layer on the surface of the brain ranging between 1.0 and 4.5 mm in thickness (average thickness is approximately 2.5 mm) depending on the region ²⁹. Despite the fact that detailed computed tomography (CT) and magnetic resonance tomography (MRI) fusion images are obtained prior to the biopsy, it is not possible to estimate the depth of the grey matter in the sampled area based on these images. In the present study, probes with a coating length of 3 mm were selected in order to enable the extraction of metabolites from grey matter with maximum precision and acceptable sensitivity. However, to further increase sensitivity without compromising extraction time, a modification was introduced to the design of the sampling device. Prior research has shown that it is possible to increase analyte extraction by increasing the surface area of the extraction phase (coating) ¹⁴, which is usually achieved by changing the device's geometry to a thin-film format. The most commonly used thin-film variant in bioanalysis is the "blade" or Coated Blade Spray format, as they can be inserted directly into the tissue ²². Unfortunately, such devices are too invasive for use in brain studies. Therefore, an alternative strategy was employed in the present study, whereby recovery was enhanced by sampling each brain structure with two fibers simultaneously (i.e., the device is comprised of four fibers). After sampling, the two fibers used to sample each structure were desorbed together, thus increasing the amount of analytes extracted within the set 4 min (Figs. 1, 2). This modified approach enabled the detection of a wide range of analytes (Figs. SA1-SA4). Nonetheless, it is important

to note that the range of extracted metabolites was still limited, and, consistent with the research conducted by Cudjoe et al. and Boyaci et al., for instance neurotransmitters were not observed in untargeted analyses^{10,11}. However, it is possible to extract neurotransmitters with SPME by optimizing the analytical protocol for that specific group of substances^{10,15}.

Zhang et al.³⁰ employed a similar spatial sampling approach to assess the concentration of pollutants in fish tissue samples, using segmented SPME fibers to simultaneously sample adipose and muscle tissues. After sampling, Zhang et al. cut off the coated parts of the fiber, and desorbed them in separate vials. Recently, another approach to spatial resolution analysis with SPME was presented by Lendor et al., who analyzed the fluoxetine profile in rat brains using SPME fibers for extraction, and desorption electrospray ionization (DESI) coupled to mass spectrometry for target analyte detection³¹. Enhanced spatial resolution was achieved by moving the DESI probe along the fiber that had been inserted into the studied tissue.

The analytical methods most commonly used in *in vivo* neurochemical studies generally do not provide spatial resolution of brain structures that is as detailed as the method presented by Lendor et al.³¹ With MD probes-even those with a diameter similar to SPME fibers (about 200µm)- it is impossible to obtain comparable results due to the method's different governing principles. Thus, sampling must be carried out in a different way, which usually results in slower spatial resolution or time delay (i.e., sampling the grey and white matter sequentially rather than simultaneously)^{26,32}. Moreover, if sampling from two locations is planned, two cannulas outfitted with MD probes must be used, which will increase the invasiveness of the procedure and impact spatial resolution. Additionally, the MD probe needs to be connected to the device that pumps the perfusate during the sampling procedure. This means that a two single-channel-syringe pumps or one two-channel-syringe pump would be required in order to analyze two brain locations, which creates a need for additional space in the surgery room to accommodate the necessary extra equipment.

Distinct differences in the metabolomic profiles of the two studied brain matters were expected, but PCA did not show clear separation (Fig. SA5). Similarly, lipidomic analysis did not result in any differentiation between the analysed structures (Fig. 4). It was observed that the samples taken from patient B12 were located very close to each other (almost overlapping), which may lead to the assumption that, in this case, sampling was performed only from one type of matter (Fig. 4). As mentioned before, it can be quite difficult to locate the precise border between white and grey matters, as they vary in thickness across brain regions²⁹. Moreover, in *in vivo* human studies conducted in clinical environments, it is not possible to sample brain tissue purely for scientific purposes and to create a standardized study group. The patients from whom samples were collected in this study constituted a heterogeneous group in terms of age, sex, type, and location of brain lesion (Table SA1), which could affect the repeatability of the obtained results. Various factors, such as age, sex, medical conditions, sampling location in the cerebral cortex, and treatment applied before neurosurgical procedures, all influence the brains' metabolome profile, and they all contributed to the relatively high variation observed in the average peak areas (Table

SA2-SA5). For these reasons, biochemical *in vivo* brain studies are almost exclusively performed on laboratory animals, wherein entire neurosurgical procedures are designed for the research at hand. The current study was conducted on a small cohort, which makes it difficult to draw solid conclusions. Indeed, our results only verify the concept and identify drawbacks that should be addressed in future studies seeking to analyze a larger number of patients.

Given the small cohort and great variability of sampled areas and patients in the current study, the chemometric and statistical analysis of the identified metabolites predictably did not reveal significant differences in the metabolome profiles of the white and grey matter (Fig. SA5, Table SA2., and Table SA3). However, the analysis did reveal that most of the pathways in which non-hydrophobic species were engaged were related to amino acid metabolism. On the other hand, the best matches for metabolic pathways were assigned to lipids (Fig. 3). This result is in line with previous reports identifying lipids species as the predominant component in brain tissue composition^{6,15,29,33}. With regards to cerebral lipids, it is worth mentioning that MRI, a clinically approved method of differentiation of brain structures and detecting lesions, is mainly based on differences in lipid content (cholesterol and phospholipids)³⁴. Prior findings have also shown that white matter contains about 60% more lipids and 10–15% less proteins than grey matter³⁴. Similar observations regarding the prominent role of lipids in brain cells have been made using other mass-spectrometry-based methods, including REIMS and DESI²⁰. Cudjoe et al., concluded that SPME could be a complementary technology to MD, as it covers a broader hydrophobic range of metabolites (e.g., lipids), while MD is suitable for highly polar species (e.g., amino acids)¹⁰. Both methods were compared by the performance of untargeted metabolomic profiling as well as targeted analysis of neurotransmitters (dopamine and serotonin) obtained from brain of living rats. Recent work by Boyaci et al.¹¹ has also confirmed Cudjoe et al.'s¹⁰ observations regarding the complementarity of SPME and MD. Solid-phase microextraction makes it possible to tailor the protocol to the metabolites of interest, as it allows researchers to employ more selective coating and desorption parameters, and it can be coupled with appropriately optimized analytical instrumentation. In the present study, the results of the metabolomic analysis indicated a need for more in-depth lipidomic analysis. In order to better assess the lipid composition of the examined tissues, two types of sorbent were tested. First, fibers coated with C18 were analyzed, as they have been used in lipidomic studies previously, and they have a high affinity towards hydrophobic species^{12,35,36}. The second type of fiber that was selected for analysis was HLB-sorbent-coated fibers, as previous studies have demonstrated their high level of performance^{36,37}. The results of our comparison showed that the peak areas of detected features were larger in the samples collected with the C18 fibers, which indicated that this sorbent offered slightly better recoveries. The areas of only a few compounds showed a statistical significant difference (about 11% in RPLC-HRMS and 4% in HILIC-HRMS analysis) (Table SB1 and Table SB2). Based on this finding, the lipidomic analysis was carried out using the C18 fiber. This is consistent with the work of Boyaci et al.¹¹, who used the C18 sorbent for their lipidomics analysis, and a mixed-mode extractive phase for their metabolomics analysis.

Despite the lack of statistically significant separation between the two types of brain matter determined by PCA (Fig. 4), the results nevertheless showed the characteristic patterns of lipids in those structures. For instance, the results showed that phospholipids were present in greater level in the white matter, which could be due to the higher number of glia cells in this structure (Table 1). It has been proven that astrocytes and oligodendrocytes function as a reservoir for energy substrates (e.g., lipids for neurons), which means that there is intensive crosstalk and metabolite transfer between glia and neurons^{38,39}. These metabolites are probably more readily available to an SPME probe. It was also observed that the average peak area for TGs was bigger in the grey matter compared to their peak areas in the white matter (Table 2, Table SA4). Since the grey matter is located on the brain surface, many nutritional substances in their free form are first transported there. The high CV value for TGs in the present study can be partially explained by variations in the individual patients' intake of nutrients, but it may also be due to the contamination of the fiber coatings by cerebrospinal fluid or blood present on the brain surface during the procedure, as both contain a wide range of substances (e.g., TGs)^{40,41}. Moreover, biological processes related to underlying disorders (e.g., obesity) may also influence the amount of TGs detected in individual patients⁴⁰. The detailed lipidome analysis results revealed that mainly phospholipids were identified in brain tissue (Table S.A.4., Table S.A.5.). This finding was unsurprising, as phospholipids are the main components of membranes and are engaged in variety of metabolic processes⁴². The level of PC(36:1) was higher in white matter than in grey matter (the white-to-grey matter ratio was 1.3 for H⁺ adduct and 1.6 for Na⁺ adduct) while the opposite was observed for PC(34:1) (i.e., the white-to-grey matter ratio was 0.8) (Table S.A.5.). Martínez-Gardeazabal et al.⁴² used matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) to map the lipidome in rat brains, and found that some lipids are more abundant in white matter than in grey matter. The authors detected a wide range of lipids, and spatial distributions of PC(36:1) and PC(34:1) that corresponded to our results. In addition, Martínez-Gardeazabal et al.⁴² observed a higher intensity of LPC(16:0) compared to LPC(18:0), which is also in agreement with our results. In contrast, Jarmush et al.⁴³ used DESI to conduct a comparative analysis of neoplastic tissue, white matter, and grey matter *ex vivo*. This approach allowed the visualization and spatial resolution of the phospholipids and sphingolipids in a studied tissue^{43,44}. Some of the lipid species detected in the samples analyzed by Jarmush et al.⁴³ (e.g., PC(32:1), PC(34:1), PC(36:2), PE(36:2p), PE(38:5p), PE(40:6p)), were also detected in the present study, however there were differences in the formed adducts. Jarmusch et al.⁴³ reported that the white and grey matter had different lipid profiles, but they did not discuss this difference from a biological perspective. Among the mentioned phospholipids, two were identified as discriminative for differentiating white and grey structures^{43,44}. Specifically, they found that the presence of PC(34:1) in grey matter was more intense than in white matter, while the opposite was true for PE(36:2p). These findings were confirmed by the results of the current study (Table S.A.4., S.A.5.). MALDI-IMS and DESI enable the mapping of lipid species, but they cannot be applied for *in vivo* studies in clinical environments because of the complex setup of the hardware. Moreover, they provide less accurate identification compared to LC-MS/MS. In contrast, REIMS enables the fast detection of lipids *in vivo*, but not the accurate identification of individual species^{21,45}. This diagnostic method is based on comparing the profile of detected signals against the existing

database. In addition, it provides results in real time, which makes it a great tool for assessing neoplasma margins during surgical procedures. However, the above-mentioned methods are not suitable for assessing structures/lesions located under the surface of the studied tissue, unless the area is exposed (e.g., during the removal of the lesion). This indicates that SPME can complement the portfolio of the existing MS-based approaches in neurological research.

This work documents the first use of SPME for the study of human brain tissue *in vivo*. Our analysis revealed some separation between the brain structures, with larger amounts of lipids being detected in the white matter than in the grey matter. Regardless, more detailed studies using larger study groups are required in order to draw solid conclusions regarding the biochemical profile of human brain tissue.

The research described herein demonstrates that it is possible to achieve spatially resolved chemical characterization of a living human brain in a fast and low invasive manner, thus not disturbing other medical procedures (e.g., biopsy). The sampling device can be customized into a personalized diagnostic tool, and the proposed protocol can be applied in numerous clinical settings when supported by imaging data. In addition, this research proves that the proposed method complements those already used in neuroscience, for example, microdialysis, push-pull analysis, and electrochemical methods. Furthermore, the findings of this research also show that chemical biopsies performed with SPME probes enable more in-depth chemical analysis of brain tissue compared to other clinically available methods (e.g., MRI, biopsy). Not only is SPME capable of extracting lipids and more polar metabolites, but its lipid-characterization capabilities are also unique compared to other *in vivo* sampling devices. The results presented in this paper indicate that the proposed tool could be potentially used as a medical device, as it causes minimum damage and features operational parameters that are compatible with surgical procedures.

Finally, it should be emphasized that more portable reading devices-rather than mass spectrometers using optical spectroscopic techniques, such as fluorescence or Raman could be used in targeted determinations in order to create a technology that is more compatible with bedside or operational theatre applications⁴⁶.

Declarations

Acknowledgements

The work was supported by the grant, entitled, “Metabolomic and lipidomic profiling of white and gray matter of cerebral cortex using novel technology based on chemical biopsy coupled to mass spectrometry,” which was provided by the Ministry of National Defence of Poland under the second edition of the Kościuszko program (506/2017/DA).

The authors would like to thank Supelco Inc. (Merck) for supplying the SPME probes for the MM and C18 fibers, and Waters for providing the HLB particles.

Author contributions are as follow: J.B. and K.B. – preparation of SPME probes; M.B., J.F.– surgical procedures and recruitment of the patients; J.B., B.B., K.B. K.Ł. – assistance in samplings and handling of samples after biopsies; J.B. – instrumental analysis, J.B., K.J., K.Ł. – statistical analysis and data interpretation; J.B. – writing original draft of the manuscript; M.B, J.B., B.B., K.B., M.H., K.J. K.Ł. J.P. – review and editing of the manuscript; M.H. – coordination and supervision of the medical side of the project; J.P. – funding, participation in the design of the analytical side of the project; B.B. – conceptualization, coordination and supervision of the project. All authors read and approved the final manuscript.

All authors have read the journal's authorship agreement and policy on disclosure of potential conflicts of interest

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Figures

Figure 1

The construction of chemical biopsy probe

Figure 2

Chemical biopsy of brain tissue with the use of SPME probe

Pathway Name	Match Status
Sphingolipid metabolism	3/25
Nitrogen metabolism	3/39
Aminoacyl-tRNA biosynthesis	4/75
Fatty acid biosynthesis	3/49
Phenylalanine, tyrosine and tryptophan biosynthesis	2/27
Glycerophospholipid metabolism	2/39
Phenylalanine metabolism	2/45
Glycosylphosphatidylinositol (GPI)- anchor biosynthesis	1/14
Linoleic acid metabolism	1/15
Alanine, aspartate and glutamate metabolism	1/24
Thiamine metabolism	1/24
Fatty acid elongation in mitochondria	1/27
Valine, leucine and isoleucine biosynthesis	1/27
Arginine and proline metabolism	2/77
beta-Alanine metabolism	1/28
Drug metabolism – cytochrome P450	2/86
Ubiquinone and other terpenoid-quinone biosynthesis	1/36
Purine metabolism	2/92
Glutathione metabolism	1/38
Valine, leucine and isoleucine degradation	1/40

Figure 3

Biochemical pathways analysis of metabolomic data. Biological pathways related to analytes detected in the samples derived from all patients. Table and chart were generated using compounds found in all LC-HRMS modes

Figure 4

Chemometric analysis of data obtained with use of hydrophilic-interaction liquid chromatography high resolution mass spectrometry (HILIC-HRMS) for lipidomic data. The parameters of PLS-DA for HILIC-HRMS showed over-fitting of the model, while separation was slightly better for the RPLC-HRMS analysis. A. principal component analysis (PCA); B. partial least squares discriminant analysis (PLS-DA), plot parameters: 1 component: R^2 : 0.659; Q^2 : -0.072; 2 component: R^2 : 0.957; Q^2 : -0.129; and reversed-phase

liquid chromatography high resolution mass spectrometry (RPLC-HRMS) for lipidomic data C. PCA; D. PLS-DA, plot parameters: 1 component: R2: 0.447; Q2: 0.060; 2 component: R2: 0.881; Q2: 0.049.

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