Doctoral Thesis

Functional magnetic resonance spectroscopic imaging of the mouse brain: overcoming current sensitivity limitations

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Functional magnetic resonance spectroscopic imaging of the mouse brain: overcoming current sensitivity limitations.

A thesis submitted to attain the degree of

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(Dr. sc. ETH Zurich)

presented by

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Material and methods
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Introduction
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Discussion

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CV
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$^1$H MRS</td>
<td>Proton magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartate</td>
</tr>
<tr>
<td>B$_0$</td>
<td>Static (main) magnetic field</td>
</tr>
<tr>
<td>B$_1$</td>
<td>Transmit/receive field</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygenation level dependent (contrast)</td>
</tr>
<tr>
<td>CBV</td>
<td>Cerebral blood volume</td>
</tr>
<tr>
<td>Cho</td>
<td>Choline</td>
</tr>
<tr>
<td>CNR</td>
<td>Contrast to noise ratio</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatine</td>
</tr>
<tr>
<td>CRLB</td>
<td>Cramer-Rao lower Bounds</td>
</tr>
<tr>
<td>CRP</td>
<td>Cryogenic coil</td>
</tr>
<tr>
<td>CSDE</td>
<td>Chemical shift displacement error</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
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<tr>
<td>FMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FMRS</td>
<td>Functional magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>FMRSI</td>
<td>Functional magnetic resonance spectroscopic imaging</td>
</tr>
<tr>
<td>GABA(\gamma)-Aminobutyric acid</td>
<td></td>
</tr>
<tr>
<td>Glc</td>
<td>Glucose</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Glx</td>
<td>Glutamate and Glutamine</td>
</tr>
<tr>
<td>Ins</td>
<td>Myo-Inositol</td>
</tr>
<tr>
<td>Lac</td>
<td>Lactate</td>
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<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl-aspartate</td>
</tr>
<tr>
<td>OVS</td>
<td>Outer volume suppression</td>
</tr>
<tr>
<td>paCRP</td>
<td>phased array receive only cryogenic coil</td>
</tr>
<tr>
<td>PCh</td>
<td>Phosphocholine</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>quadCRP</td>
<td>quadrature transmit/receive cryogenic coil</td>
</tr>
<tr>
<td>RF</td>
<td>Radio Frequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SI</td>
<td>Spectroscopic imaging</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>SVS</td>
<td>Single Voxel Spectroscopy</td>
</tr>
<tr>
<td>Tau</td>
<td>Taurine</td>
</tr>
<tr>
<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>totCre</td>
<td>total Creatine (Cr+PCr)</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>VOI</td>
<td>Volume of interest</td>
</tr>
<tr>
<td>WS</td>
<td>Water suppression</td>
</tr>
<tr>
<td>ω$_0$</td>
<td>Larmor frequency</td>
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Summary

Proton Magnetic Resonance Spectroscopy (1H-MRS) serves to detect quantitative information about several neurotransmitters, general indicators of brain metabolism, and also certain antioxidants and osmolytes non-invasively. This technique is becoming increasingly important as a diagnostic tool in clinical routine, but also as a research tool for biomedical, pre-clinical research. Particularly when applied to mice, MRS represents a unique technique able to deliver spatially resolved information about brain biochemistry and function in various genetic models of neurological disorders available only in this species. However, MRS faces several technical challenges, mainly due to intrinsically low signal to noise ratio (SNR) and long measurement times. For measurements on mice, ultra-high field systems together with dedicated hardware and measurement techniques are required to overcome these difficulties. As a consequence, and despite the attractive possibilities of the technique, MRS and particularly Spectroscopic Imaging (SI) is not yet routinely applied in mice.

In this thesis, we developed protocols for fast and reliable acquisition of highly resolved metabolite maps of the mouse brain. We used the first existing prototype of receive-only cryogenic coil array designed for the mouse brain. For very small objects, such as mice, the system noise is the dominant noise source in an MR experiment. Cooling down the receive coil to 30K using liquid Helium leads to significant noise reduction and therefore to an increased SNR by a factor of 2 to 3. This gain in SNR can generally be reinvested in higher temporal or spatial resolution for all kinds of MR experiments, and also for SI.

In a second step, optimized experimental procedures and measurement techniques dedicated to SI on the mouse brain were implemented. Conventionally, an MRS measurement involves the formation of spin echoes, which is associated with significant signal loss due to T2 relaxation compromising the sensitivity and therefore results in long acquisition times. Yet, SI experiments can be sped up by sampling the free induction decay (FID) signal immediately after a slice-selective excitation instead of the spin echo, thereby avoiding signal loss due to T2 relaxation. In order to maximize spatial resolution and minimize unwanted contribution from outside the target region, the VOI from which SI data are collected, is confined by suppressing signals arising from outside the VOI (outer volume suppression, OVS). The combination of FID sampling and localization by outer volume suppression (FIDLOVS) has been developed for high field clinical systems in order to minimize the chemical shift displacement artifacts and the signal losses due to fast T2 relaxation. Here, this technique was implemented on a 9.4T system and extensively tested on the mouse brain.

Increased SNR allows higher temporal resolution and therefore the possibility to monitor transient changes in specific neurotransmitter and indicators for brain metabolism related to brain function.
Using the optimized protocol described above combined with the superior sensitivity provided by the cryogenic coil, we were able to use SI as a functional tool on the mouse brain for the first time. In a first study, neural activity was triggered by infusing a GABA\(_A\) receptor antagonist, Bicuculline. Bicuculline blocks the inhibitory action of GABA and produces strong and sustained seizures. The metabolic events accompanying such an epileptic episode were monitored using SI with a temporal resolution of 12 minutes. Using different doses of Bicuculline, we could observe dose dependent metabolic changes, while regular BOLD fMRI indicated a failure of neurovascular coupling.

In a second study, we attempted to visualize brain activation upon electrical paw stimulation in mice by quantifying mainly changes in glutamate (Glu) and lactate (Lac) levels, using spectroscopic imaging (SI). Conventionally, neuronal activity is accessed by evaluating activity-evoked local changes in blood oxygenation levels (BOLD contrast) using fMRI methods. In mice however, the BOLD signal seems to be dominated by systemic contributions. Widespread and bilateral responses are generally observed following unilateral stimulation. SI allows the covering of extended brain regions, and hence monitoring of differential responses of brain areas, e.g. a direct comparison of activated versus non-activated regions. Our study aimed to elucidate whether SI can serve to deliver a more specific readout on the activation of respective brain regions involved in processing of sensory stimuli in mice.

1H-MRS remains a very challenging procedure when applied to mice. The small organ size puts high demands on spatial resolution, which results in acquisition times unsuitable for measuring the short term biochemical changes associated with neural activity. However, with adapted hardware and measurement protocols we were able to demonstrate the feasibility of stimulus evoked functional 1H-MRS measurements in mice for the first time.
Zusammenfassung


Im Rahmen der vorliegenden Doktorarbeit wurden Protokolle entwickelt, welche die schnelle und zuverlässige Aufnahme hoch aufgelöster Verteilungen der Metabolitenkonzentrationen (sogenannte metabolische Landkarten) im Gehirn der Maus ermöglichen. Die Messungen wurden mit dem ersten Prototypen einer speziell für Anwendungen in der Maus entwickelten cryogenen receive-only Phased-Array Spule (paCRP) durchgeführt. Bei MRI-Messungen kleiner Messobjekte, oder wie hier von Mäusen, stellt das elektronische Rauschen die dominierende Störquelle dar. Abkühlung der Spule auf 30 K mithilfe flüssigem Heliums führt zur signifikanten Verringerung des Rauschens und folglich zu einer Erhöhung des SNRs um einen Faktor 2 bis 3. Die SNR-Verbesserung ermöglicht letztendlich die Erhöhung der zeitlichen und räumlichen Auflösung bei MRI-Messungen und damit auch bei SI.

Ziel war es, die experimentellen Methoden und Mess-Sequenzen für SI am Gehirn der Maus zu optimieren. Bei MRS-Messungen wird konventionell ein Spin-Echo erzeugt, was mit Signalverlust durch T2-Relaxationsprozesse einhergeht. Die folglich verringerte Sensitivität der Aufnahme kann nur durch mehrere Messwiederholungen und konsequenterweise lange Messzeiten kompensiert werden. Um den Einfluss des Signalverlustes durch T2-Relaxation zu verringern und damit SI-Messungen zu beschleunigen besteht nun aber die Möglichkeit statt der Erzeugung eines Spin-Echo-Signals das Signal des freien Induktionsabfalls (Free Induction Decay, FID) unmittelbar nach der selektiven Anregung einer gewählten Schicht zu analysieren. Zusätzlich zur Verbesserung der zeitlichen Auflösung lassen sich dann noch die räumliche Auflösung erhöhen und gleichzeitig die Signaleinflüsse ausserhalb der Zielregion unterdrücken (Outer Volume Suppression, OVS). Die
Kombination der Analyse des FID und der Lokalisation der Zielregion durch OVS wurde für klinische Hochfeldsysteme entwickelt um Artefakte aufgrund von chemischer Verschiebung (chemical shift displacement artifacts) und Signalverlust durch T2-Relaxation zu minimieren. Diese Methode, wird als FIDLOVS bezeichnet, und wurde am 9.4T-MR-Scanner implementiert und am Mausgehirn umfangreich ausgetestet.


1H-MRS bleibt eine sehr anspruchsvolle Methode, wenn bei der Maus angewendet. Die Kleinheit der Organe, unter anderem des Gehirns, stellen hohe Anforderungen an die räumliche Auflösung. Aufgrund dessen sind die erforderlichen langen Messzeiten zur Erfassung der kurzlebigen biochemischen Ereignisse, die mit neuronaler Aktivität zusammenhängen, nicht geeignet. Dennoch, mit geeigneter Technik und optimierten Messprotokollen konnten wir zum ersten Mal die Machbarkeit solcher funktionellen 1H-MRS-Messungen an der Maus demonstrieren.
Chapter 1

Introduction
1.1. Noninvasive imaging in mice: high demands on spatial resolution and sensitivity

Mice are widely used in preclinical and basic research as a model organism for human diseases. Genetic engineering techniques have open new avenues, e.g. in developing models for chronic diseases with slow progression such as neurodegenerative diseases or autoimmune disorders. Currently, many of these approaches are limited to the mouse; hence, no other species offers as many possibilities for fundamental biomedical and pharmaceutical research. Non-invasive methods are of particular interest in characterizing such models in a longitudinal manner; i.e. techniques such as magnetic resonance imaging (MRI) and spectroscopy (MRS) are ideally suited for investigating potential morphological, physiological, or metabolic aberrations associated with disease progression. Yet, both MRI and MRS of mice are challenging due to the small dimensions and correspondingly to the high demands on spatial resolution and sensitivity. We can assume that the signal is directly proportional to the number of signal generating molecules within a volume element (voxel), and correspondingly to the voxel dimension. Hence, increasing the spatial resolution, i.e. decreasing the voxel volume, will immediately deteriorate the signal-to-noise ratio (SNR). This can be accounted for by signal averaging, which however is rather inefficient as the SNR increases only with the square root of the number of averages. Hence alternative methods for improving the sensitivity in mouse MRS become essential. But before discussing these hardware and software optimization procedures in more detail, we will first introduce some basic concepts of in vivo proton MRS.

1.2. In vivo proton magnetic resonance spectroscopy: the sensitivity challenge

In vivo proton MRS (\(^1\)H MRS) provides biochemical information in human or animal tissue non-invasively. \(^1\)H MRS is based on the physical principles of nuclear resonance. Certain nuclei such as hydrogen possess a magnetic moment and are thus sensitive to external magnetic fields. They become resonant upon absorbing electromagnetic radiation at a specific frequency. This frequency, also known as Larmor frequency (\(\omega_0\)), depends on the strength of the local magnetic field (\(B_0\)) experienced by the nucleus, and on a nucleus-specific constant called the gyromagnetic ratio (\(\gamma\)), in the following way:

\[
\omega_0 = \gamma B_0 \tag{1.1}
\]

The local magnetic field experienced by the nucleus under investigation is composed of the main magnetic field applied (static field produced by a superconducting magnet) and the local magnetic field induced by the electrons surrounding the nucleus. The intensity of this phenomenon, also called electronic shielding, is dependent on the number of electrons in the close proximity of the nucleus, and therefore varies in function of the structure and chemical composition of the molecules under investigation. These small changes in the local magnetic field experienced by a nucleus produce a shift in their resonance frequency, the so-called chemical shift \(\sigma\). Accordingly, eq. 1.1 has to be modified to
\[ \omega_{0,i} = \gamma B_0 (1 - \sigma_i). \]  

The chemical shift value \( \sigma_i \) is a sensitive indicator for the local environment of a specific nucleus \( i \) and thus constitutes an identifier of the local chemical structure. Hence, different molecular constituents are characterized by different resonance frequencies. When combined with localization techniques designed to select a specific region-of-interest (ROI) within the sample, which will be discussed later in this chapter, it becomes possible to resolve these frequencies \( \omega_{0,i} \) for this particular (ROI) and to obtain an MR spectrum containing information about the local chemical composition of tissue in a non-invasive manner. This chemical profile provides a sensitive window to the metabolic state of tissue and has been used to characterize physiological and pathological conditions in a variety of diseases. Focusing on the brain, \(^1\text{H}\)-MRS has been used to study pathological conditions such as stroke, neurodegenerative diseases, epilepsy, psychiatric diseases, metabolic disorders, or brain tumors (Daly & Cohen, 1989; Doblas et al., 2012; Geissler, Frund, Scholmerich, Feuerbach, & Zietz, 2003; Maddock & Buonocore, 2012; Martin, 2007; Saunders et al., 1995; Simister, McLean, Barker, & Duncan, 2003).

The development of \(^1\text{H}\) MRS techniques went hand in hand with the development of regular MRI procedures. In fact the potential to annotate anatomical with biochemical information was always considered a major strength of MR. However, in vivo \(^1\text{H}\) MRS is markedly more challenging compared to standard MRI of water (and lipid) protons, considering the typical relative concentrations of water an metabolite protons of about 80M versus mM, respectively: obviously in vivo \(^1\text{H}\)-MRS suffers from inherently low sensitivity. Dedicated localization techniques (to guarantee that the spectrum originates from the actual ROI with as little contribution from surrounding tissue as possible), several preparation steps (selection of the ROI, suppression of the dominating water signal, homogenization of the static magnetic field to achieve maximal spectral resolution) and signal averaging to improve the SNR (leading to long acquisition times) are required in order to obtain a spectrum of sufficient quality for quantitative analysis. This is of particular relevance for MRS studies in mice for which the sample volumes are typically small (of the order of 1µl) which directly translate into low SNR. Hence, an important aspect of the current thesis is devoted to increasing sensitivity in \(^1\text{H}\)-MRS for investigating cerebral metabolism in mice with high spatial and temporal resolution.

### 1.3. Hardware optimization to improve signal-to-noise ratio in MR spectroscopy

Sensitivity depends critically on the magnitude of the static magnetic field \( B_0 \) and the intrinsic sensitivity of the detector device.

#### 1.3.1. Increasing sensitivity by using high magnetic field strength (or more precisely high magnetic flux)
Most of the MR based techniques where first developed for clinical purposes on systems of low magnetic field strength \((B_0 \leq 3T)\) low field systems, and had to be translated to be applied on mice at ultra-high field \((B_0 \geq 7T)\). High field systems are necessary in order to maximize the signal intensity when SNR is the limiting factor, which is true for 1H MRS considering the low intrinsic sensitivity of the technique, and particularly when applied in mice due to the very small size of the regions of interest. Depending on the dominating noise source in the experiment the signals scales linearly with \(B_0^x\) (with \(x \geq 1\)) due to the increased level of polarization. In addition to the higher SNR, higher \(B_0\) values provide also increased chemical dispersion, i.e. better spectral separation of individual resonances, which should lead to improved quantifications (see 1.5).

1.3.2. Increased sensitivity by optimizing the detector design

In order to produce an MR signal, a short transient radio-frequency field (an RF pulse) is applied to deflect the net macroscopic magnetization, which results as the vector sum of all nuclear magnets in the sample, from its equilibrium alignment along the static magnetic field, imposing a rotating transverse component to the magnetization vector. In the laboratory coordinate frame, the rotating magnetization leads to a time-variant magnetic field at any given point, which according to Faraday’s law of electromagnetic induction, will induce a voltage and accordingly a current in a loop of conductive wire, the detector coil. Assuming that following this pulse all protons within the sample have been excited, the time dependent current induced in the coil is composed of various frequencies \(\omega_{0,i}\) and phases \(\phi_i\) (that depend on the initial condition for a specific magnetization vector \(i\) at the begin of the detection period). This time dependent signal is related to the spectrum of the system via a Fourier transform (R. R. Ernst, 1966).

The NMR coil, used to transmit RF radiation to the sample and to detect RF signals emitted by the sample, is a device containing one or several coil elements composed of inductances and capacitors assembled in a resonant circuit. The value of the capacitance and inductance of the coil determine its resonance frequency, which should be tuned to the Larmor frequency. For example, for a coil comprising a single inductance \(L\) and capacitance \(C\) the resonance frequency becomes \(\omega_0 = 1/\sqrt{LC}\). Any coil system assembled from lossy electrical components presents a certain electrical resistance \(R\), which depends on the coil temperature, and acts as a potential source of noise in the recorded MR signal. The noise originating from the coil is not the only noise source in an MR experiment, but its relative contribution to the total noise increases for small coil dimensions (theoretical aspects will be discussed in more details in chapter 3), a condition typically encountered in mouse MR applications.

Cooling down receiver coils has been one very efficient way to improve SNR in high resolution NMR applications (Kovacs, Moskau, & Spraul, 2005; Styles et al., 2011; Styles, Soffe, & Scott, 1989) and more recently also in small animal MRI experiments. Several studies have assessed the practical gain in SNR provided by low temperature detection with cryogenic coils (CRP) in comparison to RF coils.
operating at room temperature for different magnetic field strength. For commercially available CRPs (Cryoprobes, Bruker BioSpin) the SNR is enhanced by a factor of 2 to 2.5 in phantom studies and up to a factor of 3 for certain in-vivo applications (Baltes, Radzwill, Bosshard, Marek, & Rudin, 2009; Ratering, Baltes, Nordmeyer-Massner, Marek, & Rudin, 2008). Cryoprobes are nowadays installed on MR systems operating at field strengths ranging from 4.7T to 15T. Until recently, all cryogenic coils were designed as single loop or quadrature transmit/receive surface coils.

MR coils can be designed in different ways, and are commonly separated into volume resonators and surface coils. A volume resonator is designed such that the RF field distribution within the resonating structure is a homogeneous as possible. This warrants that each location within the sample experience the same excitation field $B_1(t)$ and correspondingly yields the same SNR of the signal detected. In contrast surface coils produced an inherently inhomogeneous field $B_1(t,r)$, $r$ indicating the location, as the field amplitude decreases with increasing distance from the electrical conductor. Correspondingly different locations within the sample will experience different amplitudes of the RF excitation field, which will lead to local differences in sensitivity. A surface coil can be optimally adjusted to produce a certain flip angle $\beta = \gamma B_1 t_p$, with $t_p$ being the duration of the excitation pulse, only for one particular location within the sample at the time. Also in receiving mode, regions close to the coil will be sensed with higher intensity compared to regions further away. For both, volume and surface resonators, the efficiency in transmitting (or receiving, following the principle of reciprocity) is mainly depending on their dimension as the electromagnetic field they are able to produce is inversely proportional to their radius (Biot-Savart law), hence dimensions should be kept minimal. In addition, the signal consists of the contribution of all magnetic nuclei within a ROI, while all nuclei within the sensitive volume of the coil contribute to the noise. Hence, in order to maximize the SNR for a particular MR application, it is desirable to design a coil with the minimum affordable dimensions considering the size of the volume of interest. For this reason, surface coils are generally the preferred receiver designs provided the ROI is close to the objects surface as e.g. for MR studies of the brain. In comparison, volume resonators need to engulf the whole sample or body part, and are therefore bigger and less efficient.

However, a small surface coil has a limited field-of-view (FOV) and penetration depth. Its high efficiency is therefore limited to small volumes, often not sufficient to cover the entire three dimensional region of interest. The concept of bringing several small and simultaneously acquiring coils closely together to form a coil array was introduced in 1990 (Roemer, Edelstein, Hayes, Souza, & Mueller, 1990). This kind of coil system offers the SNR of a small surface coil, but simultaneously covers extended regions of interest. Each coil element is attached to a separate receiver chain, and the data is combined subsequently. Array coils are nowadays widely used, and led to the development of parallel acceleration techniques (Dydak, Weiger, Pruessmann, Meier, & Boesiger, 2001; Griswold et al., 2002; Pruessmann, Weiger, Scheidegger, & Boesiger, 1999).
Commonly, surface coils and in particular array coils are used as receivers only, in combination with volume resonator for homogeneous excitation. In order to avoid interaction between transmit and receive coils, the volume resonator must be actively detuned from the Larmor frequency during signal reception, while the surface coil must be actively detuned during transmission. Active detuning can be handled using an externally controlled switching circuit, a strategy that is incompatible with phased array coil, in which several (detector) coils must be active simultaneously. The mutual inductance elicited by neighboring elements must be suppressed via additional hardware implemented in the resonant circuit itself. This more complex design has prevented until recently the development of cryogenic array coils, as conventionally used MR compatible components used for decoupling of room-temperature array coils might not be suited for cryogenic operation. Moreover, the additional hardware necessary for decoupling of coil elements acts like a resistor places in series with the coil circuit, introducing a potentially non-negligible noise source when considering that the coil noise constitutes the major source of noise in coils designed with very small radii. Therefore, the performance of a phased array coil at low temperature might not reach expectations. One aspect of this thesis was to evaluate the performance of the first existing receive only cryogenic phased array coil prototype during its development. The coil was extensively tested in terms of SNR, and its general compatibility with the current needs of demanding procedures such as fMRI, MRS, MR angiographies and high resolution imaging was evaluated in-vivo on mice (see Chapter 3 & 4).

1.4. Data acquisition strategies to increase sensitivity in 1H-MRS

Prior to discussing strategies to enhance sensitivity in 1H-MRS, we have to consider some basic aspects of data acquisition in MR spectroscopy: localization, optimizing magnetic field homogeneity to minimize line-width and thus increase SNR, and water suppression.

General procedures are briefly described here, and more details especially for the particular case of the mouse brain will be given in the different chapters of this thesis.

1.4.1. Localization techniques

The metabolic composition of tissue is heterogeneous especially across the brain; therefore the origin of metabolite signals in space has to be known. Localization schemes are commonly separated into single voxel and multi-voxel strategies with a volume element (voxel) being a homogeneous ROI giving rise to one spectrum. Typically the volume investigated in single voxel spectroscopy (SVS) is smaller, which translates, in general, into better spectrum quality. Smaller volume infers more efficient water suppression, less contamination from signal sources outside this volume (e.g. lipids) and better magnetic field homogeneity resulting in narrow lines and hence better spectral resolution (see below). In comparison, multi-voxel spectroscopy, also called spectroscopic imaging (SI) enables the acquisition of full metabolite maps, i.e. allows probing the heterogeneity in metabolite distribution.
across larger tissue domains. In this case the volume of interest is typically considerably larger, which leads to inferior water suppression and magnetic field homogeneity.

For both methods spatial localization is based on spatially varying magnetic field gradients added to the main magnetic field $B_0$ to create spatially dependent resonance frequencies along one axis. A certain group of protons (corresponding to a slice in space) can then be selected according to its resonance frequency by using the appropriate frequency selective RF pulse. When this same procedure is repeated in multiple spatial dimensions, a volume of interest can be selected at the intersection of the frequency selected slices. Based on this principle, several approaches to select a volume in three dimensions have been proposed; amongst the most popular techniques one finds methods such as PRESS, STEAM, ISIS and SPECIAL (see below). All these techniques are based on the formation of spin echoes.

An ensemble of spins that has been excited will lose its phase coherence due to interactions with neighboring spins (T2 relaxation or spin-spin relaxation) and local magnetic field inhomogeneities (T2* relaxation) that both will lead to modulation of resonance frequencies. While the interaction with neighboring spins is a stochastic process and coherence is inevitably lost, the part of the signal that is lost due to constant field perturbations can be recovered by applying a 180° pulse, also referred to as refocusing pulse, which is applied after an echo delay TE/2. All the magnetization vectors (corresponding to the incoherent groups of spins) are mirrored along the axis in the x-y plane around which the RF pulse has been applied, while the $B_0$ inhomogeneities and therefore the phase shifts occurring in the time interval between excitation and refocusing pulse are constant. As a consequence, spins will be realigned forming a spin echo at TE. The echo amplitude will be reduced by the contribution due to T2 relaxation, which accounts for stochastic processes. Hence, in order to maximize the sensitivity it is essential to keep TE minimal.

Echo formation by a 180° pulse is the most efficient way to produce an echo with full signal intensity. However, Erwin Hahn has demonstrated in 1950 (Hahn, 1950) that a pair of 90° pulses will also lead to echo formation though at a reduced amplitude. Furthermore, in a sequence of three identical 90° pulses, three regular or ‘primary’ echoes will be generated from every possible combination of pulses, and two additional or ‘secondary’ echoes will also be measured. One of them is referred to as stimulated echo and is of particular interest for spectroscopic measurements.

All currently used SVS techniques are based on the formation of spin echoes. In the following we discuss some commonly used acquisition schemes.

**Point Resolved Spectroscopy (PRESS)**

PRESS (P. A. Bottomley, 1987; P. Bottomley, 1985; Jung, 1996a, 1996b) is a double spin echo method in which one slice selective excitation is performed as described above, followed by two slice
selective refocusing pulses. The signal is collected in the form of a spin echo after a time TE corresponding to twice the total time delay between the refocussing pulses. PRESS provides the maximum signal intensity considering the signal losses due to T2 relaxation and a good suppression of signals coming from outside the volume of interest. As already stated, for maximizing the sensitivity TE should be kept minimal.

**Stimulated Echo Acquisition Mode (STEAM)**

STEAM (Jens Frahm, Merboldt, & Hänicke, 1987) is based on three successive slice selective 90° pulses and the acquisition of the so-formed stimulated echo. When the first two pulses are executed with a time delay TE/2, and the third pulse is separated from the second pulse by the time TM, then the stimulated echo occurs after a time TE + TM. STEAM allows very short echo times and therefore is less prone to B1 inhomogeneities and chemical shift displacement effects (see below). However it intrinsically provides only half of the signal amplitude compared to PRESS.

**Image Selected In vivo Spectroscopy (ISIS)**

Unlike PRESS or STEAM, ISIS (Ordidge, Connelly, & Lohman, 1986) cannot provide a spectrum in a single acquisition. For the localization of a volume in three dimensions at least 8 steps are required. A set of eight pulse sequences with one, two, three or no slice selective inversion pulses prior to a 90° excitation pulse are combined in different ways in order to result in the signal of the volume of interest only. ISIS is very efficient for the measurement of fast relaxing metabolites, but is nowadays rarely used for proton spectroscopy.

**SPin ECho, full Intensity Acquired Localized (SPECIAL)**

The SPECIAL sequence (Mlynárik, Gambarota, Frenkel, & Gruetter, 2006) combines a one dimensional ISIS localization scheme with a regular localised spin echo experiment (intersection of the frequency selected slices of the 90° and 180° pulses) to achieve complete localisation in a minimum of two steps. The main strength of SPECIAL is that it can provide the full signal intensity attributed to regular spin echoes while short echo times comparable to those achieved with STEAM can be used.

**Multi-voxel acquisition**

In multi-voxel spectroscopy, also called spectroscopic imaging (SI) or sometimes chemical shift imaging (CSI), signals from multiple locations are acquired simultaneously. Generally two-dimensional, the experiment starts with a slice selective excitation. The position of individual voxels within the slice is encoded using the principle of phase encoding (as it is applied in regular MR imaging experiments), in which magnetic field gradients are switched prior to the acquisition in order to introduce a position dependent phase into the signal. If a nxn voxel grid is defined in space,
complete localisation is achieved after nxn phase encoding steps, therefore at least nxn acquisitions are necessary leading to rather long acquisition times. The achieved spatial resolution, or voxel size, is determined by the sensitivity (mostly dependent on the setup and the position of the selected slice respective to the coil) and the invested measurement time, similarly as for SVS. Also, SVS and SI are equally efficient in terms of SNR per unit time for the same voxel dimensions.

As phase encoding can be used in two or three spatial dimensions for localization, there is theoretically no need for the selection of a volume of interest. However, due to slice selective excitation a number of unwanted signals (such as those originating from fatty tissues) are contributing to the signal, introducing artefacts. SI is therefore almost always combined with a PRESS, STEAM or SPECIAL volume selection prior to phase encoding. Ideally, the volume of interest defined as such is a large squared area containing only the tissues of interest (excluding skull and skin tissues). This volume of interest is generally also used for shimming.

1.4.2. Chemical shift displacement error

When magnetic field gradients $G(x)$ are used for localization, each frequency is associated to a specific position in space according to

$$\omega(x) = \gamma \cdot (B_0 + G(x) \cdot x). \tag{1.3}$$

However, as described in eq.1.2 individual metabolite signals are also differentiated within one spectrum according to their particular resonance frequency $\omega_i$, which is slightly shifted compared to the water resonance (chemical shift $\sigma_i$). As a consequence, when a frequency selective pulse is applied the slices excited for two signals with a chemical shift difference $\Delta \sigma$ will be shifted by a distance $\Delta x$, i.e. it cannot be distinguished whether a signal at a specific frequency arises from a resonance $i$ of a compound located at $x$ according to

$$\omega_i(x) = \gamma \cdot (B_0 - \sigma_i \cdot B_0 + G \cdot x) \tag{1.4}$$

or from resonance $j$ at a position $x'$ such that

$$\Delta x = x - x' = \frac{(\sigma_i - \sigma_j) \cdot B_0}{\gamma} = - \frac{\Delta \sigma \cdot B_0}{\gamma} \tag{1.5}.$$  

Hence, the chemical shift displacement error (CSDE) for a given metabolite is defined by its chemical shift divided by the amplitude of the magnetic field gradient applied. Because the chemical shift of a metabolite is an intrinsic feature of the molecule and the frequency offset in Hz given for certain field strength, the CSDE must be minimized by increasing the amplitude of the applied magnetic field gradient. This implies that in order to excite a voxel of a defined dimension the pulse bandwidth must be increased proportionally. Yet, an increased pulse bandwidth goes along with an increased pulse peak power, which is usually the limiting factor, either due to technical limitations of the
instrumentation or, in a clinical setting, due to restrictions regarding the specific absorption rates of tissue.

1.4.3. Increasing SNR by homogenization of the static magnetic field: shimming

Signals of 20 or more metabolites can be identified in a typical state-of-the-art spectrum acquired at high magnetic field (>7T) (Duarte, Lei, Mlynárik, & Gruetter, 2012), some of them overlapping or resonating as multiplets (spin-spin coupling). Many metabolites display signals at more than one frequency due to several distinct proton groups in one molecule. To ensure maximal spectral resolution and hence reliable quantification it is essential to avoid inhomogeneous line broadening $\Delta \omega$ due to variation in $B_0$ values across the sample (see eq. 1.1), i.e. to achieve optimal homogeneity of $B_0$. Even though the intrinsic magnetic field homogeneity is high, local field distortions will inevitably occur as soon as a patient, an animal or a sample is introduced in the magnet due to tissue specific values of the magnetic susceptibility.

Several procedures have been developed to improve the homogeneity of the static magnetic field; they are referred to as shimming procedures. The main magnetic field inhomogeneities are typically handled by adding magnetic field gradients generated by separate shim-coils (included in the magnet bore or gradient system) to the main magnetic field, once the patient, animal or sample is positioned. The standard approach assumes that any field distortion can be accounted for by a linear combination of additional fields of spherical harmonical symmetry, which should be intrinsically orthogonal, i.e. display minimal interference. Typically first (linear) and second order (quadratic) terms are considered, some systems also allowing adjustments of higher order shims (third or fourth order). Finding the optimal set of shim gradients can be achieved in an operator interactive manner (Chmurny & Hoult, 1990) or via automatic shim procedures (R Gruetter, 1993; Rolf Gruetter & Tkáč, 2000; Miyasaka, Takahashi, & Hetherington, 2006b). As field distortions are specific for each sample field shimming has to be carried out for each sample independently. Optimal shim values lead to minimal inhomogeneous broadening of individual resonance lines and correspondingly to increased SNR values. As under fully relaxed conditions the intensity of the signal (signal area) depends on the number of nuclei only, reducing the line widths translates in increased intensity.

Besides shimming, the signals of water and lipids need to be suppressed before the acquisition of the desired signal, mainly because they produce artifacts in the spectrum (such as baseline distortions) which renders accurate quantification difficult. For MR spectroscopic studies of the brain, water suppression and lipid saturation are typically achieved by frequency selective saturation of the water resonance combined with outer volume suppression (Haase, Frahm, Hänicke, & Matthaei, 1985; Tkáč, Starčuk, Choi, & Gruetter, 1999), as lipids are predominantly located outside the skull, i.e. spatially separated from the region of interest. These modules are implemented in the MRS pulse sequence and
are repeated for every phase encoding or averaging step as the water/lipid signals recover due to $T_1$ relaxation.

1.5. Potential and issues of MRS at high magnetic field strength

As already mentioned, the use of high magnetic fields is necessary to increase the signal intensity under conditions when SNR is the limiting factor, which holds for $^1$H MRS considering the low intrinsic sensitivity of the technique, and particularly when applied in mice due to the very small size of the regions of interest. In principle, higher field strengths represent a big advantage for 1H MRS not only because of higher SNR, but also because of increased chemical dispersion, which eventually will allow resolving resonances that typically overlap at low field strength. For brain 1H MRS those include the signals of glutamate (Glu), glutamine (Gln), and GABA, as well as the resonances of creatine (Cr) and phosphocreatine. In addition, spectra of strongly coupled systems such as Glu and Gln become simpler as the chemical shift difference of coupling spins increases as compared to the scalar coupling constant (Avdievich, Pan, Baehring, Spencer, & Hetherington, 2009; Mekle et al., 2008; Tkác et al., 2001), which will facilitate the quantification. On the other hand, at high field shimming requires high correction fields of complex geometry, a requirement that may exceed the performance of high power shim systems currently available. Also first and second order shims may not be sufficient to achieve reasonably narrow line width in the mouse brain to fully exploit the potential of the high magnetic field, as the VOI is typically very close to the interface between tissue and air, creating strong susceptibility artifacts. The large frequency dispersion also puts high demands on the performance of RF amplifiers as the bandwidth of the pulse has to cover the entire spectral range. Hence pulses become short, which translates into high peak power. Thus, the use of conventional SVS sequences such as PRESS involving 180° refocusing pulses may become unsuited due to limitations in pulse bandwidth, which will produce high CSDE. Moreover, due to increased gradients of magnetic susceptibility across the tissue, metabolite signals experience stronger differences in the local magnetic field $B_0$ and therefore dephase faster at high field ($T_2^*$ relaxation), enforcing the use of short echo time sequences. Dedicated sequences have been developed to overcome these difficulties among which SPECIAL, localization by adiabatic selective refocusing (LASER or SEMI-LASER, T. W. Scheenen et al., 2008; T. W. J. Scheenen et al., 2008; Shen and Rothman, 1997)) probably delivered the most convincing results (Duarte, Do, & Gruetter, 2014; Lei, Berthet, Hirt, & Gruetter, 2009; Lei, Poitry-Yamate, Preitner, Thorens, & Gruetter, 2010; Miyasaka, Takahashi, & Hetherington, 2006a; Tkac, Dubinsky, Keene, Gruetter, & Low, 2007; Tkáč et al., 2004; Weiss, Melkus, Jakob, & Faber, 2009). In particular when using SPECIAL, the acquisition of full signal intensity enabled shorter measurement times or the definition of smaller volumes, while very short echo times (as low as 1ms) helped to overcome the problem of fast relaxing metabolites signals.
1.6. The neurochemical profile as indicator of brain function

The neurochemical profile measured in the brain using 1H MRS is highly dependent on the location of the region of interest (Tkáč et al., 2004). Highly resolved metabolite maps obtained in the human or rodent brain in-vivo reflect anatomical structures and are therefore believed to also reflect the function attributed to each brain region to a certain extent. The concentrations of metabolites have been shown to evolve in time during development (Tkáč, Rao, Georgieff, & Gruetter, 2003) and during the progression of certain diseases (Clarke & Lowry, 2001; Lei et al., 2009; Lutkenhoff et al., 2010; Miller et al., 1993; Tkc et al., 2007; Van De Looij, Chatagner, Hippi, Gruetter, & Sizonenko, 2011). The chemical profile comprises the signals of important excitatory (Glu, aspartate (Asp)) and inhibitory neurotransmitters (GABA), indicators of glial activity (Glu and Gln) and general indicators of metabolism (lactate (Lac), Cr and PCR), whose changes in time could be linked to neural activity. In mice, neural activity can be triggered using established protocols for sensory stimulation or via the injection of a neuroactive compound. Commonly used MR-based methods to access brain function (fMRI) are based on hemodynamic readouts, measuring changes in blood oxygenation (BOLD) or cerebral blood volume (CBV) as surrogates of neural activity. These hemodynamic readouts depend on the integrity of neurovascular coupling, which may not be warranted under pathological conditions. In addition, cerebral hemodynamics are not only regulated by the brain metabolic demands but might also be influenced by systemic cardiovascular and respiratory effects or direct vasomotor actions of drugs. In fact, it has been reported that in mice activity-evoked BOLD and CBV responses appear to be dominated by systemic hemodynamic alterations caused by the stimulus (Schroeter, Schlegel, Seuwen, Grandjean, & Rudin, 2014). The systemic hemodynamic response translates into widespread and non-specific ‘activity’ patterns, rendering any attempt on quantification of neuronal activity superfluous. Thus, alternative readouts of brain function that are not based on cerebral hemodynamics and therefore should be less susceptible to systemic confounds, become highly attractive. 1H MRS has been successfully applied to detect metabolic events accompanying neural activity in humans (Lin, Stephenson, Xin, Napolitano, & Morris, 2012; Mangia et al., 2007; Prichard et al., 1991; Sappey-Marinier et al., 1992) and rats (Xu, Yang, Li, Zhu, & Shen, 2005), using paradigms such as visual or sensory stimulation. However, due to the limitations regarding sensitivity and hence temporal resolution described in the previous sections, its efficiency for detecting short term biochemical changes in mice has not been demonstrated yet.

References


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Chapter 2

Objectives and outlines
The aim of this work was to develop a reliable experimental protocol for the acquisition of metabolite maps at a temporal resolution sufficient to study functional processes in the mouse brain at 9.4T. As discussed in the Introduction section, spectroscopic imaging (SI) is a particularly slow procedure due to: (i) the low intrinsic SNR due to the low concentration of the metabolites under investigation (typically 1:10000 compared to water). Data averaging is therefore required, which significantly slows down the process due to its inefficiency (SNR increases with the square root of averages). (ii) The in-plane localization is based on two-dimensional phase encoding as no frequency encoding can be applied since the frequency dimension is required for spectral resolution. Each phase encoding step requires a separate data acquisition; hence the total acquisition requires at least $N_x \times N_y$ steps that have to be recorded sequentially.

Achieving the sensitivity requires optimization of animal preparation, imaging hardware, and data acquisition strategies. This defines the specific objectives of this PhD thesis:

1. Development of an animal preparation (anesthesia, physiological monitoring) that warrants minimal physiological fluctuation in the course of the experiment.
2. Optimization of imaging hardware for optimal sensitivity. This relates in particular to improving the noise figure of the RF detector.
3. Optimization of acquisition protocols (pulse sequences) in order to minimize signal loss to T2/T2* relaxation and chemical shift displacement effects to ensure that metabolite signals originate from almost identical ROIs.

The gain in sensitivity should allow achieving a temporal resolution that is sufficient to monitor changes in the metabolite profiles in response to a pharmacological or physiological challenge. Correspondingly the fourth objective becomes:

4. Demonstrate the feasibility of functional MRSI in mice to capture region-specific changes in metabolite profile induced by a pharmacological stimulus affecting large brain areas and a specific physiological stimulus (sensory stimulation of the forepaw).

These objectives are reflected in the outline of this thesis:

**Chapter 3** deals with the development of detector systems of significantly increased sensitivity. This is achieved by reducing the thermal noise of the coil electronics, i.e. by the design of cryogenic coils. In particular the development and optimization of the first prototype of a 2x2 array coil system operating as receiver only and specially designed for the mouse head will be described. The prototype was extensively tested on phantoms and mice for several MR procedures including SVS and SI.

**Chapter 4** describes high resolution SI acquired with standard localization schemes but using the cryogenic array coil as receiver, and is thought to enlighten the possibilities in terms of spatial and temporal resolution that can be achieved with such a system. Yet in the course of these experiments
several deficiencies of these acquisition schemes emerged, which will be dealt with in the following chapter.

Chapter 5 deals with the optimization of the data acquisition procedures to further enhance sensitivity and reduce potential artefacts due to CSDE in mouse functional SI. We have adapted a fast SI method (FIDLOVS, Henning et al., 2009), based on the acquisition of the FID signal recorded immediately after slice selective excitation, whereas the volume of interest is selected by suppressing potential signals originating from locations outside the VOI (outer volume suppression). This method has been developed for high field clinical systems, to minimize the CSDE and the signal losses due to fast T2 relaxation. The performance of FIDLOVS has been evaluated in assessing relative metabolite concentrations at baseline as well as with regard to the sensitivity of the method in detecting metabolic alterations elicited by the administration of a neuroactive compound.

Chapter 6 evaluates the potential of FIDLOVS to monitor small changes in endogenous tissue metabolism elicited by sensory stimuli. This more physiological stimulus should trigger a very specific and localized response, primarily in the somatosensory cortex contralateral to the stimulated hind paw. For high stimulus amplitudes or even nociceptive stimuli additional brain areas might be involved. Similar studies have been reported in humans e.g. using visual stimulation paradigms and in rats using electrical paw or mechanical whisker stimulation, however up to now not in mice, due to the numerous technical challenges mentioned above. In these studies SVS voxel protocols have been used, which record metabolic information from a single region-of-interest (ROI), a severe limitation as a) cerebral processing typically involves multiple brain areas and b) no conclusions can be drawn regarding the specificity of the metabolic response. The aim of this study was to evaluate the sensitivity of the implemented SI protocol and to probe for the specificity of the measured response, considering the difficulties encountered when using standard fMRI protocols (based on hemodynamic readouts), where the response seems to be dominated by systemic effects (Schroeter et al., 2014). The well documented variability encountered in quantitative functional MR experiments in mice is very high and presents a real challenge. Achieving reproducible experimental conditions requires sophisticated optimization of the experimental setup including the restriction of motion.

In the appendix, we review the requirements for stable and reproducible functional SI experiments based on the observations made in the process of setting up the experiments described in chapter 5 and 6. The mouse is a fragile organism, and maintaining its physiology under anesthesia constitutes a major challenge. For functional (MR) studies, irrespective of whether the readout is hemodynamic (BOLD, CBV) or metabolic, unstable physiology appears to contribute to a large extend to the observed variability, an aspect that appears often underestimated.
By combining the increased sensitivity provided by the receive only cryogenic array coil with FID-based sampling of spectroscopic data we were able to develop a protocol with sufficient temporal resolution to monitor metabolic changes upon chemical and sensory stimulation in mice. In the future, we aim to apply this method to study changes in metabolism due to pathology, both with regard to baseline metabolism and functional SI in response to a challenge, which might be more sensitive than baseline values themselves. The ability of carrying out such studies in mice is highly attractive given the large number of genetically engineered mouse models of human diseases available for biomedical research.

References


Chapter 3

A four-element cryogenic phased array receive only RF probe for mouse MR imaging and spectroscopy

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Adapted from:


Abstract

Structural and functional imaging of the mouse brain requires high spatial resolution and can lead to long acquisition times or insufficient SNR. The development of commercially available cryogenically cooled RF coils has been demonstrated beneficial for small animal MRI, by improving the SNR by a factor 2 to 3 on average at 9.4T. All cryogenic RF coils developed until recently operate in transmit/receive mode and are either linearly polarized or in quadrature. Yet, the inhomogeneous excitation by a surface coil is a disadvantage for several applications, e.g. whole brain 3D imaging, imaging of deep brain structures or spectroscopic imaging. Progress in coil technology and electronics enabled the realization of a receive-only array coil operating under cryogenic conditions. Here we show phantom and in-vivo data acquired with the first existing cryogenic coil array composed of more than two elements. The objective was to compare its performance to that of a similar receive only coil setup operating at room temperature (RT) and to that of the previously designed quadrature cryogenic transmit/receive coil.
3.1. Introduction

In MRI/MRS experiments, two dominant noise sources contribute to the total noise: the noise generated within the radiofrequency (RF) coil and the noise originating from the sample. Electromagnetic noise arises from thermal agitation of charge carriers in dissipating media, i.e. of electrons in the conducting structures of the receiver circuit and electrical charges (ions) distributed within the magnetically coupled sample. Under most conditions in MRI/MRS, sample noise is dominating. Yet, for RF coils with a small diameter (≤30mm), the noise introduced by the sample is strongly reduced and becomes comparable to the noise introduced by the coil. As thermal agitation depends on the temperature within a material, lowering the coil temperature can lead to significant decrease in the overall noise figure and correspondingly to an increase in signal-to-noise ratio (SNR) (Darrasse & Ginefri, 2003; Hall et al., 1988; Hoult & Richards, 1976; Styles et al., 1989). In the past decade, the development of meanwhile commercially available cryogenically cooled RF coils (Baltes et al., 2009; Ratering et al., 2008) has been demonstrated beneficial for small animal MRI. For mice, a coil diameter of 2 cm is sufficient to cover the entire brain as a region of interest. In humans, comparable coil diameters could potentially be encountered only in a complex phased array configuration composed of many elements, which would render the design of a clinical head coil rather challenging.

Commonly, surface coils and in particular array coils are used as receivers only in combination with a volume resonator for homogeneous excitation. The problematic interaction between transmit and receive coils is generally avoided by actively detuning one of the two coils while operating the other one using an externally controlled switching circuit (Bendall, Connelly, & McKendry, 1986). However, when operating several (detector) coils simultaneously, for example in the case of an array coil, mutual inductances must be suppressed via additional hardware implemented in the resonant circuit itself. The common inductive decoupling method where adjacent coils overlap in a way that cancels the mutual inductance to minimize the coupling is generally insufficient. Common strategies include capacitive decoupling and the use of low input impedance amplifiers in order to reduce undesired currents flowing in the individual coil elements (Duensing, Brooker, & Fitzsimmons, 1996; Lee, Giaquinto, & Hardy, 2002; Roemer et al., 1990). Until recently, these more complex design requirements have prevented the development of receive only cryogenic coil arrays. Operation at low temperatures put severe constrains in terms of design and materials that can be used. Commonly used MR compatible components might introduce susceptibility artifacts at very low temperatures, seriously reducing the scope of possible applications. Additional hardware also introduces noise in the individual elements, and the achieved coil performance at low temperature might not reach expectations. While for a single loop coil the SNR enhancement due to cryogenic cooling can theoretically be estimated for a given coil size operating at a certain frequency, estimating the performances of a cryogenic array coil is less straight forward.
One of the first commercially available cryogenic coil designed for in-vivo mouse imaging was developed by Bruker (Bruker BioSpin AG, Fällanden, Switzerland) (Baltes et al., 2009) for a 400MHz system. This cryogenic quadrature transmit/receive coil was compared with a similar RT (receive only) coil in terms of SNR and showed an enhancement by a factor of 2.5 on average. This result is in good agreement with the theoretical predictions (Darrasse & Ginefri, 2003; Ratering et al., 2008).

More recently, a receive-only array coil has been designed by the same company. The coil is composed of four elements arranged in a 2x2 geometry, while the overall coil dimension remained unchanged compared to the previous quadrature coil design. Hence, the array coil should provide higher SNR compared to its predecessor.

In this study we show phantom and in-vivo data acquired with the (to our knowledge) first existing cryogenic coil array composed of more than two elements. The objective was to compare its performance to that of a similar receive only coil setup operating at room temperature (RT) and to that of the previously designed quadrature cryogenic transmit/receive coil.

3.2. Theoretical Background

The SNR is defined as the ratio of the signal divided by the standard deviation of the noise and can therefore be increased either by maximizing the signal for a particular MR experiment or by minimizing the noise. According to the principle of reciprocity, an electromotive force of the amplitude $\xi$ induced in a detector coil by a magnetic moment $\vec{M}$ rotating with a frequency $\omega$ at a given voxel position within the sample can be expressed as follows:

$$\xi = -\frac{\partial}{\partial t} \left( \vec{B}_1 \cdot \vec{M} \right) = \omega \cdot \left( \frac{\vec{B}_1 \cdot \vec{M}}{I} \right)$$  \hspace{1cm} (3.1)

where $\vec{B}_1$ is the RF magnetic field generated at the voxel position when a current $I$ is supplied to the coil. The dominant noise sources contributing to the total noise observed in an MR experiment are coil noise and sample noise. The noise can be regarded as originating from the thermal noise of the lossy elements in the receiver chain and represented by resistances connected in series with the coil circuit. Each resistance is associated with a certain temperature. The voltage variance induced by a resistance $R$ at a temperature $T$ is given by:

$$n^2 = 4 \, k_B \, T \, R$$  \hspace{1cm} (3.2)

where $k_B$ is Boltzmann’s constant. For two resistances connected in series, e.g. the sample resistance $R_S$ and the coil resistance $R_C$ given for the temperatures $T_S$ and $T_C$, the total RMS voltage spectral noise density $n$ which appears in the detection coil can be written as:
For a given MR experiment, the SNR can be derived from equation (3.1) and (3.3) and from the parameters related to the imaging sequence:

\[ n = \sqrt{n_s^2 + n_c^2} = \sqrt{4 \cdot k_B \cdot (R_S \cdot T_S + R_C \cdot T_C)} \]  

(3.3)

For a given MR experiment, the SNR can be derived from equation (3.1) and (3.3) and from the parameters related to the imaging sequence:

\[
\text{SNR} \propto \frac{\omega \cdot (B_t / I)}{\sqrt{4 \cdot k_B \cdot (R_S \cdot T_S + R_C \cdot T_C)}} \cdot M_T \cdot V_{voxel} \cdot \sqrt{\frac{N_{FE}}{BW}} \cdot \sqrt{\frac{N_{PE}}{N_A}}
\]  

(3.4)

with \( M_T \) indicating the transverse magnetization component giving rise to the signal, \( V_{voxel} \) the voxel volume, \( BW \) the bandwidth of acquisition, \( N_{FE} \) the number of samples in the frequency encoding direction, \( N_{PE} \) the number of phase encoding steps and \( N_A \) the number of averages. The first term of expression (3.4) reflects the contribution of the RF detection (RF sensitivity) while the second part comprises terms characteristic for the MR pulse sequence. Given that a specific MR experiment implies constraints in terms of spatial and temporal resolution and is conducted at fixed field strength, we are interested in optimizing the first term of equation (3.4).

The RF sensitivity can be enhanced by increasing the magnetic coupling coefficient \( B_t / I \). The coupling coefficient is derived from the Biot-Savart law and for a circular RF coil of radius \( r \) becomes:

\[
\frac{B_t}{I} = \frac{\mu_0}{2r}
\]  

(3.5)

where \( \mu_0 \) stands for the magnetic permeability constant. The coupling coefficient and correspondingly the signal can be increased by decreasing the size of the coil. As small coils do not necessarily cover the entire region of interest, the design of coil arrays might be considered (Roemer et al., 1990).

Another approach to improve the RF sensitivity term is to reduce the noise contribution \( \sqrt{4 \cdot k_B \cdot (R_S \cdot T_S + R_C \cdot T_C)} \) by reducing the resistances \( R_S \) and \( R_C \) and/or by reducing the coil temperature \( T_C \) (as the sample cannot be cooled down for in-vivo applications).
Figure 3.1: a) Scheme of a circular surface copper coil of \( \ell \) turns with a radius \( r_{\text{coil}} \) positioned at a distance \( d \) from a lossy sample characterized by the electrical conductivity \( \sigma \). b) Relative SNR as a function of \(^1\text{H} \) resonance frequency for RF coils operating at 293K (gray), 30K (black) and 0K (dotted). c) Gain in SNR for a receiver coil operating at 30 K (black) and 0K (dotted) as compared to RT detection. Parameters used for the simulation were \( r_{\text{coil}}=10 \text{ mm}, n=1, \sigma=0.66 \text{ S/m}, \xi r_{\text{coil}}/r_{\text{wire}}=40, T_S=310\text{K (37°C)}, T_C,RT=293\text{K}, T_C,LT=0\text{K}, 30\text{K}, 293\text{K}, d=1\text{mm}, \rho_c(293\text{K})=1.49\cdot10^{-7} \Omega\cdot\text{m}, \text{temperature coefficient } \alpha=0.003.

In the review paper of Darrasse and Ginefri (Darrasse & Ginefri, 2003), analytical expressions for a circular RF coil of radius \( r_{\text{coil}} \) consisting of \( \ell \) turns of a wire with radius \( r_{\text{wire}} \) placed at a distance \( d \) from a homogeneous semi-infinite sample with an electrical conductivity \( \sigma \) (Fig. 3.1a) can be found for the two noise contributions in equation (3.3) and (3.4). The resistances \( R_S \) and \( R_C \) are then given by:

\[
R_S = \frac{2}{3\pi} \cdot \sigma \cdot \mu_0 \cdot \omega^2 \cdot n^2 \cdot r_{\text{coil}}^3 \cdot \arctan \left( \frac{\pi \cdot r_{\text{coil}}}{8 \cdot d} \right) \tag{3.6}
\]

\[
R_C = \sqrt{\frac{1}{2} \cdot \rho_c \cdot \mu_0 \cdot \omega \cdot \ell^2} \cdot \left[ \frac{\xi}{r_{\text{wire}}} \cdot \frac{r_{\text{coil}}}{r_{\text{wire}}} \right] \tag{3.7}
\]

In equation (3.7) the coil resistivity is given by \( \rho_c \) (which in itself is inversely proportional to the temperature), while \( \xi \) denotes the influence of the proximity effect given by the RF current distribution around the coil wire. Both resistances can be diminished by lowering the coil radius or the frequency \( \omega \). It can be seen that the sample resistance \( R_S \) (and hence the noise power) increases with the third power of the coil radius (approximately defining the sampling volume for surface coil) and the square of the resonance frequency, while the coil resistance \( R_C \) increases only as the square root of \( \omega \) and is typically constant with the coil radius as one would typically scale the wire radius \( r_{\text{wire}} \) together with the coil radius \( r_{\text{coil}} \). Hence, it becomes obvious that the relative contribution of coil noise to the total noise increases for small values of \( r_{\text{coil}} \), as typically encountered in mouse MRI applications, and also for low values of \( \omega \). Hence, for \( R_C T_C \geq R_S T_S \) lowering the coil temperature becomes attractive as the term \( R_C T_C \) is then the dominant contribution in equation (3.3) and will lead to significant gain in SNR (Fig. 3.1b,c). The limit of the noise predominance domain of either sample or coil noise depends
mainly on the frequency, the coil radius and to a smaller amount on the coil resistivity. Typically, at 400 MHz and considering a single loop copper coil, the two terms become equal for coil radii of about 10 mm at room temperature and 6 mm for a coil cooled down to 30K. For coil radii exceeding these values, the gain in sensitivity achieved through cryogenic cooling is intrinsically limited due to the dominance of the sample noise. For coil radii smaller than the critical value, the sensitivity can still be improved by further cooling the coil, by reducing the intrinsic coil resistance (i.e. by using superconducting material Black et al., 1993) or by further diminishing the coil size.

These simple considerations hold for small circular RF coils. Similar principles may be applied for coil arrays designed to cover larger sample volumes. While the SNR gain of phased array coils is undeniable in clinical MR applications, it is not always obvious for small animal imaging. When coil dimensions become very small, contributions from additional noise sources become non-negligible: electronic components such as capacitors, transmission lines, preamplifiers, cables, and soldering material may substantially contribute to the overall noise figure thereby diminishing the expected SNR gain achieved by reducing the coil size (i.e. reducing the sample noise). For cryogenic coils, the noise originating from preamplifiers (including its transmission line) become a substantial contributor to the total noise, therefore preamplifiers has to be operated at low temperatures as well, typically at ≤50K to take full advantage of coil cooling. Moreover, the distance between the coil and the preamplifier should be minimal, as any additional cable length will add noise to the system. While cryogenic phased array coils appear very attractive, the complexity of design inherently involving additional lossy electronic components may limit the potential gain in sensitivity and hence the additional benefit as compared to a single-loop or a quadrature surface coil.

**Figure 3.2: Potential designs for cryogenic surface coils.** A single loop coil is the simplest design. The sensitivity of a surface coil can be increased by increasing the number of coil elements while maintaining the same overall coil dimension. Quadrature (2 elements) and array coils (multiple elements) are possible designs.
3.3. Materials and Methods

MRI System and coil setups

All experiments were carried out on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) small animal MR system operating at 400 MHz. The system is equipped with a BGA 12AS HP gradient system with a maximum gradient strength of 440mT/m and minimum rise time of 70 μs. The shim system allows shimming up to the second order.

Both cryogenic coil probes (CRP) used in this study are operating at 30K with integrated cooled preamplifier (operating at 77 K). For each CRP, constant cooling is achieved using a closed loop system connected to a remote cryo-cooler, allowing both systems (array and quadrature) to be cold simultaneously. The quadrature transmit/receive CRP (quadCRP) possesses two side-by-side overlapping coil elements (each of them is 16x20 mm²) with an overall coil size of 27x20 mm². The receive only phased-array CRP (paCRP) possesses 4 elements arranged in a 2x2 geometry with an overall coil size of 27x20 mm².

![Figure 3.3: Sensitivity profile of both cryogenic surface coils.](image)

Signal and sensitivity profiles of the four individual coil elements (1,2,3 and 4) of the paCRP (a) and the sum-of-square (SOS) combination in a horizontal view (b). Both CRPs have different sensitivity profiles (c). However, the quadrature coil is used also as a transmit coil, and is therefore inhomogeneous in transmission (d). The line in (d) represents the depth, at which the reference attenuation of is adjusted.

The paCRP is operated in combination with a quadrature volume resonator (86 mm inner diameter) for homogeneous transmission. The room temperature phased-array coil had the same size and geometry as the paCRP and was used in combination with the same volume resonator. A guiding tube inserted in the magnet ensured reproducible positioning and fixation of the CRPs in the magnet. As both coil arrangements had the exact same outer dimensions, changing coils during an experiment was easy and fast. Animals are prepared on a separate support that is subsequently inserted in the magnet when the coil has already been mounted in the bore (as opposed to a regular RT setup, in which the coil is positioned on the animal before inserting it in the magnet).
Phantom validation

A cylindrical phantom of diameter 20mm was chosen to match the shape of the inner surface of the coils and was filled with physiological saline (0.9% NaCl) and Dotarem (concentration: 0.5 mmol Gd/mL from Guerbet, Aulnay-sous-Bois, France). For SNR comparison, same images were acquired with each coil in axial orientation with gradient echo (FLASH) and spin echo (RARE) sequences. The following parameters were used: **FLASH (Fast Low Angle Shot, (J Frahm, Haase, & Matthaei, 1986))**: Repetition time/echo time TR/TE = 380/7 ms, pulse angle $\alpha = 40^\circ$, field-of-view FOV: 20 x 20 mm, matrix dimension Mtx = 256 x 256, slice thickness SLTH = 0.5 mm. **RARE (Rapid Acquisition with relaxation enhancement (Hennig, Nauerth, & Friedburg, 1986))**: TR/TE = 3271/8ms, FOV: 20 x 20 mm, Mtx: 256 x 256, SLTH = 0.5 mm. When using the cryogenic transceive system (quadrature coil), images were acquired several times using different RF power levels adjustments in order to optimize the pulse angles for different positions.

Surface coils have inhomogeneous sensitivity profiles. The SNR gain provided by the cryogenic array coil was investigated in three axial slices (as shown in figure 3) corresponding to different sensitivity patterns. The SNR was determined by dividing each pixel value by the standard deviation of the noise level, originating from a region of interest placed in the outer corner of each slice.

In Vivo experiments

All *in vivo* experiments were carried out in strict adherence with the Swiss law for animal protection. Female C57BL/6 mice (Janvier, Le Genest-St Isle, France) at 3-4 months of age and ca. 23 g were anesthetized using isoflurane, intubated and artificially ventilated (80 breaths per minute, bpm) using a small animal ventilator (CWE, Ardmore, USA) for the entire duration of the experiment. Stereotactic fixation was used to ensure reproducible positioning of the animal on the support. The body temperature was kept at 37°C throughout the duration of the experiment. For high resolution imaging and angiography, a catheter was placed in the tail vein for intravenous injection of pancuronium bromide (1 mg/kg; Sigma-Aldrich, Steinheim, Germany) as a bolus in order to avoid movement during acquisition and for the administration of the contrast agent (Endorem, Roissy, France).

3.4. Results

Phantom validation

All four coil elements of the novel paCRP were shown to work independently and no mutual inductance has been observed among them (Figure 3a). No coupling to the transmit coil has been observed either. The coil has been intensively tested regarding potential susceptibility artifacts using sensitive sequences such as echo-planar imaging (EPI) or FISP (fast imaging with steady-state precession, Oppelt et al., 1986), delivering satisfying image quality (data not shown). The data
measured in all four coil elements were combined to yield the full MR image using the sum-of-squares (SOS, Roemer et al., 1990) method (figure 3b). The coil elements 1 and 2 (Fig.3.3a) were approximatively 20% less sensitive compared to coil elements 3 and 4. As a result the SNR gain achieved with the paCRP was found to be rather inhomogeneous when compared to a RT setup of analogous dimensions (Figure 4). A factor 2.5 was observed in a position corresponding to the maximal sensitivity of element 3 and 4 (slice 1), a factor 2 on average was measured in the middle slice, while a factor 1.5 – 2 was found under element 1 and 2 (slice 3). While the noise levels and the Q-values seem to be constant across all coil elements, element 1 and particularly element 2 seem to be significantly less sensitive (by approximately 20%) than elements 3 and 4. The exact cause of the sensitivity loss is not known, but seems to be related to the design of the coil as it has been observed in 4 different prototypes.

Figure 3.4: SNR gain provided by the cryogenic array coil compared with an identical RT setup. Three slices have been considered for the SNR analysis (a). The SNR gain is not homogeneous, slice 1 (corresponding to elements 3 and 4) shows a higher SNR gain compared to slices 2 and 3.

The paCRP was also compared to its predecessor, a quadCRP with same overall coil size operated in transmit/receive mode. Both coils have inherently inhomogeneous sensitivity profiles (figure 3c), with several maxima corresponding to the position of each coil element. Moreover, an additional sensitivity gradient is introduced when using the quadCRP as it is used also for transmission. The optimal flip angle can be reached only in one particular position in space, which is generally selected by varying the pulse power for a particular pulse duration and shape (figure 3d). Inhomogeneous excitation translates into inhomogeneous SNR patterns, as shown in Figure 3. When the pulse angle was adjusted to be optimal at the surface of the sample, a more rapid decrease in SNR was observed when using the quadCRP, especially when acquiring spin echoes due to the use of 180° pulses. Consequently, a significant SNR gain was achieved in regions far away from the coil surface when using the paCRP, mainly through homogeneous transmission. However, due to the already mentioned differences in sensitivity of elements 1 and 2, the SNR gain was found to be inhomogeneous across the FOV.
Figure 3.5: SNR gain provided by the cryogenic array coil compared to a cryogenic quadrature coil of same overall size. The SNR gain is strongly dependent on the MR sequence and on the settings of the reference attenuation (marked by the red line). In slice 1 (corresponding to the elements 3 and 4 of the cryogenic array coil) the SNR gain is substantial for both spin echo and gradient echo sequences. A drop in the SNR gain is observed for slices 2 and 3, due to seemingly less efficient elements 1 and 2. When the reference attenuation is set deeper in the sample (b), the SNR gain is below one at the surface. However, the array coil setup (operating in cross-coil mode with a volume resonator) provides much better SNR in deeper located regions due to the homogeneous excitation provided by the volume coil.

As depicted for slice 3 in Figure 5a, the SNR gain is negative at the surface of element 1 and 2. When the pulse is adjusted to be optimal at a deeper position (Figure 3b), the advantage of the phased array setup is particularly obvious when using spin echo sequences.

In vivo comparison

Good SNR is essential for in vivo measurements. Especially in mice, the small organ size puts high demands on spatial resolution and often several averaging steps are necessary in order to reach sufficient SNR levels. Prolonged measurement times are unwanted in particular for functional or metabolic studies, in which high temporal resolution but also stable physiology is required. The SNR gain provided by the cryogenic coil can be reinvested in higher spatial resolution (though a SNR gain
by a factor of 2 would translate in 20% reduction in linear voxel dimensions only) and increased contrast-to-noise ratio (CNR), enabling better delineation of structures as shown in Figure 6. GE images of the mouse brain acquired at 9.4 T revealed a distinctly superior image quality compared to the room-temperature coil setup when using any of the two CRPs and the same imaging parameters. The magnified inserts depict significantly more structural details in the images acquired with the CRPs compared to the room-temperature coil due to superior CNR. High resolution MR imaging on mice is generally applied to study changes in the morphology of tissues or organs. Particularly in the brain, structural changes can be associated with development/aging or with the progression of certain diseases. Optimally, these scans are three-dimensional with isotropic voxel dimensions to map accurately small brain structures. The gain in sensitivity provided by the paCRP compared to the quadCRP is not obvious in tissue areas near the coil surface. However, the cross-coil setup providing homogeneous excitation is an advantage when looking at deeply located brain structures, such as the brain stem (a factor 1.4 SNR gain was measured in this particular case).

High CNR is particularly important for MR angiography (MRA), as illustrated by a study applied to a mouse model of Alzheimer’s disease (transgenic arcAβ mice) involving time-of-flight MRA (TOF-MRA) and contrast-enhanced MR microangiography (CE-mMRA) (Klohs et al., 2011, 2012). These mice show age-dependent parenchymal and vascular deposition of amyloid-β peptide and cerebrovascular dysfunction, thereby mimicking aspects of Alzheimer’s disease. Quantitative analysis of contrast-enhanced microangiograms revealed a reduction of intracortical microvessels in 24-month-old arcAβ mice compared to age-matched controls, while no significant differences were seen between arcAβ mice and wild-type controls at 4 months of age. In this study, the cryogenic quadrature coil was...
used together with a 3D FLASH protocol for the pre and post-contrast images. A slab of $15 \times 12 \times 2.2$ mm$^3$ with a spatial resolution of $60 \times 60 \times 61$ μm$^3$ was placed in the cortex. Using the receive-only paCRP the same protocol could be extended to the entire brain (not feasible using the transceiver quadCRP). An example is displayed in Figure 3.7a. The same experiment was performed using the RT array coil (figure 3.7b). The increased SNR (and CNR) enabled the detection of a higher number of individual vessels; particularly those of smaller diameter (~100 μm) could be detected more reliably in the angiograms obtained with the CRP (figure 3.7c).

The use of CRPs is also very attractive for magnetic resonance spectroscopy (MRS) as the signals measured are weak and several averaging steps are required to obtain spectra of sufficient quality. In particular when applied to mice, MRS is very challenging due to the small volumes of interest (VOI) involved (typically around 1-2 μl). Not surprisingly, measurement times often exceed 60 minutes. The gain in SNR by a factor 2 to 3 provided by CRPs translates into a reduction of measurement time by a factor of 4 to 9 with regard to data acquisition using a room-temperature coil of equal dimensions. Alternatively, the SNR gain can be invested into improved spectral quality for the same acquisition time. Figure 3.8a shows spectra acquired from a 2μl voxel within the mouse hippocampus using the paCRP and the RT phased-array coil subsequently in the same animal applying point-resolved spectroscopy techniques (PRESS, Bottomley et al 1984). The SNR gain observed with the CRP immediately translates in more accurate quantification as indicated by the Cramer-Rao lower bands (CRLB, Figure 3.8b), especially for small metabolite signals such as GABA and glutamine. Accurate metabolite quantification is a critical aspect in these studies. Typically, pathologies or genetic/pharmacological interventions will lead to altered metabolite levels. These alterations may be in the order of a few percent to a few tens of percent of the basal levels. In this respect, it is attractive to invest the SNR gain provided by CRP receivers into an improved spectral quality as this immediately translates into better quantification. Alternatively, the sensitivity gain may be used to
increase the temporal resolution, which might enable carrying out dynamic studies on tissue metabolism in response to a challenge.

The multiple receiver acquisition also allows applying acceleration of data acquisition using parallel imaging strategies such as GRAPPA (Griswold et al., 2002) or SENSE (Pruessmann et al., 1999), e.g. in the acquisition of 3D high-resolution images. Since the images obtained using 3D acquisition revealed excellent SNR, acceleration based on multiple receiver techniques could be applied.

Figure 3.8: Single voxel spectroscopy on the mouse brain. A 2 μl PRESS voxel was placed in the mouse hippocampus and recorded with phase-array RT and paCRP setup (a). The following parameters were used: TR/TE=2500/13 ms, number of scans: 512, acquisition time: 21 min. CRLB were estimated using LCmodel. Due to increased SNR, the quantification of metabolites is more reliable using the cryogenic coil (b).

SENSE reconstruction was successfully performed with an acceleration factor up to 2 using the paCRP. A 3D RARE sequence with a resolution of 60μm³ was first used to acquire a full-FOV image (Figure 3.9a, left). The same scan was repeated with a reduction factor (R) of 1.5 (Figure 3.9a, middle) and 2 (Figure 3.9a right), by reducing the FOV in the first phase encoding direction. Sensitivity maps were derived from low resolution images acquired with the surface coil and the volume resonator. The reconstructed images were subtracted from the full FOV image to highlight the structural differences (Figure 3.9,b). The SNR loss was estimated to be 25% for R=1.5 and 35% for R=2. The GRAPPA algorithm was applied using a 3D FLASH sequence with 60μm³ resolution acquired in 45 min. Fig. 39.c shows the comparison of two coronal cross-sections through the mouse brain recorded without acceleration (left) and with a GRAPPA acceleration factor of 2 (right). 64 reference lines were acquired, leading to a total scan time reduction of 15 min and an SNR decrease of approximately 35%. Further acceleration led to artifacts due to missing reference lines and poor SNR.
In vivo high-resolution images were acquired with the full FOV (a, R=1) and with reduced FOV (R=1.5 and R=2)). The unfolding of images was performed using SENSE reconstruction (a). (b) Difference maps obtained from the subtraction of the SENSE reconstructed data from the original full FOV image. R= 1.5, R= 2. (c) Coronal cross-sections through the mouse brain recorded without acceleration (left) and with a GRAPPA acceleration factor of 2 (right).

3.5. Discussion

CRPs are nowadays commercially available (Cryoprobe\textsuperscript{R}, Bruker BioSpin) and are increasingly used by research groups throughout the world. They constitute an attractive option for increasing the SNR in MRI and MRS studies, either as an additional SNR boost at high fields for the ultimate SNR, or as a cost-effective alternative to increasing the magnetic field strength, which requires expensive magnets. For an optimal SNR gain at very low temperature, the coil noise must dominate the other noise sources in the system, of which the most prominent being the sample noise. Since the sample resistance increases with the third power of the coil radius (while the coil resistance remains almost constant), cryogenic coils have first been designed for application in the mouse brain, where coil radii typically do not exceed 1cm. Since the engineering of a CRP is very demanding, systems developed until now are based on a rather simple design: single loop CRPs or quad CRPs working as transmit and receive systems. A major engineering challenge was thermal insulation, which ensures protection of the living animal from the cryogenic temperatures while keeping the distance between the cold coil and the animal surface minimal (of the order of 1mm). This problem could be overcome and led to coil designs that are relatively straightforward to handle and can be inserted into and taken out of the magnet bore in the cold state, which largely facilitates operation. Nowadays, the feasibility of more complex CRP designs is being investigated. Firstly, it would be advantageous to benefit from the homogeneous excitation provided by a separate volume resonator, given the coils can be decoupled from each other. Homogeneous excitation is valuable for every MR application. Using surface coils, sufficient homogeneity for many applications can be achieved using adiabatic pulses (Garwood &
DelaBarre, 2001). Secondly, the development of phased-array coils would constitute the next logical step to further increase the sensitivity of the receive system, as a larger coil would be composed of multiple smaller coils thereby reducing sample noise contributions. Moreover, building array coils would constitute the only way to make CRPs matching the large FOVs encountered in clinical applications. As clinical MRI scanners operate at lower field strengths (typically 1.5-3T), the potential of cryogenic cooling would be higher in these conditions.

In this work the first receive-only paCRP prototype was characterized in terms of SNR and its applicability demonstrated for in vivo imaging of the mouse brain. Results were unambiguous concerning the advantage of homogeneous excitation. Compared with a quadCRP transceiver system, a significant SNR gain has been measured, independently from the MR sequence used both in phantoms and mice for regions at a distance of the order of the coil radius from the coil plane. Nevertheless, the performance of the paCRP suffered from technical imperfections. Arranged in a 2x2 geometry, two elements were consistently (measured in all available prototypes) less sensitive than the others, leading to significant losses in SNR. A speculative explanation for this phenomenon is the interference with the additional hardware components necessary for the array operation. The dense packing of electronic components may be responsible for deviations from ideal circuitry behavior. As a consequence, the SNR gain provided by the paCRP is inhomogeneous when compared with an equivalent, well balanced RT setup. Considering only the two best elements, an SNR gain of a factor of 2.5 was achieved, which is closer to the theoretical expected value.

The transceiver quadCRP is composed of two elements working in quadrature mode, with same overall coil size as the cryogenic array coil. The array coil should therefore offer higher SNR at the surface, considering smaller individual elements. However, this has not been observed here. In particular near the surface, the quadCRP seems to be (at least) equivalent to the array coil in terms of SNR. Array coils are particularly beneficial when the sample losses are dominant. In human MRI applications, phased array coils have been shown to be advantageous in terms of SNR and parallel acceleration techniques are nowadays routinely used in clinics (Chung & Muthupillai, 2004; De Vries et al., 2005; Golay, de Zwart, Ho, & Sitoh, 2004; Weiger, Boesiger, Hilfiker, Weishaupt, & Pruessmann, 2005; Weiger, Pruessmann, & Boesiger, 2000; Zech et al., 2004). In small animal imaging the benefits of array coils are less obvious. Considering the very small coil dimensions used, sample losses are not dominant anymore and every noise source becomes equally important. The additional hardware and cables used in array coils in order to decouple the individual elements acts like a resistor placed in series with the coil. The contribution of the additional resistance is negligible for large coils, for which sample noise is dominant, but will become an important contributor to the total noise for small coils and therefore degrade the beneficial effects of coil cooling (Doty, Entzminger, Kulkarni, Pamarthy, & Staab, 2007). Nevertheless, paCRP provide the potential for parallel acceleration, which represents a great advantage also in small animal imaging.
3.6. Outlook

The paCRP characterized in this study was the first four-channel paCRP receiver prototype operating in cross-coil mode with a volume resonator for homogeneous excitation. Significant progress in optimizing the coil performance with regard to the overall sensitivity has been made in the course of the project, yet the problem of the sensitivity difference among the individual elements could not be resolved (though somewhat improved). The paCRP has meanwhile reached a development status that it is now routinely used for most MRI and MRS studies of mouse brain.

More recently, the same cryogenic phased array coil has been developed at a larger scale for the rat brain. Preliminary results show an SNR gain of about 2.5. If this technology can be further extended to larger arrays (composed of more elements), it would be conceivable to build similar coils for human applications. Used at lower field strength, the SNR gain is expected to be higher, allowing tremendous scan time reduction.

References:


Chapter 4

High resolution spectroscopic imaging in mice using a cryogenic receive-only phased array coil

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Adapted from

High resolution spectroscopic imaging of the mouse brain using a cryogenic 2x2 phased array coil at 9.4T.


Abstract

Spectroscopic imaging (SI) in the mouse brain puts high demands on spatial resolution. In order to resolve brain structures such as cortex, thalamus or hippocampus, voxel dimensions of the order of 1 μl are required, leading to long acquisition times. Cryogenically cooled detectors have been shown to provide supplementary SNR that could be reinvested in enhanced spatial resolution without increasing the measurement times. However, up to now cryogenic coils have been commercially available only in the form of transmit/receive systems, a setup that is inadequate for SI applications due to the inhomogeneous distribution of the transmit B1-Field across the field-of-view. In this study, we used a novel cryogenic receive-only phased array coil that operates in combination with a volume resonator for homogeneous excitation, enabling for the first time the acquisition of SI data with voxel sizes of 0.68 μl volume within two hours acquisition time. The SI protocol was applied in a mouse model of glioblastoma, delivering highly resolved metabolite maps showing regionally specific metabolic aberrations associated with this type of brain disease.
4.1. Introduction

Brain pathologies are accompanied by structural, functional and metabolic changes, which nowadays are used as markers to identify a particular disease type or stage, or to evaluate treatment effects. MRI, offering high soft tissue contrast in a non-invasive way has become an indispensable diagnostic tool in clinical routine, mostly used to identify structural changes associated with a particular pathology (Filippi & Rocca, 2011; Pyatigorskaya, Gallea, Garcia-Lorenzo, Vidailhet, & Lehericy, 2014; Ramani, Jensen, & Helpern, 2006; Rees, 2003; Wozniak & Lim, 2006). However, structural changes in the brain might become apparent only at an advanced stage of a disease, and their slow evolution might be limiting when evaluating the efficacy of a treatment. Also, structural malformations may be subtle and thus difficult to identify, rendering precise diagnosis difficult (e.g. psychiatric disorders, Nugent et al., 2006; Sala et al., 2004; Woods et al., 1995). Identifying a disease at an early stage is attractive in view of a potential therapeutic intervention. Alterations in cerebral metabolism are believed to precede structural changes or even to be at the origin of many neurodegenerative and psychiatric diseases (Choi, 1988; T. Ernst, Itti, Itti, & Chang, 2000; Lau & Tymianski, 2010). This renders non-invasive approaches for studying brain metabolism such as MR spectroscopy or spectroscopic imaging (SI) very attractive for basic biomedical research aiming at elucidating mechanistic aspects but also with regard to drug discovery and development. Studies in humans are challenging because a disease is rarely recognized in an early, potentially pre-symptomatic, phase. With the availability of transgenic mouse models mimicking aspects of human (brain) pathologies, it is nowadays possible to investigate the initial phase of a disease, before the first symptoms appear.

The brain is a complex organ connecting specialized substructures, which vary in their neurochemical profile. Pathological dysfunction may therefore involve multiple brain areas at a variable degree. Also, many brain disorders are of diffuse nature and affect multiple brain areas. For example amyloid depositions in Alzheimer’s patients are found across large brain areas. Therefore, methods such as SI enabling metabolic investigations covering extended regions of interest offer significant advantages over techniques that provide focal information only. However, SI in mice is very challenging, the principal limiting factor being the spatial resolution achievable in view of the inherently low SNR. Resolving brain structures such as cortex, thalamus, and hippocampus requires voxel dimensions of the order of 1 μl. Such high spatial resolution implies long measurement times, as on one hand SI relies on phase encoding in two or three spatial dimensions, but also because several averaging steps will be necessary to achieve an SNR sufficient for quantitative analysis of the individual spectra. As a consequence, the use of SI for monitoring biochemical changes at the order of minutes, e.g. for studying alterations in the functional state of the brain, is largely impossible when using conventional acquisition methods and hardware. SNR increase by use of low temperature detection may overcome the hardware limitation to some extent. It has been demonstrated that the use of cryogenic transceiver coils for in vivo imaging of the mouse brain improved SNR by a factor of 2.5 at 9.4 T (Baltes et al.,...
2009). Yet this setup was found inadequate for SI applications due to the inhomogeneous distribution of the transmit B1-Field across the field-of-view (FOV).

In the present study, we report on the feasibility of metabolic imaging of the mouse brain using a novel cryogenic receive-only phased array coil that operates in combination with a volume resonator for homogeneous excitation. Our goal was to investigate the possibilities and limitations in terms of spatial resolution for SI on the mouse brain. To demonstrate spatial selectivity of the SI approach, we have used a mouse glioblastoma model, with tumor cells injected into the right striatum of the animals. The SNR enhancement provided by the cryogenic receiver coil was invested into improved spatial resolution, enabling for the first time the acquisition of SI data with voxel sizes of 0.68 μl volume using a standard PRESS (P. A. Bottomley, 1987; P. Bottomley, 1985; Jung, 1996a, 1996b) protocol. Highly resolved metabolite maps of the brain could be generated in healthy and pathological conditions, on one hand accurately reflecting the normal anatomy of the brain, and on the other hand depicting the expected aberrations in a mouse glioblastoma model.

### 4.2. Materials and methods

**MRI System and coil setup**

All experiments were performed at 9.4T (BioSpec 94/30, Bruker BioSpin MRI GmbH, Ettlingen, Germany). The system is equipped with a BGA 12AS HP gradient system with a maximum gradient strength of 440mT/m and minimum rise time of 70 μs. The shim system allows shimming up to the second order. A 4-element receive-only cryogenic phased array surface coil (2x2 geometry, overall coil size 20x27mm²) operating at 30K (Bruker BioSpin AG, Fällanden, Switzerland) was used in combination with a linearly polarized 72mm ID room temperature volume resonator for transmission.

**In Vivo experiments**

All *in vivo* experiments were carried out in strict adherence with the Swiss law for animal protection. All mice were anesthetized using isoflurane, intubated and artificially ventilated (80 breaths per minute, bpm) using a small animal ventilator (CWE, Ardmore, USA) for the entire duration of the experiment. Stereotactic fixation was used to ensure reproducible positioning of the animal on the support. The body temperature was kept at 37°C throughout the duration of the experiment. For the intracranial tumor model, 6 CD-1 nude mice, 9- to 11-weeks of age, were injected with two microliters of stably transfected GL261 brain tumor cells (10⁴ cells/mL, ~3 mm deep into the brain, 1 mm anterior to the bregma and 1.5 mm right to the midline). SI was performed 17 days after injection. The growth and location of the tumor in the individual animals varied substantially across animals (figure 1).
Brain tumor cells were injected in 6 nude mice the same day and in the same amount. However, tumor development varied across animals resulting in variable size and shape of the glioblastoma at day 17. The PRESS excitation volume (purple box) was positioned to comprise only brain tissue in order to avoid large contributions from extracranial lipids. As a consequence, it had to be adapted according to the position of the tumor. In some of the mice glioma tissue was included only in parts. Nevertheless, SI experiments were carried out in all 6 animals.

**Figure 4.1: Glioma-bearing mice (N=6).**

**MR imaging and spectroscopy**

Prior to SI, anatomical images were acquired for accurate positioning of the SI slice. T2 weighted images (RARE, TR/TE = 3500/14 ms, 66 μm², slice thickness: 0.5mm) were acquired in axial and horizontal directions, followed by a reference image corresponding to the SI slice geometry. For imaging of the tumor bearing mice, anatomical images were slightly T1 weighted (RARE, TR/TE = 1000/8 ms, 66 μm², slice thickness: 0.5mm) in order to better highlight the tumor. For SI, generally a slice of 1.5mm thickness was placed horizontally, including striatum, thalamus, ventricles, hippocampus and parts of the cortex. For SI on the tumor bearing mice, the position of the slice was sometimes slightly adapted to englobe more of the tumor tissue. Shims up to the second order were adjusted on a ~ 8x8 voxel using previously acquired fieldmaps. VAPOR was used for water suppression. The acquisition was weighted using a Hanning filter. Detailed protocols were:

- Control mice: signal type: spin echo (PRESS), TR/TE = 2000/26 ms, FOV: 17x17 mm, Mtx: 20x20, slice thickness: 1.5 mm, number of averages: 11, acquisition time: 100 min.
- Tumor bearing mice: signal type: spin echo (PRESS), TR/TE = 2000/26 ms, FOV: 17x17 mm, Mtx: 25x25, slice thickness: 1.5 mm, number of averages: 8, acquisition time: 120 min.

The separate datasets obtained from the individual channels were phased and weighted using a water reference signal prior to combination in the time domain. Metabolite maps were obtained by integrating over the metabolite signals (ParaVision). Further analysis was performed using LCModel (Provencher, 2001). After SI, T1 weighted contrast enhanced images (Dotarem, 0.5mmol gadolinium/mL, Guerbet S.A, France) were acquired.
4.3. Results

Highly resolved metabolite maps of the mouse brain have been recorded using a novel receive only cryogenic phased array coil. The metabolite distributions accurately reflected the anatomy of the brain (figure 2, A) with high metabolite levels (Cre, Cho, NAA, Glu, Tau) in brain tissue (cerebral cortex, thalamus, striatum), while the respective levels were low in ventricular structures. Metabolite concentrations varied across brain regions (Fig. 2 B). This is reflected by the signal for NAA, generally considered as neuronal marker: while the ratio NAA/(Cr+PCr) was approximately 2 in cerebral cortex, it was only 1 in the striatum and 0.7 in thalamus. Metabolite maps acquired in glioma bearing mice show aberrations from the normal metabolite distribution in the tumor area (figure 3). Ins and Cho concentrations were found to be increased in the tumor tissue, while NAA and Cre levels were decreased. The SNR of Ins (the weakest metabolite signal under investigation in this study) was found to be above 6 on average (figure 4, A). All metabolite levels were quantified with respect to Cr + PCr using LCmodel assuming the amount of Cr+PCr to remain constant over time in cerebral tissue.
The Cramer-Rao Lower Bounds (CRLB) for the metabolite signals of interest were estimated to be below 20% in healthy tissue, which is essential for reliable quantification. As expected, NAA signals were strongly reduced in the tumor tissue, and therefore more difficult to quantify (figure 4, B). Results (Figure 5) of the group quantification (6 tumor bearing mice compared with 6 healthy control animals) showed a significant increase in Ins and Cho and a strong decrease in NAA signals. The normal appearing brain tissue in tumor bearing mice displayed normal metabolite levels, as revealed by the comparison with naïve mice, indicating that increased intracranial pressure did not (yet) affect metabolism.

Figure 4.3: SNR and CRLB of the SI data acquired in 2 hours with a nominal voxel size of 0.68 μl. SNR in the tumor tissue compared to the contra lateral region (A). CRLB of the curve-fit (LC Model) in the tumor tissue, the contra lateral side, and the striatum of healthy animals (healthy control) (B). The NAA signal is difficult to quantify in the tumor region, due to very low metabolite concentration. (N = 6 animals/group, error bars represent standard deviations).

Figure 4.4: Quantified values of Ins, NAA, and totCho relative to totCr. Significant changes were measured in Ins, NAA and totCho levels in the tumor tissue compared to healthy tissue (in the same animal) and in the striatum of healthy control animals. Metabolite concentrations are expressed as ratio to totCr. It is important to take into account that totCr values are reduced in tumor tissue as reflected by Fig.5. (N = 6 animals/group, error bars represent standard deviations).
4.4. Discussion

The use of a receive-only cryogenic phased array coil yielded excellent spectral quality in SI experiments even for voxel dimensions as small as 0.68 μl per voxel. The metabolite distributions across the FOV reflect the brain anatomy in healthy animals and also reveal slight differences in metabolite profiles across different brain regions in line with published data in mice using single volume spectroscopy (SVS; Tkáč et al., 2004). Neoplastic tissue in glioma bearing mice shows major changes in metabolite levels, in particular increased levels of Cho and Ins indicative of proliferative and inflammatory activity as well as low levels of NAA indicative of displacement of neural tissue. Interestingly also the total PCr concentration (PCr+Cr) was significantly decreased in tumor tissue. We did not observe an elevation of Lac levels, which is a common feature of many tumors including glioblastomas. Our data reveal that even for voxel volumes of less than 1μl, accurate quantitative analysis of the metabolite composition in brain and tumor tissue is possible.

It is difficult to compare SI studies in mice. Most reports using proton MR spectroscopy in mice are based on SVS, which in general yields better data quality. However, several well designed SI studies can be found in the literature (Alf et al., 2012; Boska et al., 2005; Hamans et al., 2013; Weiss et al., 2009). To our knowledge, an SI dataset with a spatial resolution of 0.68 μl per voxel acquired within two hours has not been reported before, demonstrating the potential of the cryogenic array coil, together with an efficient data combination strategy. Even at this spatial resolution, SNR was sufficient to generate maps for metabolite occurring at relatively low concentration such as Ins, Glu and Tau.

In our study we invested the gain in SNR provided by the cryogenic coil in improved spatial resolution and maintained SNR at a level to ensure accurate quantitative analysis. As a result, acquisition times using the PRESS protocol were rather long, though not exceeding 2h. This is mainly due to high
spatial resolution (phase encoding) and signal averaging with 11 and 8 averages collected. While this temporal resolution may be sufficient for quasi-stationary conditions as targeted in the current study (the tumor proliferation rate is slow compared to the 2.5h acquisition window), it would not allow dynamic studies of metabolic adaptations to a challenge, e.g. the administration of a drug.

The PRESS protocol used here, involving two 180° refocusing pulses, suffers from other drawbacks which render it suboptimal for measurements performed at high field on such small objects. One of the major issues encountered is the chemical shift displacement error (CSDE), particularly pronounced at high field strength. For a chemical shift difference of 1ppm (NAA vs Cr+PCr) the difference in resonance frequencies at 9.4 T arises to 400Hz. Assuming a given voxel size of 8mm in the x direction and a maximal pulse bandwidth of 5 kHz, the CSDE then amounts to 0.6 mm. The coil setup used in this study, comprising a linearly polarized volume resonator for homogeneous excitation (designed for rat body imaging), was not efficient enough in transmission. In particular, severe limitations regarding the bandwidth of the 180° refocusing pulses increases the susceptibility to chemical shift artifacts. On average, a maximal pulse bandwidth of 3.5 kHz was reached, leading to an average displacement of more than 2 mm between Ins and Lac. This might explain why no increased Lac levels were found in the tumor. CSDE render quantification problematic as the volume of the metabolite and the reference are not identical. Hence methods that minimize this effect are highly desirable (Henning et al., 2009). Moreover, due to T2 decay of the signals the use of shorter echo times would be advantageous. The STEAM (Jens Frahm et al., 1987) protocol, involving only 90° pulses, would help addressing these issues by reducing the CSDE and allowing using shorter echo times. However, STEAM inherently provides only half of the signal intensity compared to PRESS, and would therefore require longer measurement times. An alternative solution to perform high resolution SI on the mouse brain is given by SPECIAL (Mlynárik et al., 2006), providing full signal intensity, short echo times and good localization. Yet the methods still involves the formation of a spin echo using a 180° pulse with the limitations outlined above.

In summary, the gain in sensitivity provided by the receive-only cryogenic phased array coil is a significant advantage for measuring SI on mice, where spatial and temporal resolutions are limited due to low SNR. However, datasets acquired using a conventional PRESS sequence suffer from important CSDE due to inefficient transmission through a large volume coil. Alternative methods such as STEAM (providing only half on the signal) or FIDLOVS, comprising only 90° pulses would be better adapted for this particular setup.
References


Chapter 5

Metabolic changes assessed by MRS accurately reflect brain function during drug-induced epilepsy in mice in contrast to fMRI-based hemodynamic readouts.

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Abstract

Functional proton magnetic resonance spectroscopy (1H-MRS) enables the non-invasive assessment of neural activity by measuring signals arising from endogenous metabolites in a time resolved manner. Proof-of-principle of this approach has been demonstrated in humans and rats; yet functional 1H-MRS has not been applied in mice so far, though it would be of considerable interest given the many genetically engineered models of neurological disorders established in this species only. Mouse 1H-MRS is challenging as the high demands on spatial resolution typically result in long data acquisition times not commensurable with functional studies. Here, we propose an approach based on spectroscopic imaging in combination with the acquisition of the free induction decay to maximize signal intensity. Highly resolved metabolite maps have been recorded from mouse brain with 12 min temporal resolution. This enabled monitoring of metabolic changes following the administration of bicuculline, a GABA-A receptor antagonist. Changes in levels of metabolites involved in energy metabolism (lactate and phosphocreatine) and neurotransmitters (glutamate) were investigated in a region-dependent manner and shown to scale with the bicuculline dose. GABAergic inhibition induced spectral changes characteristic for increased neurotransmitter turnover and oxidative stress. In contrast to metabolic readouts, BOLD and CBV fMRI responses did not scale with the bicuculline dose indicative of the failure of neurovascular coupling. Nevertheless fMRI measurements supported the notion of increased oxidative stress revealed by functional MRS. Hence, the combined analysis of metabolic and hemodynamic changes in response to stimulation provides complementary insight into processes associated with neural activity.
5.1. Introduction

Neural activity involves changes in the levels and turnover of metabolites associated to neurotransmission as well as energy turnover, which can be monitored non-invasively in vivo using proton magnetic resonance spectroscopy (1H-MRS, Duarte et al., 2012). The application of 1H-MRS as a functional tool remains however very limited due its inherent low sensitivity, which compromises spatio-temporal resolution. To date, there are no reports on functional 1H-MRS in mice. Yet, numerous models of neurological disorders are established in this species only, and phenotyping them with regard to structural and functional abnormalities becomes increasingly important. While structural MRI is widely used in this context, functional neuroimaging in mice remains challenging: fMRI based readouts assessing activity induced hemodynamic changes in the blood oxygenation (BOLD) or cerebral blood volume (CBV) in mice appear to be dominated by systemic hemodynamic effects ( Schroeter et al., 2014). Therefore, alternative readouts of brain function such as functional MRS, which is not based on cerebral hemodynamics and therefore should be less susceptible to systemic confounds, become highly attractive.

Commonly, the sensitivity issue in functional 1H-MRS studies in humans and rats is addressed by using 1H single voxel spectroscopy (SVS) with relatively large voxels (typically 10-50 μl) to achieve a temporal resolution of several minutes (Hyder et al., 1996; Just et al., 2013; Xu et al., 2005). Localization in SVS techniques involves the formation of spin echoes, which is associated with significant signal loss due to T2 relaxation compromising the sensitivity. A further limitation of SVS is that it provides only local information on the voxel selected, while for functional studies it might be attractive to cover larger brain areas in order to monitor differential responses of brain areas, e.g. comparing responses in activated versus non-activated brain regions. This can be achieved by spectroscopic imaging (SI) techniques, which provide 2- or 3-dimensional spatially resolved spectral information on an extended volume-of-interest, however, at the expense of significantly prolonged measurement times (Alf et al., 2012; Boska et al., 2005; Miyasaka et al., 2006; Mlynárik et al., 2008; Weiss et al., 2009).

Yet, SI experiments can be sped up by sampling the free induction decay (FID) signal immediately after a slice-selective excitation instead of the spin echo, thereby avoiding signal loss due to T2 relaxation. To maximize spatial resolution and minimize unwanted contribution from outside the target region, the VOI from which SI data are collected, is confined by suppressing signals arising from outside the VOI (outer volume suppression, OVS). The combination of FID sampling and localization by outer volume suppression (FIDLOVS) has been developed for high field clinical systems in order to minimize the chemical shift displacement artifacts and the signal losses due to fast T2 relaxation (Henning et al., 2009). This SI acquisition scheme was shown to provide higher sensitivity compared to standard SVS experiments.
In the present study, FIDLOVS was adapted for functional MRS studies of the mouse brain at 9.4T. We have evaluated its accuracy in assessing relative metabolite concentrations and tested its sensitivity in detecting metabolic alterations elicited by the administration of a neuroactive compound. High signal intensity resulting from FID acquisition combined with the superior sensitivity provided by a cryogenic phased array coil enabled serial SI acquisitions of an array of 17x17 voxels of a volume of 1 µl with a temporal resolution of 12 min. Following the application of bicuculline, an antagonist of the γ-amino-butyric acid A (GABA-A) receptor, which blocks the inhibitory action of GABA and therefore mimics epilepsy, dose-dependent changes in regional levels of neurotransmitter acids such as glutamate (Glu), glutamine (Gln), GABA, and also of lactate (Lac), creatine (Cr) and phosphocreatine (PCr) could be quantified. Stimulation-induced changes in metabolite levels have been compared with conventional readouts of neural activity based on BOLD and CBV fMRI.

5.2. Material and methods

Animal preparation

All in vivo experiments were carried out in strict adherence with the Swiss law for animal protection. Female C57BL/6 mice (Janvier, Le Genest-St Isle, France) at 3-4 months of age and ca. 23 g were anesthetized using isoflurane. Anesthesia was induced with 3% isoflurane and maintained at 1.5% in an oxygen/air (20% / 80%) mixture. Mice were intubated and artificially ventilated (80 breaths per minute, bpm) using a small animal ventilator (CWE, Ardmore, USA) for the entire duration of the experiment. Stereotactic fixation was used to ensure reproducible positioning of the animal on the support. The body temperature was kept at 37°C throughout the duration of the experiment. A catheter was placed in the tail vein for intravenous injection of pancuronium bromide (1 mg/kg; Sigma-Aldrich, Steinheim, Germany) as a bolus in order to avoid movement during acquisition and for the administration of bicuculline. The numbers of animals for the different treatment groups are indicated in the figure legends.

Bicuculline and administration paradigm

Bicuculline (Molekula, Gentaur, Aachen, Germany) was dissolved in 0.1N HCl and diluted to a final concentration of 1 mg/ml with physiological saline solution. The pH was adjusted to pH 6.5 with 0.1 N NaOH. In the SI and fMRI experiments bicuculline solution was administered at two different doses (1 mg/kg and 1.5 mg/kg); vehicle injections served as a control. Animal numbers per dose and treatment are indicated in the respective figure legends. Drug infusion was performed using an infusion pump (Harvard Apparatus, Holliston, USA) with the total dose applied within one minute in a volume of 0.12 ml solution.
**MRI Hardware**

All experiments were carried out using a BioSpec 94/30 (Bruker BioSpin MRI GmbH, Ettlingen, Germany) small animal MR system operating at 400 MHz and equipped with a BGA 12AS HP gradient system with a maximum gradient strength of 440mT/m. A four-element receive-only cryogenic phased array surface coil (2 x 2 geometry, overall coil size 20 x 27 mm²) with the coil system operating at 30 K (Bruker BioSpin AG, Fällanden, Switzerland) was used in combination with a circularly polarized 86 mm volume resonator for transmission.

**Spectroscopic imaging, SI**

For SI experiments two slice orientation, coronal and horizontal, have been selected with a slice thickness of 1.37 mm. Experiments for the different slice orientations have been carried out in different animals, to keep the total experimental time per mouse minimal. The coronal slice was spanning a region from Bregma +1.34 mm to Bregma +0.02 mm according to a stereotaxic mouse brain atlas (Paxinos and Franklin, 2004) for high resolution SI of a cortical region-of-interest (ROI) comprising 4 to 5 voxels. The horizontal section included striatum, thalamus, hippocampus and ventricles. First and second order shim gradients were adjusted in the volume of interest using field maps. Prior to excitation, water suppression (WS) pulses (Tkáč et al., 1999) were applied interleaved with saturation pulses for OVS. Efficient saturation was achieved using 6 hyperbolic secant shaped pulses with phase and amplitude modulation of the B1 field, applied with a bandwidth of 20 kHz. After WS and OVS, a frequency selective 90° sinc3 pulse was applied (BW= 6000 Hz) in combination with a slice selective gradient for excitation of a slice of 1.37 mm nominal thickness. The resulting FID signals were collected after an acquisition delay of 1.29 ms, repetition time of 2500 ms, spectral width = 8 kHz with 4096 data points (spectral resolution 2 Hz / point). Parameter settings for the coronal slice were: field of view of 15 x 15 mm², matrix size of 17 x 17, resulting in the following spatial resolution: 0.88 x 0.88 x 1.37 = 1 μl (the k-space was weighted with a Hanning function), and 12 min acquisition time. Parameter settings for the horizontal slice were: field of view of 22 x 14 mm², matrix size of 22 x 14, resulting in the following spatial resolution: 1 x 1 x 1.37= 1.37 μl (k-space was weighted with a Hanning function), and 12.8 min acquisition time. For each animal, the MRSI protocol was repeatedly applied, one time pre-injection and four times post-injection of bicuculline.

**fMRI**

Twelve adjacent coronal slices of 0.5 mm slice thickness spanning all the regions covered in the MRSI experiments have been recorded. The first slice was positioned at Bregma +2 mm according to a stereotaxic mouse brain atlas (Paxinos and Franklin, 2004). The local field homogeneity has been optimized in the area of interest using previously acquired field maps. BOLD and CBV fMRI data
prior and during infusion of bicuculline were acquired using a gradient-echo echo-planar imaging (GE-EPI) sequence with the following parameters: field of view of 23.7 x 14 mm², matrix size of 90 x 60, yielding an in-plane voxel dimension of 263 x 233 µm, repetition time of 1000 ms, echo time of 10 ms, average number of 1, yielding a temporal resolution of 1 s, with interleaved acquisition of slices. A data set comprised 3600 repetitions, resulting in a total acquisition time of 60 min. Bicuculline was infused after recording baseline data for 12 min. For CBV fMRI experiments, after acquisition of a pre-contrast agent scan, the paramagnetic iron oxide nanoparticle based intravascular contrast agent Endorem® (Laboratoire Guerbet SA, Roissy, France) was injected at a dose of 30 mg Fe/kg. CBV-weighted fMRI scans were started 15 min after injection of Endorem: at this time the contrast agent concentration has reached a steady state (Mueggler et al., 2001).

Data analysis

All data are given as mean across animals ± standard deviation.

LC model (Provencher, 2001) has been used for the analysis of SI data. Spectral processing includes zero and first order phase correction and baseline correction. No further pre-processing was applied to the data prior quantification. Relative quantification was performed using total creatine (Cr+PCr) as an internal reference. The concentrations obtained from each voxel were averaged to shaped regions of interest (ROI) in the following brain areas: thalamus, hippocampus, cortex and striatum. Four to five voxels were averaged in the cortex (resulting in a ROI size of approximatively 5µl), four voxels in the thalamus (two on each side), and two voxels each in hippocampus and striatum. Statistical analysis was performed in R 3.0.1 (The R Foundation for Statistical Computing, Vienna, Austria). A linear mixed model was designed for each metabolite using lme4 package (Bates D, Maechler M, Bolker BM and Walker S (2014). “lme4: Linear mixed-effects models using Eigen and S4.” ArXiv e-print; submitted to Journal of Statistical Software), with the relative metabolite concentration as a response variable, bicuculline dose and post-injection time and their interaction as fixed effects and the individual animal intercepts as random effects. The assumption of normally distributed residuals was tested with QQ-plots, Tukey–Anscombe plots for the homogeneity of the variance and skewness, and scale location plots for homoscedasticity. A normal distribution was considered plausible for all metabolites tested. Contrasts were applied with multcomp package (Hothorn et al., 2008) to perform the following comparisons for each time post-injection: ‘1.5 mg/kg vs. veh’, ‘1.5 mg/kg vs. 1 mg/kg’, ‘1 mg/kg vs. veh’. Multiple comparison corrections were performed within multcomp package. Statistical significance is indicated in the figures as follows: p-value ≤ 0.001 ‘***’, ≤ 0.01 ‘**’, ≤ 0.05 ‘*’, ≤ 0.1 ‘•’.

For analysis of fMRI data, MATLAB based software Aedes (http://aedes.uef.fi) was used to define ROIs according to a stereotaxic mouse brain atlas (Paxinos and Franklin, 2004) for the somatosensory cortex, thalamus, and hippocampus. ROI time courses were analyzed using custom-written MATLAB code (The MathWorks, Natick, MA). The first 100 repetitions were dismissed to account for the T1
relaxation effect. Prior to BOLD signal analysis, the linear trend was removed from the time series raw data and the signal changes expressed as percentages relative to baseline. Changes in CBV were derived according to $\Delta \text{CBV} = \Delta R_{2,CA^*}(t)/\Delta R_{2,CA^*}(\text{bsl}) = \ln[S(t)/S(\text{bsl})]/\ln[S(\text{bsl})/S_{\text{pre}}]$ with $S(t)$ being the signal intensity at time $t$, $S(\text{bsl})$ the average signal intensity during baseline (after contrast agent administration) and $S_{\text{pre}}$ the steady state signal prior to Endorem infusion (Kim et al., 2013).

5.3. Results

SI maps of mouse brain at baseline and reliability of quantitative assessment of metabolite levels.

Horizontal and coronal SI maps showing the spatial distribution of the metabolites Glu and Lac in mouse brain at baseline with 1 μl spatial resolution have been recorded in 12 min (Fig. 1A). Single voxel spectra for cortical, thalamic, hippocampal and striatal voxels allow resolving the resonances of more than 10 metabolites (Fig. 1B, 2A). The spectral quality is documented by values for line widths and SNR of individual metabolite signals as derived from the LCModel analysis (data not shown). Obviously the value for SNR depended on the performance of the baseline correction and should therefore only be considered for qualitative interpretation. As expected, SNR and line width of metabolite signals were not homogeneous across the various brain regions. For example the line widths in thalamus were approximately half of their respective values in the striatum, indicating inferior magnetic field homogeneity for the latter. This is also reflected by SNR values, which were comparable in thalamus, hippocampus and cortex, however lower in the striatum. Representative spectra (real part) of the unprocessed data and LCModel fits are shown in Fig. 1B. For all regions analyzed except the striatum, the resonances of Glu, Gln and GABA in the range 2.2 to 2.5 ppm could be resolved and therefore quantified independently. In the thalamus, in the cortex and sometimes in the hippocampus the resonances of Cr and PCr could be individually quantified (Fig. 1D). Although baseline correction was occasionally imperfect (e.g. in the hippocampal spectrum, Fig. 1B), LCModel yielded good fitting results as reflected by minimal residuals (Fig. 1B). Another quality measure is the Cramer Rao Lower Bound (CRLB) for individual metabolite signals (Fig. 1C). At least 10 metabolites signals could be reliably quantified, including those of Glu, Gln, GABA and Lac. For the Glu signal, the CRLB were below 5% for thalamic, hippocampal and cortical voxels with small variability across animals, and about 7% for a striatal voxel. For GABA and Gln resonances CRLB were slightly higher ranging between 10 and 15% on average in all brain regions with high variability in the striatum. The resonances of PCr and Cr at 3 ppm could be well separated in the thalamus, and for the majority of the animals also in the cortex and hippocampus (Fig. 1C). This was not the case for the striatal voxel due to inferior field homogeneity.
Figure 5.1: Representative spectroscopic imaging (SI) data of mouse brain recorded with 1 μl spatial resolution and 12 min temporal resolution. (A) Metabolite maps for Glu and Lac in coronal and horizontal orientation. Slice thickness is 1.37 mm. (B) Representative spectra (real part) extracted from four regions of interest in the mouse brain: cortex, thalamus, hippocampus, and striatum. The red curve represents the fitted spectrum as derived from the analysis using LCModel with the trace in the insert at the top indicating the residuals. (C) Cramer-Rao lower bounds (CRLB) for seven metabolites/neurotransmitters, as derived from LCModel, are given for ROIs located in cortex, thalamus hippocampus and striatum. Values indicated mean ± s.d. averaged from of N=9 animals. (D) Enlarged part of the spectra showing the Cr and PCr resonances around 3.93 ppm. While the two resonances could be clearly resolved for cortex and thalamus, and to a lesser extent in hippocampus, this was not the case for the striatal ROI.

The striatum is a heterogeneous region, and the shim procedure applied in this study, optimized for large volumes of interest, was obviously not efficient enough to achieve satisfactory line width in this region. In particular during stimulation some line broadening was observed (see below). Hence, data quality in this region was considered not to be sufficient to accurately reveal changes in metabolite levels during stimulation with bicuculline, and spectra originating from the striatal voxels were not analyzed further.
Infusion of bicuculline induces region-specific transient changes in neurotransmitter and metabolite levels

Administration of bicuculline prompted transient changes in the signals of Glu, Gln, GABA, alanine (Ala), aspartate (Asp), Lac, Cr and PCr signals extracted from a voxel located in the somatosensory cortex (Fig. 2). This is reflected by the spectra (Fig. 2A) and by the temporal profiles of individual metabolite signals (Fig. 2B). Changes in concentrations are given relative to the baseline level. Glu was significantly decreased by almost 20% (spectrum post1), and remained low until the end of the experiment. Correspondingly, Gln levels tended to increase (spectra post2 to post4) though the change was not reaching statistical significance. Concentrations of Ala were increased (p < 0.1 reaching a maximum 24-36 min (spectra post2, post4) after the infusion of bicuculline. Lac levels strongly increased approximately 3-fold during the first 12 min after stimulation (post1), but rapidly decreased to a new steady state at about 180% of the baseline value (spectra post2, post4), and had returned almost back to the baseline by the end of the experiment (spectrum post4). A similar time course was observed for Cr: the concentration almost doubled immediately after the infusion of bicuculline (spectrum post1), followed by a rapid and continuous decrease over the subsequent post-scans. As expected, PCr evolved in the opposite direction. GABA levels were significantly increased (spectrum post3; p < 0.05). These results are in line with previously observed metabolic changes in rats upon administration of bicuculline at similar doses (Chapman et al., 1984, 1977; Patel et al., 2005).

The stimulus-evoked neurotransmitter and metabolic changes also displayed different characteristics depending on the brain region analyzed as reflected by the respective metabolite maps at different time points following the bicuculline administration (Fig. 3A). Cerebral cortex was most affected by the GABA antagonist, in particular visible in maps displaying the changes in Glu and Lac levels. Immediately after infusion of bicuculline at 1.5 mg/kg, Lac was found to be increased by up to 200% on average in the cortex, and by 100% in the thalamus and hippocampus (Fig. 3B).

Also the temporal evolution of the Lac response varied regionally. While remaining elevated until the end of the experiment in the cortex, thalamic and hippocampal Lac levels returned back to baseline much faster. The level of Lac in the thalamus even displayed an undershoot compared to baseline values towards the end of the experiment, though this was not significant.
Figure 5.2: Metabolic response upon stimulation with bicuculline. (A) Spectra extracted from one voxel located in the somatosensory cortex, before, during and after administration of bicuculline with the assignment of resonances indicated. The red curve represents the fitted spectrum as derived from the analysis using LCModel with the trace in the insert at the top indicating the residuals. (B) Evolution of metabolite levels in the cortical ROI following administration of bicuculline and vehicle, respectively. Values are given as percentage change relative to the baseline value. Each square represents the average signal intensity of 4 to 5 cortical voxels of one animal (bicuculline: N=6, red; vehicle: N=3, grey). The mean of all animals is represented by the solid line.
It should be noted that infusion of bicuculline is followed by a significant vascular response (see below), and therefore accompanied with changes in magnetic susceptibility. This response translated into line broadenings of approximately 4 Hz in cortex and 8 Hz in striatum, respectively. Nevertheless these changes in susceptibility had minor consequences on the reliability of the quantitative results provided by LCModel. This is also reflected by the quality of spectra of a cortical voxel obtained before, during and after bicuculline infusion (Fig. 2A).

Changes in neurotransmitter and metabolite changes scale with bicuculline dose

To further assess the robustness of the SI technique we evaluated bicuculline-induced changes in neurotransmitter and metabolite levels as a function of the drug dose (Fig. 4). Shown are the levels at the time point, at which the effect for a specific metabolite was most pronounced (post1 for Glu, Lac, PCr and Cr, and post3 for Gln, GABA, Ala and Asp). For all metabolite signals except for Asp a clear dose-dependence could be demonstrated with the higher dose of bicuculline (1.5 mg/kg) leading to larger changes in Glu, Gln, Lac, Cr and PCr signal intensities. For Asp both doses yielded similar effects, which may be due larger uncertainties in concentration determination due to relatively high CRLB values.

Hemodynamic fMRI readouts do not correlate with bicuculline dose

BOLD and CBV fMRI data sets were acquired as additional readout of brain activation elicited by bicuculline administration. While administration of vehicle solution did not cause any changes in BOLD signal intensity (Fig. 5A) and as previously shown also not in CBV (Mueggler et al., 2001), bicuculline injection caused significant transient changes in both BOLD and CBV signals.” Both responses displayed a biphasic pattern, with a short initial response lasting typically 1 to 2 min and slow second phase of 20 to 30 min duration.

Bicuculline prompted a transient increase in CBV in all brain regions analyzed and for both doses evaluated (Fig. 5B). In contrast the BOLD responses show a differential pattern both with respect to the region of interest analyzed and with regard to the bicuculline dose (Fig. 5A).
Figure 5.3: Spatially resolved changes of Glu and Lac signals upon administration of bicuculline. (A) Metabolite maps for Glu, Lac and PCr revealing region specific signal responses upon administration of 1.5 mg/kg bicuculline. The color bar for the pre-stimulation map scales metabolite levels relative to total Cr signal. The post-stimulation metabolite maps show percentage change to the respective baseline level concentration (blue: decrease, red: increase). (B) Changes in metabolite levels in percent of baseline values in the cortical, hippocampal and thalamic ROI for Glu, Lac, and PCr. Bicuculline induced metabolic changes associated with oxidative stress (Lac and PCr) appear more pronounced in the cortical ROI, while the decreases in Glu levels appear rather balanced across the three regions. Values are given as mean ± s.d.
For 1 mg/kg bicuculline the BOLD signals in the cortex and thalamus increased to a maximal value of approximately 1.5%. Within 15 min the cortical signal returned to baseline level. In contrast, the elevated BOLD signal in thalamus persisted for more than 30 min. Hippocampal BOLD signal changes showed a similar pattern as observed in the cortex though of reduced amplitude. Whereas the temporal profile of the BOLD signal change in the thalamus was comparable for both doses of bicuculline, the BOLD responses in cortex and hippocampus displayed a different time course for the two doses. For the animals treated with 1.5 mg/kg bicuculline, a strong short-lasting BOLD signal increase was observed during the first 1-2 min of bicuculline infusion followed by a strong decrease below baseline values. The signal returned to baseline only after 30 min. Interestingly, the trend observed for the BOLD signal in the cortex and hippocampus was not reflected by the CBV response. Both the doses of bicuculline triggered a strong increase in CBV signal with a return to baseline within ca. 20 min. While for thalamus the CBV response scaled with the bicuculline dose, this was not the case for cortical and hippocampal ROIs. For these brain structures the CBV response scaled with drug dose only during the initial two minutes. Yet, these high CBV levels could not be maintained; CBV responses at 1.5 mg/kg bicuculline rapidly returned to baseline and were weaker than those measured for the 1 mg/kg dose for time points later than 2-3 min after onset of infusion.

Figure 5.4 Changes of metabolite levels relative to baseline values as a function of the bicuculline dose. Values indicate maximal signal change measured for the respective metabolite. Data are shown as box plots comparing changes averaged from 4-5 cortical voxels for 1 mg/kg bicuculline (N=6, orange), 1.5 mg/kg bicuculline (N=6, red), and vehicle solution (N=3, black).
Figure 5.5: Individual time courses of BOLD (A) and CBV (B) fMRI responses in the cortical, hippocampal and thalamic ROI upon administration of bicuculline in two different doses (1 mg/kg/min, orange; and 1.5 mg/kg/min, red), and vehicle (black), respectively. Bicuculline was injected after recording baseline signals for 12 min.

5.4. Discussion

Functional MRS constitutes an attractive non-invasive approach for studying brain function in vivo complementing regular BOLD fMRI. In fact, as the method does not depend on the integrity of neurovascular coupling it is less susceptible to the status of the cerebral vasculature and to interference by potential cardiovascular confounds. In humans and rats, SVS was successfully applied to characterize neurotransmitter and metabolic changes using visual (Frahm et al., 1996; Lin et al., 2012; Mangia et al., 2007; Prichard et al., 1991) and sensory (Xu et al., 2005) stimulation paradigms. Common observations include changes related to the increased energy demand of active brain areas such as elevated concentrations of Lac indicative of glycolytic energy production as well as a decreased ratio of PCr/Cr reflecting replenishing of ATP levels via the creatine kinase reaction (Erecinska and Silver, 1989; Sauter and Rudin, 1993). Stimulus-induced spectral changes also include altered levels of the neurotransmitters Glu and Gln. MRS-based metabolic and neurotransmitter readouts are attractive measures of brain function as they more intimately related to neural activity than hemodynamic readouts.

Studies of brain function using MRS methods however face several challenges, in particular when applied to mice, which would be attractive in view of the large number of genetically engineered
mouse lines available for mechanistic studies or as models of human disease. Matching the constraints imposed by the time scale of biochemical changes associated with neural activity evoked by sensory or physiological / pharmacological stimulation with MRS techniques puts high demands on temporal and spatial resolution. Especially when targeting voxel dimensions of the order of 1 µl, which is appropriate for mouse studies, the use of conventional spin echo-based 1H-MRS techniques is of limited value due to their low inherent sensitivity. Acquiring an FID signal with very short acquisition delays (1.2 ms in this study) instead prevents signal loss due to T₂ relaxation thereby leading to substantial improvements in SNR. Incorporation of the FID sampling into an SI protocol furthermore allows sampling of extended brain areas enabling the investigation of regional differences in metabolism. Accordingly the FIDLOVS technique used in this study enabled to simultaneously resolve activity-induced changes in cerebral metabolism across several brain regions with a spatial resolution of 17 x 17 voxels of 1 µl volume and a temporal resolution of 12 min suited e.g. for analyzing the metabolic response to the administration of a neuroactive compound. We have selected bicuculline as a reference drug, as its effect on the brain metabolism has been extensively studied both using MRS and biochemical analyses (Blennow et al., 1979; Chapman et al., 1984, 1977; Patel et al., 2005, 2004; Söderfeldt et al., 1983).

The bicuculline-induced metabolic alterations measured using the FIDLOVS technique are in good agreement with results from previous studies performed in rats using a similar dosage regimen. In these studies seizure onset was marked by a strong increase in Lac concentrations reflecting enhanced glycolytic activity. The increased energy demand became apparent from the decrease of the PCr signal intensity indicating increased flux through the phosphocreatine kinase forward reaction PCr+ADP+H⁺ = Cr+ATP, which transfers the phosphate group from PCr to ADP to replenish ATP levels in order to match its high rate of consumption (Sauter and Rudin, 1993). Glu levels have been reported to be decreased following bicuculline stimulation, while the progressive increase of Asp, Ala and Gln levels has been attributed to ammonia detoxification in tissue mainly via amination of Glu (Chapman et al., 1977; Patel et al., 2005). GABA levels have been found to progressively increase as well, presumably due to de-novo synthesis (Chapman and Evans, 1983; Chapman et al., 1984).

It is reasonable to assume that the bicuculline response depends on the anesthetic regimen chosen, in particular when using anesthetics acting on the GABAergic system such as isoflurane, which might to some extend counteract the bicuculline effect. However, the purpose of the study was to analyze whether metabolic effects in contrast to hemodynamic responses were proportional to the response elicited by pharmacological stimulation even if this was moderated by the anesthetic. The metabolic responses measured in this study scaled with the bicuculline dose. In particular at high doses the administration of bicuculline prompted seizure activity, which was associated with levels of metabolic activity and oxidative stress that exceeded typical conditions of physiological stimulation, e.g. via sensory input. While for cortical and hippocampal voxels we observed a good correlation of
metabolite levels with the bicuculline dose, this was not the case for the functional readouts based on hemodynamic measures. In this context the dampening of the hemodynamic response due to the vasodilatative effects of isoflurane has to be considered, though their extent depends on the dose of inhalant used. At a dose of 1.5% isoflurane we usually measured only weak isoflurane effects on changes in CBV and BOLD (Schroeter et al., 2014). This is in agreement with previous observations in resting state and stimulus-evoked fMRI studies (Liang et al., 2012; Liu et al., 2011). The BOLD fMRI response in cortex and hippocampus did not scale with the bicuculline dose and correspondingly with neuronal activity. Instead, in particular for 1.5 mg/kg, a strong decrease in the cortical BOLD signal was observed following an initially positive response of short duration. Similarly, the cortical and hippocampal CBV responses did not correlate with the bicuculline dose apart from the initial 2 min, though for all brain regions examined a positive CBV response has been measured. The different sensitivity of BOLD and CBV readouts to the vessels size might contribute to their differential behavior to the bicuculline infusion. Yet, this dependence alone cannot explain the negative BOLD response to a pharmacological stimulus, which is known to induce high neuronal activity as derived from EEG recordings [Sauter and Rudin, 1993]. It has been reported that epileptic seizures can produce a vascular break-down (Ingvar and Siesjö, 1983; Mueggler et al., 2001; Theard et al., 1995), which decreases the value of hemodynamic readouts. Combining the compromised cortical CBV response observed at the high bicuculline dose with the information derived from SI indicating oxidative stress (Lac and PCr signals), we attribute the BOLD signal decrease to increased levels of deoxy-hemoglobin in venous blood, as the stimulus-induced increase in blood supply is not capable of matching the increased energy demands. Also for the lower dose of 1 mg/kg the change in BOLD signal was significantly smaller than expected considering the strong CBV response. This ‘limited flow’ interpretation is supported by the data obtained for thalamus: in this case the CBV response scales with the bicuculline dose, i.e. the thalamus vascular system is largely capable of coping with the increased demands. This is in agreement with earlier reports describing higher baseline CBV values in thalamus than in cortical structures (Schroeter et al., 2014). As a consequence, we observe a positive BOLD response that also scales with the stimulus strength. This underlines that under conditions of flow limitation, hemodynamic readouts become poor indicators of neuronal activity. In contrast, even under conditions of vascular failure metabolite levels were found to correlate with the stimulus strength and hence constitute an attractive indicator of neural activity. It should be mentioned though that bicuculline infusion produces strong and sustained neuronal activity as well as strong associated metabolic changes and is therefore peculiarly suited for such SI studies. In general, spectroscopic data suffers from high variability as indicated by CRLB for the individual metabolites. Hence, for milder stimulation paradigms inducing only weak changes in metabolite levels, large groups of animals might be necessary in order to observe significant effects.

The use of SI techniques is confronted with several technical challenges, in particular when applied without volume selection. High performance of OVS is critical as insufficient saturation may yield
artifacts in the form of lipid contamination and baseline distortions resulting in quantification errors. Broadband frequency-modulated saturation pulses and careful animal preparation in terms of positioning and movement prevention are necessary to minimize these artifacts. Intubation and mechanical ventilation of the animals in combination with muscle relaxants is inevitable for achieving stable experimental conditions. Optimizing of animal preparation procedures allows maintaining the physiological variables of a mouse within the narrow boundaries and thereby reducing variability in data. Although SI techniques offer the advantage of covering extended brain regions, shimming over large volumes of interest becomes challenging: field homogeneity may vary significantly across the volume-of-interest and even be insufficient in some regions. Typical line width reported for mouse brain with SVS at 9.4T are of the order of 10 and 15 Hz, resulting in CRLB of 4-5% for Glu, and 10-15% for GABA (Tkáč et al., 2004), depending on the brain region comprising the voxel. Our data acquired in 12 min with SI shows comparable shim quality for voxels located in cerebral cortex, thalamus and hippocampus, and inferior values for striatal voxels.

In summary, we have demonstrated that functional $^1$H-MRS can be used to monitor stimulus induced metabolic changes in mouse brain. This was achieved by optimizing an FID-based SI method for functional measurements on the mouse brain enabling the recording of changes in cerebral metabolism with higher spatial (17 x 17 voxels of 1 µl volume) and temporal resolution (12 min). Using a well-characterized pharmacological stimulus (bicuculline) we observed changes in neurotransmitter and metabolite indicative of increased neurotransmitter turnover and oxidative stress that were in agreement with previous reports and that varied across brain regions. Metabolic responses were found to scale with the bicuculline dose in contrast to conventional fMRI readouts (BOLD and CBV changes), which did not reliably correlate with the stimulus strength, in particular for cortical and hippocampal volume-of-interest, indicating coupling mismatch between energy consumption and supply at high bicuculline doses. Under such circumstances hemodynamic-based fMRI readouts appear to be poor indicators of neural activity. The combined analysis of metabolic (SI) and functional changes (fMRI) in response to a stimulus provides complementary insight into underlying processes in a temporal-spatially resolved manner and represents an attractive approach when studying pathological conditions with suspected impairment of neurovascular coupling.

References


Chapter 6

Functional SI: mapping glutamate and lactate levels in the mouse brain during electrical stimulation of the hind paw

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This work is currently in preparation

Abstract

Investigation of physiological changes and metabolic events associated with brain activity in the mouse constitutes a major challenge. Conventionally, neuronal activity is accessed by evaluating activity-evoked local changes in blood oxygenation levels (BOLD contrast) using fMRI methods. In mice however, the BOLD signal change seems to be dominated by systemic contributions even when using only innocuous stimulus strengths. Widespread and bilateral responses are generally observed following unilateral stimulation. MRS is sensitive to a different aspect of neuronal activation compared to regular BOLD fMRI and therefore provides a valuable complementary tool for investigating the brain at work. The aim of this study was to evaluate the sensitivity of a FID based spectroscopic imaging (SI) protocol for the measurement of metabolic alterations elicited by unilateral electrical paw stimulation at different amplitudes in mice. Dose-dependent changes in levels of glutamate and lactate were investigated in terms of amplitude and directionality. Moreover, the specificity of the metabolic response was investigated by comparing contra- and ipsilateral somatosensory cortices.
6.1. Introduction

Functional magnetic resonance imaging (fMRI) and spectroscopy (MRS) capture different aspects associated with neuronal activation and thus constitute valuable complementary tools for studying the brain at work. Investigations of physiological and metabolic adaptation to changes in brain activity in the mouse constitute a major challenge. fMRI measurements cannot be considered as reliable readout for neuronal activation. For example the quantification of changes in the conventional blood oxygen level dependent (BOLD) and cerebral blood volume (CBV) related fMRI signals upon sensory (e.g. electrical) paw stimulation turned out problematic due to stimulus-induced alterations in cardiovascular parameters potentially associated to arousal (Schroeter et al., 2014). Such influences of systemic physiology on cerebral hemodynamic response have been shown to mask specific stimulus-elicited fMRI signals. Strategies to minimize the contribution from systemic changes in hemodynamic parameters might include modified stimulation protocols (Schlegel et al., 2015) but also the use of non-vascular activity readouts.

Functional proton magnetic resonance spectroscopy (1H-MRS) measuring signals arising from endogenous metabolites and neurotransmitters in a time-resolved manner constitutes an alternative readout of brain function, which is not based on cerebral hemodynamics and therefore should be less susceptible to systemic confounds. Of the various metabolite signals identified in the MR spectrum, several are directly associated with neurotransmitter (glutamate, Glu; glutamine, Gln; gamma-aminobutyric acid GABA) or energy turnover (lactate, Lac; phosphocreatine, PCr; creatine, Cr). Quantitative assessment of changes of these metabolite signals changes, in particular in glutamate (Glu) being the major excitatory neurotransmitter, can be used as an indication for neuronal activity. In humans and rats, single voxel MR spectroscopy (SVS) was successfully applied to characterize neurotransmitter and metabolic changes during visual (Frahm et al., 1996; Lin et al., 2012; Mangia et al., 2007b; Prichard et al., 1991), motor and sensory (Just et al., 2013; Xu et al., 2005) stimulation. Common observations include changes related to the increased energy demand of active brain areas as reflected by elevated concentrations of Lac indicative of glycolytic energy production, a decreased ratio of creatine (Cr) to PCr reflecting replenishing of ATP levels via the creatine kinase reaction (Erecinska and Silver, 1989; Sauter and Rudin, 1993), and altered levels of the neurotransmitters Glu and Gln.

Previously, we proposed a 1H-MRS approach based on spectroscopic imaging (SI) for mapping metabolite and neurotransmitter distribution in the mouse brain at 1 µl spatial and 12 min temporal resolution. Following the administration of the GABA-A receptor antagonist bicuculline at doses that trigger epileptic seizures we were able to quantify dose-dependent changes in levels of metabolites involved in energy metabolism (Lac, PCr) and of neurotransmitters and their precursors (Glu, Gln, GABA) in a region-dependent manner (Seuwen et al., 2015). In contrast to the metabolic readouts, fMRI responses did not scale with the bicuculline dose indicative of a coupling mismatch between energy consumption and supply. Nevertheless, fMRI measurements were useful for interpretation of
functional MRS data supporting the notion of increased oxidative stress highlighting the complementary nature of the two readouts.

In the present study, we evaluated the sensitivity of our established SI approach when applying stimuli in a physiological range, i.e. electrical stimulation of the mouse hind paw. The use of FIDLOVS (Henning et al., 2009), a fast SI method, which we adapted for functional MRS experiments of mouse brain, together with a cooled detector system at 9.4T enabled serial SI acquisitions of arrays of 17x17 voxels of 1μl volume with a temporal resolution of 12 min. Application of an electrical stimulation paradigm resulted in changes in Lac and Glu signals in the primary cortical somatosensory or motor area that were found to scale with the stimulus strength. The unilateral stimulation of the hind paw led to larger changes in Glu in the corresponding contralateral somatosensory cortical region. In contrast BOLD fMRI responses were of widespread bilateral nature even though they were found to correlate with the stimulus amplitude. This indicates that MRS-based metabolic and neurotransmitter readouts are attractive measures of mouse brain function when investigating activation upon peripheral stimulation –and appear to reflect neural activity more intimately than hemodynamic readouts.

6.2. Materials and methods

Animal preparation

All in vivo experiments were carried out in strict adherence with the Swiss law for animal protection and approved by the cantonal veterinary office. Female C57BL/6 mice (Janvier, Le Genest-St Isle, France) at 3-4 months of age and ca. 23 g were anesthetized using isoflurane. Anesthesia was induced with 3.5% isoflurane and maintained at 1.5% in an oxygen/air (20% / 80%) mixture. Mice were intubated and artificially ventilated (80 breaths per minute, bpm) using a small animal ventilator (CWE, Ardmore, USA) for the entire duration of the experiment. Stereotactic fixation was used to ensure reproducible positioning of the animal on the support. The body temperature was kept at 37°C throughout the duration of the experiment. A catheter was placed in the tail vein for intravenous injection of pancuronium bromide (1 mg/kg; Sigma-Aldrich, Steinheim, Germany) as a bolus in order to avoid movement during acquisition and during the electrical stimulation. For the electrical stimulation, a pair of bipolar platinum needle electrodes (Genuine Grass Instruments, West Warwick, USA) was inserted subcutaneously into the right hind paw with a distance of 2 mm between the two needles. The numbers of animals for the different stimulation amplitudes and MRI readouts, respectively, are indicated in the figure legends.

Electrical stimulation

The pair of needle electrodes placed under the skin of the right and/or left hind paw was connected to a current stimulus isolator (A365D, World Precision Instruments Inc., Sarasota, USA). The stimulus
delivery was controlled with custom-written LabVIEW software (National Instruments, Austin, USA) and synchronized to the onset of the fMRS and fMRI imaging sequence by a trigger signal from the MR scanner. A fixed set of stimulation parameters (current amplitude = 1.0, 2.0, or 3.0 mA, pulse duration = 0.5 ms, pulse frequency = 5 Hz) was chosen and used in all experiments. Generally, the stimulation paradigm consisted of ten cycles of 40 s stimulus period and 20 s post-stimulus period; in the fMRI experiment the block design started with a resting period of 12 min (baseline) followed by the ten stimulation cycles.

**MRI hardware**

All experiments were carried out using a BioSpec 94/30 (Bruker BioSpin MRI GmbH, Ettlingen, Germany) small animal MR system operating at 400 MHz and equipped with a BGA 12AS HP gradient system with a maximum gradient strength of 400 mT/m and minimum rise time of 70 μs. The shim system allows shimming up to the second order. A four-element receive-only cryogenic phased array surface coil (2x2 geometry, overall coil size 20x27 mm²) with the coil system operating at 30 K (Bruker BioSpin AG, Fällanden, Switzerland) was used in combination with a circularly polarized 86 mm volume resonator for transmission.

**Spectroscopic imaging, SI**

Prior to SI, anatomical images were acquired for accurate positioning of the SI slice and the volume of interest. T2 weighted images (RARE, TR/TE = 3500/14 ms, 66 μm², slice thickness: 0.5mm) were acquired in axial and horizontal directions, followed by a reference image corresponding to the SI slice geometry. A coronal slice spanning a region from Bregma +1.34 mm to Bregma +0.02 mm was selected according to a stereotaxic mouse brain atlas (Paxinos and Franklin, 2004) for high resolution SI of a cortical region-of-interest (ROI) comprising 4 to 5 voxels. SI was performed using FIDLOVS (Henning et al. 2009). First and second order shim gradients were adjusted in the volume of interest using field maps. Prior to excitation, water suppression (WS) pulses (Tkáč et al., 1999) were applied interleaved with 7 saturation pulses delimiting the volume of interest. Efficient saturation was achieved using hyperbolic secant shaped pulses with phase and amplitude modulation of the B₁ field, applied with a bandwidth of 20 kHz. After WS and OVS, a frequency selective 90° sinc3 pulse was applied (BW= 6000 Hz) in combination with a slice selective gradient for excitation of a slice of 1.37 mm nominal thickness. The resulting free induction decay (FID) signals were collected after an acquisition delay of 1.29 ms, repetition time of 2500 ms, spectral width = 8 kHz with 4096 data points (spectral resolution 2 Hz / point). Parameter settings for the coronal slice were: field of view of 15x15 mm², matrix size of 17x17, resulting in the following spatial resolution: 0.88x0.88x1.37 = 1 μl (the k-space was weighted with a Hanning function), and 12 min acquisition time. For each animal, the SI protocol was repeated three times for baseline measurements, one time during stimulation, and three times after stimulation.
fMRI

Twelve adjacent coronal slices of 0.5 mm slice thickness spanning all the regions covered in the MRSI experiments have been recorded. The first slice was positioned at Bregma +2 mm according to a stereotaxic mouse brain atlas (Paxinos and Franklin, 2004). The local field homogeneity has been optimized in the area of interest using previously acquired field maps. BOLD fMRI data prior and during electrical stimulation were acquired using a gradient-echo echo-planar imaging (GE-EPI) sequence with the following parameters: field of view of 23.7x14 mm2, matrix size of 90x60, yielding an in-plane voxel dimension of 263x233 µm, repetition time of 1000 ms, echo time of 10 ms, average number of 1, yielding a temporal resolution of 1 s, with interleaved acquisition of slices. A data set comprised 2160 repetitions; resulting in a total acquisition time of 36 min. Electrical stimulation was started after recording baseline data for 12 min.

Data analysis

All data are given as mean across animals ± standard deviation.

LC model (Provencher, 2001) has been used for the analysis of SI data. Spectral processing includes zero and first order phase correction and baseline correction. No further pre-processing was applied to the data prior quantification. Relative quantification was performed using total creatine (Cr+PCr) as an internal reference. Statistical analysis was performed using a two-sample t-test (Matlab), to perform the following comparisons: ‘1 mA vs. control’, ‘1 mA vs. 2mA’, ‘1 mA vs. 3mA’, ‘2 mA vs. control’ ‘2 mA vs. 3mA’, ‘3 mA vs. control’; for both metabolites, Glu and Lac. Statistical analysis was also performed to compare the responses in left and right somatosensory cortices. Statistical significance is indicated in the figures as follows: p-value ≤ 0.001 ‘***’, ≤ 0.01 ‘**’, ≤ 0.05 ‘*’.

For analysis of fMRI data, MATLAB based software Aedes (http://aedes.uef.fi) was used to define ROIs according to a stereotaxic mouse brain atlas (Paxinos and Franklin, 2004) for the somatosensory cortex, thalamus, and hippocampus. ROI time courses were analyzed using custom-written MATLAB code (The MathWorks, Natick, MA). The first 100 repetitions were dismissed to account for the T1 relaxation effect. Prior to BOLD signal analysis, the linear trend was removed from the time series raw data and the signal changes expressed as percentages relative to baseline.

6.3. Results

BOLD fMRI response upon unilateral paw stimulation

Mean BOLD signal changes in percent of baseline values (ΔBOLD) elicited by electrical hindpaw stimulation are shown for stimulus amplitudes of 1 mA, 2 mA, and 3 mA, respectively (Fig. 1). Time courses were extracted from the primary somatosensory hindpaw cortical region (S1HL). ΔBOLD
depended in a *linear* manner on the stimulus strength. In all cases the BOLD signal displayed an ‘initial peak’ of approximately 60s duration that could not be sustained but leveled off to a ‘steady state’ value for the rest of the stimulation period, which was not different from the baseline value for the lowest stimulus amplitude of 1mA. Both the ‘initial peak’ and the ‘steady state level depended on the stimulus strength (Fig. 1).

Signal increases returned to baseline only slowly after the last of the ten stimulation blocks. The time courses of ΔBOLD extracted from Ctrl and ipsilateral regions indicated that BOLD responses were not confined to contralateral S1HL, thalamus, and S2 (secondary somatosensory hindpaw cortex; data not shown), irrespective of the applied stimulus strength used, but showed a rather widespread pattern. The average positive BOLD responses calculated from values between onset of the first stimulus period and return to baseline level in the analyzed regions ipsilateral to stimulation were almost identical to those in the corresponding contralateral ROIs (Fig. 1). This observation has been reported for mice already earlier (Bosshard et al. 2010, 2012; Schroeter et al. 2014). In a previous work (Schroeter et al. 2014) we show that specific fMRI responses associated to the stimulus may be masked because of influences by stimulus-induced cardiovascular changes, which indicate an arousal response.

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**Figure 6.1:** Analysis of BOLD signal changes (ΔBOLD) elicited by electrical paw stimulation. Individual time courses of representative animals show stimulus-evoked ΔBOLD extracted from the contralateral S1 hind paw region (A). Ipsilateral signal changes look almost identical (data not shown). Data shown as Mov. Avg. (n=15) ± STD. The gray rectangle indicates the simulation period from 720-1320s.
Figure 6.2: spectra originating from a single voxel located in the left somatosensory cortex of the mouse brain before (black line) and during stimulation (red line) with 1 mA (A), 2 mA (B) and 3 mA (C). A Glu increase is observed when stimulating with 2 mA and 3 mA. Lac is significantly increased when stimulating with 1 mA. Post processing includes phase correction (zero and second order), zero filling and truncation of all spectra. Line broadening (0.2 – 0.8 Hz) was generally applied to the spectra acquired during stimulation to match the line width of the NAA signal acquired at rest to compensate for the BOLD effect. However, especially when applying 3 mA the spectra recorded during stimulation appeared to have broader line width compared to the spectrum recorded at rest. Therefore the line broadening was applied to the spectra at rest, resulting in a lower general data quality (C), as reflected by the separation of the PCr/Cr resonances at 3.9 ppm.
Metabolite maps were acquired with a spatial resolution of 1 μl and a temporal resolution of 12 min before and during electrical stimulation of the right hindpaw. Highly resolved spectra extracted from a single voxel located in the left somatosensory cortex allowed resolving at least 10 metabolites, including Glu and Lac (Fig. 2). In order to compensate for the BOLD effect, line broadening (0.2 – 0.8 Hz) was generally applied to the spectra acquired during stimulation according to the line width of the NAA signal acquired at rest. However, especially when applying 3 mA the spectra recorded during stimulation appeared to have broader line width compared to the spectrum recorded at rest. Therefore the line broadening was applied to the spectra at rest, resulting in a lower general data quality (Fig 2C). Therefore, the spectral resolution was decreased with increasing stimulus amplitude as reflected by the separation of the PCr/Cr resonances at 3.9 and the taurine (Tau)/PCr+Cr signal at 3.2ppm. The Cramer Rao Lower Bound (CRLB) estimated using LCModel were 3% on average for Glu, and 10% for Lac (during baseline). One voxel was selected from each side, the contra- and ipsilateral somatosensory cortex, for quantitative analysis before and during stimulation. In general, Glu and Lac levels were increased during stimulation as compared to baseline values, with the amplitude of these changes being dependent on the stimulus strength. Whereas application of a stimulus strength of 1 mA resulted in no significant change in Glu and 50 % increase on average in Lac in the contralateral somatosensory cortex, amplitudes of 2mA and 3mA led to increased levels of Glu (8 % and 10 %, respectively), however the changes in Lac at 2mA were less pronounced (20 %) relative to those at 1mA, and when applying 3mA no significant changes were observed at all. In contrast, Glu levels displayed a clear dose dependent effect with significant differences from baseline values for current amplitudes of 2 and 3mA. Glu changes were found to be higher in the primary somatosensory cortex contralateral to the stimulated paw as compared to the side ipsilateral to the stimulated paw (Fig 4). A spectrum extracted from a voxel located in the right somatosensory cortex and compared with the corresponding spectrum from the activated region on the contralateral (left) side. For a stimulation amplitude of 2mA a trend towards an increased Glu response in the contra- as compared to the ipsilateral region emerges, which became highly significant for an amplitude of 3 mA. The Lac response appears less specific. A general increase was measured in the cortex when stimulating with 1mA and 2mA with no significant difference between contra- and ipsilateral side. However, the Lac decrease observed when stimulating with 3mA is restricted to the contralateral somatosensory cortex.
Figure 6.3: Percentual Glu (A) and Lac (B) level changes during electrical stimulation of the right hind paw in the mouse. Glu is unchanged (or decreased) during stimulation with 1mA, and increased in the left somatosensory cortex (S1HP_L) for both stimulus strengths 2mA and 3mA. No significant changes were observed on the ipsilateral side (S1HP_R). Surprisingly, the strongest Lac increase was measured during stimulation with 1mA. Asterisks indicate statistically significant difference at p≤0.05 (*) and p≤0.01(**).
Figure 6.4: Glu responses: comparison between left (S1_HP_L) and right (S1_HP_R) somatosensory cortices. A localized unilateral metabolic response indicates specificity. The Glu response was more pronounced in the contralateral side when stimulating with 2mA and 3mA. A highly significant difference was measured when using 3mA.

Figure 6.5: Lac responses: comparison between left (S1_HP_L) and right (S1_HP_R) somatosensory cortices. The Lac response seems less specific. A general increase was measured in the cortex when stimulating with 1mA and 2mA, no significant difference was observed between contra- and ipsilateral side. However, the Lac decrease observed when stimulating with 3mA is restricted to the left somatosensory cortex and could therefore be attributed to neural activity.
6.4. Discussion

In a previous study we have demonstrated that functional 1H-MRS can be used to monitor metabolic changes in the mouse brain upon stimulation with bicuculline, a GABA-A receptor antagonist inducing strong and sustained neuronal activity in the form of epileptic seizures. This was achieved by optimizing an FID-based SI method for functional measurements on the mouse brain, enabling the recording of changes in cerebral metabolism with higher spatial (17×17 voxels of 1 μl volume) and temporal resolution (12 min). We found that the metabolic changes measured with MRS scaled with the dose of the neuroactive compound and therefore with neuronal activity in contrast to conventional fMRI readouts (Seuwen et al. 2015). fMRI is based on the measurement of local hemodynamic changes as surrogate of neural activity and the approach relies on the integrity of neurovascular coupling, which might be compromised under pathological conditions. Also alterations in peripheral hemodynamics might affect fMRI signal. It has been reported that especially in mice, activity-evoked BOLD and CBV responses appear to be dominated by systemic hemodynamic alterations caused by the stimulus (Schroeter et al., 2014). Widespread and bilateral responses are observed following an even mild unilateral sensory stimulus, rendering the BOLD signal unspecific as readout for neuronal activity in this species. Thus, alternative readouts of brain function that are not based on cerebral hemodynamics become highly attractive. The SI approach proposed earlier is particularly interesting in this regard as it allows measuring changes in levels and turnover of metabolites associated to neurotransmission and energy turnover in a spatially resolved manner, two key metabolic processes related to neural activity. Hence, fMRS constitutes an attractive strategy for differentiating active from supposedly non-active regions.

The major challenge when applying time-resolved SI lies in its insufficient temporal resolution limited by intrinsic poor sensitivity. The strategy used here combines the high sensitivity provided by a cryogenic phased array receive-only coil with a SI method based on FID acquisition with short acquisition delay, yielding nearly full signal intensity. This approach delivered satisfactory results when applied with bicuculline, a rather harsh stimulation paradigm inducing intense and diffuse neuronal activity. Yet, peripheral sensory stimulation is a more physiological paradigm triggering discrete and localized metabolic events. Moreover, as prolonged stimulation paradigms must be used (when compared to regular BOLD fMRI) neurons might habituate to the stimulus. The first question addressed in this work was therefore to determine whether the proposed SI approach was suitable in terms of sensitivity and temporal resolution for measuring the weak changes in metabolite levels associated with electrical hind paw stimulation. The sensitivity of the SI readout was tested by applying several stimulus amplitudes, with the intention of characterizing the paradigm in terms of minimal applicable stimulus strength and dose dependent response. We measured dose dependent changes in Lac and Glu levels in mice upon unilateral electrical stimulation of the hind paw using stimulus strengths of 1 mA, 2 mA and 3 mA. Glu levels were not affected at a stimulus amplitude of
1 mA, but significantly increased when using 2 mA and 3 mA. The dose dependence of Lac was unexpected as a maximal and significant increase has been observed at the lowest amplitude value of 1 mA, a less pronounced increase at 2 mA, while there was even trend towards a decrease at 3 mA. In contrast to observed changes when applying bicuculline in our recent study, Lac increases upon sensory paw stimulation were much smaller, and measured Glu values did not decrease but increase during the activation period eventually indicating a more physiological response.

As no comparable studies have been carried out in mice to date we compare our findings with 1H-MRS studies in rats and humans reporting changes in Glu and Lac levels upon sensory stimulation though there is considerable variability across these studies. Changes in Lac levels in humans upon visual stimulation range from strong to moderate increase (Frahm et al., 1996; Lin et al., 2012; Maddock et al., 2006; Mangia et al., 2007a, 2007b; Prichard et al., 1991; Sappey-Marinier et al., 1992). In rats, a study involving electrical forepaw stimulation reported decreased Glu levels and no significant changes in Lac concentration (Xu et al., 2005), while another study reported increased Glu concentrations and strongly elevated Lac levels during stimulation of the trigeminal nerve (Just et al., 2013). The latter study reported changes in Glu levels of 8% upon stimulation, similar to the values found in our study though the changes reported for lactate were considerably larger in the rat study. These discrepancies might be attributed to differences in experimental conditions including different stimulation paradigms and in the case of animal studies the use of different anesthetic agents or anesthesia levels. It has been reported earlier that anesthesia, both with regard to the type of anesthetic used and depth of anesthesia, have a profound influence on function both in response to a stimulus (Schroeter et al. 2014, Schlegel at al. 2015) and at rest (Grandjean et al., 2014). Our results regarding Lac levels were unexpected and deserve further consideration. We found a significant increase in lactate levels for the lowest stimulation amplitude in line with these reports. Elevation of Lac results from increased glycolytic activity and hence indicates increase neural activity. At this current amplitude there was no persistent BOLD signal throughout the stimulation paradigms. Even though it has been demonstrated that with prolonged repeated stimulation levels of Lac tend to increase less (reflecting neuronal adaptation; Mangia et al., 2007b), one could intuitively expect stronger increase in Lac levels with increasing the stimulus strength. Yet, this was not observed; in fact, Lac levels showed a negative correlation with stimulus amplitude for the range evaluated in this study. For 2 and 3 mA stimuli we observed BOLD responses of 2 and 3.3% throughout the duration of the stimulation indicative of a strong hemodynamic response, i.e. and increase in CBF and CBV, which on the one hand may lead to faster clearance of tissue Lac. Alternatively, increased CBF will enhance oxygen delivery to the tissue resulting in a decreased contribution of glycolysis to the energy production, which might explain the decrease in Lac level with increasing stimulus amplitude for the range of stimuli used in this study. For very strong stimuli, the neurovascular reactivity might not be able to cope with the energy demands of the tissue thereby causing oxidative stress and correspondingly a strong increase in Lac levels (Seuwen 2015). Finally there is increasing evidence that Lac is used as an
energy substrate by neurons (Mangia et al., 2003; Pellerin and Magistretti, 1994; Wyss et al., 2011). Cerebral Lac levels depend in a complex manner on cerebral metabolic and physiological processes, which limit their suitability as surrogate of brain activity.

The evolution of Glu levels during stimulation and the observed dose-response behavior appears to better correlate with neural activity. Elevated Glu levels were measured in humans during visual stimulation by Mangia et al. (Mangia et al. 2007a) and later confirmed in a study by Lin et al. (Lin et al. 2012). Recently, a raise in Glu levels by approximately 8% in response to electrical stimulation of the trigeminal nerves in rats was reported (Just et al. 2013). These changes were postulated to reflect an increased flux through the malate-aspartate shuttle, as corresponding decrease in aspartate (Asp) was measured. However, in the study of Xu et al. (Xu et al. 2005) involving forepaw electrical stimulation in rats, a decrease in Glu and a corresponding increase in Gln was measured, both attributed to Glu-Gln cycling. Interestingly, no changes in Lac levels were measured. One explanation for these fundamentally different responses could be the application of a different stimulus paradigm; however our data recorded in mouse are similar to the results by Just et al. (Just et al. 2013) while the stimulation paradigm used was similar to the one of Xu et al. (Xu et al. 2005). These discrepancies rather seem to point towards a different baseline physiological state of the animal, leading to a different way of processing the stimuli. Basal levels of blood oxygenation (ventilated vs. non-ventilated animals) together with the impact of different levels of anesthesia during preparation and during the experiment might have played an important role. Nevertheless, Glu appears to be a good indicator for the metabolic processes associated with neural activity as it shows a monotonous dependence on the stimulus amplitude.

A major motivation for the current study was the intriguing observation that the BOLD response to unilateral hindpaw stimulation in mice lacked regional specificity, i.e. involved large cortical domains in both hemispheres. This was attributed to systemic stimulus induced hemodynamic changes that overrule the specific signals reflecting sensory processing (Schroeter et al. 2014). One of the possible solutions to overcome this limitation was the use of non-hemodynamic functional readouts such as tissue metabolism. The SI approach is of particular interest in this regard as it allows the simultaneous recording of activation induced metabolic changes across large brain areas, enabling the identification of regions displaying stimulus induced activity in comparison with non-activated reference regions. A specific response should be restricted to the region of neuronal stimulus processing, for the paradigm used in this study predominantly the contralateral somatosensory S1 area. In fact, our results clearly indicate a specific Glu increase in the contralateral S1 territory, which reached statistical significance for a stimulus amplitude of 3 mA with values of $\Delta$Glu (S1contra) = 10±5% as compared to $\Delta$Glu (S1ipsi) = -5±3%. Also in this regard, the Glu signal seems to be a more reliable readout for neural activity than the Lac signal. The Lac response is less confined and probably reflects the general increase in CBF affecting both the oxygen delivery to Lac efflux from the tissue throughout the brain.
There seems to be a significant difference between decreased Lac levels in the activated regions and unchanged or slightly elevated Lac levels in the somatosensory cortex ipsilateral to the stimulated paw. This result might support the hypothesis of the astrocyte-neuron-lactate shuttle (ANLS, Pellerin and Magistretti, 1994), in which activated glutamatergic neurons use lactate released by astrocytes as their energy substrate (for review see Castro et al., 2009; Dienel and Hertz, 2001; Dienel, 2012; Schurr, 2006), which would affect the contra, but not the ipsilateral side.

Relatively strong stimulus amplitudes (> 1 mA) are required to measure significant Glu responses in mice due to small voxel volumes (1µl) and high demands on temporal resolution. As expected, achieving sufficiently high sensitivity and reproducibility and therefore a reliable quantification of changes in metabolite concentrations in the mouse brain turned out to be a challenging task. In this study, Glu levels could be quantified by LCModel with a minimal error of 3%, which implies that the Glu changes triggered by the stimulus should be higher than 3% in order to become significant for small group sizes. Variability arises on one hand from issues with the quantification, which is difficult for data obtained with the FID based method used here, for which definition of the volume of interest is achieved via OVS. Insufficient saturation might lead to contamination of the spectra by unwanted lipid signals, and often a substantial residual water signal was observed. Moreover the acquisition delay that needed to be chosen as short as possible in order to increase the signal intensity comes at the expense of significant signal contribution arising from macromolecules. As a result, estimation of baseline was found often problematic and might have led to errors in the quantification. A substantial amount of the variability observed probably originated from instable physiological conditions of the animal. Particularly the influence of the basal metabolic state, which depends on the anesthetic regimen, seemed to affect the metabolic changes measured. The most limiting factor is the low sensitivity of MRS and SI approaches: for example electrical stimulation with 1 mA during 10 min is a stimulus that most likely represents a stressful experience for mice, yet hardly evoked an fMRS response and higher current amplitudes were required to elicit significant metabolic changes. In contrast an amplitude of 1mA prompted a strong BOLD response. Hence, the scope of fMRS applications appears rather limited to paradigms involving strong stimuli that with potentially strong side effects on the mouse’s physiological state. On the other hand, the metabolic response is more intimately linked to neural activity than the BOLD response and hence is less prone to artifacts introduced by systemic hemodynamic changes that may overrule cerebral autoregulation (Schroeter et al. 2014). The improved specificity of stimulus-induced metabolic changes as compared to hemodynamic response is an attractive feature of SI – at least in mice.

In conclusion, we monitored cerebral metabolic changes associated with neural activity elicited by a sensory stimulus in mice in a temporo-spatially resolved manner using SI. Upon electrical stimulation of the hindpaw Glu levels were found to be increased in the somatosensory cortex contralateral to the stimulation, but remained unchanged in the respective ipsilateral territory. The BOLD fMRI response
lacked this spatial specificity, but was bilateral nature, due to the effect of systemic hemodynamic changes elicited by the stimulus. A limitation of functional MRS is its intrinsic low sensitivity, which limits the spatial resolution to typically 1μl and the temporal resolution to 10 minutes. Also the use of strong stimuli of rather long duration is required. Nevertheless, metabolic mapping using SI could become an interesting alternative to BOLD fMRI especially when applying noxious stimuli, for which strong systemic responses would be expected in mice.

References


Chapter 7

Discussion
Spectroscopic imaging (SI) offers the unique possibility to investigate brain neurochemistry in vivo and in a spatially resolved manner. At high field, up to 20 metabolite signals, including excitatory and inhibitory neurotransmitters and their precursor (Glutamate (Glu), GABA, glutamine (Gln)) as well as general indicators of energy metabolism (lactate (Lac), glucose (Glc), creatine (Cr) and phosphocreatine (PCr)) can be recorded from the brain and subsequently quantified (for review see Duarte et al., 2012). Metabolite levels can then be evaluated in healthy tissues and compared with tissues affected by diseases; thus they can serve as biomarkers to identify certain neurological disorders, or can be used to monitor disease progression and response to therapeutic interventions. As a non-invasive procedure, MRS can also be applied as a tool for fundamental research in longitudinal studies, a considerable advantage when studying slowly progressing neurological disorders in animal models.

In recent years there has been increasing interest in developing adequate protocols dedicated to SI in the mouse brain in view of the various genetically engineered mouse lines available for basic biomedical research. However, this method is particularly challenging when applied to mice. Due to the small organ size, high demands in terms of spatial resolution are put on a method with intrinsically low sensitivity. A simple though inefficient strategy to match the requirements concerning the spatial resolution is data averaging, which increases SNR by the square-root of the number of averaging steps. Yet, this typically leads to impracticable measurement times even at higher field strength (7T onwards). Moreover the method is prone to artefacts due to motion which may affect field inhomogeneity, unwanted contamination with lipids or large residual water signals and inconsistent position of the volume of interest. An efficient method to counteract the low intrinsic signal-to-noise ratio (SNR) is the use of a high (magnetic) field system, as the sample polarization scales at least with the field strength. On the other hand, a high B0 field amplifies field inhomogeneity and CSDE, and introduces new technical difficulties related to short T2 relaxation times, imposing the use of short echo times and therefore the appearance of prominent macromolecule signals, rendering the adequate baseline definition required for quantitative analysis difficult. Nevertheless, even for high field systems SNR has remained the limiting factor for SI applications to study brain metabolism in mice until now. In particular the temporal resolution provided by conventional acquisition methods and hardware is insufficient for functional studies aiming at quantifying instant and short-term neurotransmitter and metabolite changes in response to neural activity.

In an MR experiment, the SNR can be improved by ameliorating the detector system and/or by adapting the measurement protocol. Sequence parameters such as bandwidth, spatial resolution, echo time and repetition time can be set to provide maximal SNR, however they are often constrained by the application itself. Therefore the SNR is usually improved by performing more averaging, a rather inefficient procedure in terms of temporal resolution. The detector coil can be improved by increasing
its intrinsic sensitivity as a receiver, or by lowering its noise contribution to the system under investigation. The sensitivity of detectors can be optimized by assembling several smaller coils in a coil array (Roemer et al., 1990), providing the sensitivity of a small coil (increased filling factor) over extended regions of interest. The noise originating from the coil is directly proportional to its temperature, and can therefore be reduced very efficiently by cooling down the coil wires and preamplifiers (Baltes et al., 2009; Kovacs et al., 2005; Ratering et al., 2008; Styles et al., 2011, 1989). This translates into an overall improvement of the SNR, provided the coil noise is a relevant noise source, i.e. comparable to the intrinsic noise generated by the sample itself.

The objective in this thesis was to investigate the possibilities in terms of spatial and temporal resolution of SI applied to the mouse brain using a cryogenic phased array coil (array CRP) at 9.4T. The SNR improvement provided by the cold detector should be reinvested in spatial and temporal resolution. Accelerated acquisition strategies eventually allowing functional studies should be implemented and evaluated during stimulus evoked brain activity.

The array CRP described in chapter 3 has been a major contributor to the success of the development of fast SI procedures enabling the first applications of fMRS in mice. The gain in SNR by a factor 2 to 3 provided by CRP translates into a reduction of measurement time by a factor of 4 to 9 with regard to data acquisition using a RT coil of equal dimensions. However, the benefits of the array coil compared to its predecessor, a quadrature transceiver coil, are less evident. Theoretical estimations would predict an increase of coil sensitivity by a factor of 2 near the coil surface of the array coil (assembled with coil elements of half the size compared with the previous quadrature coil), while maintaining the same overall coil dimension and therefore also the same field of view (FOV). However with appropriate settings of pulse gains for the transceiver quadrature coil we observed a rather heterogeneous SNR gain (phased array CRP over quadrature CRP) ranging from comparable to negative. As already discussed in chapter 3, this might be explained by the additional hardware and cables used in the array coil to decouple the individual elements, which act like resistors placed in series with the coil (thereby decreasing the Q-factor of the coil). The contribution of this additional resistance is negligible for large coils, when the sample noise dominates the coil noise. However, for small coils the noise originating from this resistance becomes significant and most probably degrades the beneficial effects of building smaller coil elements, when compared with a simpler quadrature coil design. Nevertheless, the homogeneous transmission provided by the volume resonator using the array CRP setup is an advantage in most applications, including SI. Based on phantom and in vivo data, similar SNR can be achieved using the quadrature CRP in brain tissues near the coil surface; however, for deeper located brain structures the SNR drops rapidly compared with the array CRP. As for SI applications metabolite maps are acquired over extended regions of interest, the array CRP setup (i.e. the cross coil operation using homogeneous excitation) is to be preferred regarding global SNR. Yet, due to the complex design of the CRP, a large and therefore rather inefficient volume resonator had to be chosen
for transmission. Serious limitations in the maximal achievable pulse bandwidth have been encountered, as discussed in chapter 4 of this thesis. Considering the increased chemical dispersion at high field, large CSDE have been observed using a regular PRESS sequence (Bottomley, 1985, 1987), enforcing the use of alternative measurement methods.

Considering the high temporal resolution required for fMRS experiments and the coil setup chosen (providing maximal SNR but lacking efficiency in transmission), FIDLOVS (Henning et al., 2009) has been considered as optimal for two reasons:

1. It provides full signal intensity. The FID is collected after a very short acquisition delay, minimizing signal losses due to T2 relaxation.
2. One single 90° pulse is used for slice selective excitation. Thus, the CSDE caused by insufficient pulse bandwidth is minimized and restricted to one spatial dimension.

An alternative to FIDLOVS in terms of signal intensity would have been SPECIAL (Mlynárik et al., 2006). This method combines a one dimensional ISIS localization step (Ordidge et al., 1986) with a regular localized spin echo experiment (intersection of the frequency selected slices by the 90° and 180° pulses), allowing the acquisition of an echo after a delay as short as 1ms. Yet the method still involves the formation of a spin echo using a 180° pulse, leading to a strong CSDE along one spatial dimension, especially when using the array CRP coil setup described above. Moreover, a minimum of two steps is necessary to achieve complete localization. Compared to FIDLOVS, the minimum achievable scan time would therefore be doubled (considering that no averaging was performed in the studies described in chapter 5 and 6). Therefore, FIDLOVS appears to be the optimal SI method when temporal resolution is critical. In addition SI allows the simultaneous coverage of an extended region-of-interest yielding an overview on the metabolic activity across multiple brain areas including regions potentially not involved in a specific task that might serve as a control. This was considered essential when evaluating the specificity and validity of the measured metabolic changes during brain activity. In comparison, most fMRS studies to date have been performed using single voxel spectroscopy (SVS), in which independent of the method used signal averaging is necessary. In this case, a more efficient surface transceiver coil allowing large pulse bandwidth and minimal CSDE could be used. For such applications SPECIAL would be time-wise nearly as efficient as FIDLOVS, however providing information from one single voxel only.

The mechanisms underlying metabolic processes associated with neural activity are complex and not yet fully understood. They rely on a fragile equilibrium between energy producing and energy consuming processes, the type and availability of metabolic substrates, the basal physiological condition, the stimulus type and strength, and in anesthetized animals on modulation of these effects by the anesthetic. In principle, 1H MRS can be used to assess metabolic events accompanying neural activity by measuring changes in levels of Glu, Gln, GABA, Lac, Asp, Cr and PCr. However, the
method measures metabolite levels that are averaged across the voxel and therefore comprise contributions from different compartments, i.e. different types of cells (e.g. neuronal and astrocytic Glu and Gln), as well as extra-, intra- and subcellular compartments. $^1$H MRS only capture net changes of metabolite concentrations, averaged over several minutes. It is therefore difficult to link the observed changes to a specific metabolic process in a quantitative manner. Moreover, the temporal resolution is insufficient to resolve transient biochemical changes elicited by a short-term sensory stimulus. For fMRS applications, stimuli must be continuously or repeatedly applied in order to match the duration of one scan, which could cause neuronal adaptation and therefore complicate the interpretation of results.

Using the described measurement protocol changes in Glu levels elicited by sensory and pharmacological stimulation were found to correlate well with the stimulus intensity (chapter 5 and 6) and could therefore be considered as a quantitative marker for neural activity. Yet, the simple translation ‘increased activity equals increased glutamate levels’ does not hold: chemical stimulation using bicuculline causing epileptic seizures in response to GABAergic disinhibition produced a strong decrease in Glu levels (Chapter 5), while the more physiological electrical hind paw stimulation triggered a more subtle increase in Glu levels (Chapter 6). The decrease of Glu levels accompanied by increases in Gln, Ala and Asp following bicuculline treatment has been associated to ammonia detoxification of tissue by amination of Glu (Chapman et al., 1977; Patel et al., 2005). An alternative explanation would be Glu efflux from brain tissue. It has been reported that ischaemic stress led to Glu efflux from cerebral cortex in rat brain slices, a process that is inhibited by GABA and GABA mimetics. On the other hand, this inhibiting effect was lost in the presence of bicuculline (Nelson et al., 2000). We observed significant oxidative stress following the administration of bicuculline as indicated by transient increases in PCr, increases in Lac and strong negative BOLD changes indicative of high levels of deoxyhemoglobin, which might have prompted Glu efflux and hence a rapid drop in cerebral Glu levels in line with the changes in PCr and Lac. Following bicuculline clearance the Glu levels were slowly re-established. In contrast, electrical paw stimulation was not associated with signs of oxidative stress (no in PCr, normal BOLD response) and thus Glu efflux. Under those circumstances, neural activity had prompted a positive Glu response. The different outcomes regarding Glu levels for two conditions illustrates the complex interplay of neurotransmitter cycling, maintenance energy homeostasis and detoxifying metabolic processes that complicates the interpretation of $^1$H MR spectroscopic data. Also the brain is not a self-contained compartment, hence there may be net changes in metabolite levels, e.g. alteration in cerebral blood flow may affect the metabolite levels. Carefully designed studies under different conditions combining both fMRS and fMRI as readouts of triggered brain activity could provide a basis for elucidating underlying processes. Further efforts are crucial for a better understanding of the link between the metabolic readouts or fMRI signal changes and neural or metabolic activity.
Nevertheless, it appears that by maximizing the SNR through improving both, the detector efficiency and the measurement protocol, monitoring changes in brain metabolism in mice elicited by an external stimulus becomes possible. The metabolic response, though not fully understood, appears more specific than the fMRI readout known to be altered by systemic hemodynamic changes, eventually masking the specific stimulus induced neural response particularly in mice (Schroeter et al., 2014). As a non-invasive method, fMRSI could be of high value for biomedical research, when applied to monitor the evolution of brain pathologies in animal models and possible therapeutic effects of novel treatment approaches.

References


Chapter 8

Conclusion and outlook
In this thesis, the array receive only cryogenic coil (CRP) was crucial to all fMRS experiments as it allowed to increase the temporal resolution by at least a factor of 4 compared with a regular RT setup, enabling the first attempts of fMRSI in mice.

The characterization and optimization of the first existing prototype of array CRP designed especially for the mouse brain revealed an SNR gain of 2 to 3 compared with an identical room temperature (RT) coil. This led to better contrast-to-noise ratio (CNR) in high resolution MR imaging and MR angiography of the mouse brain and more precise quantification of metabolites in spectra originating from small voxels (~1μl). Moreover, compared with a quadrature transceiver CRP, we measured a significant SNR gain in phantoms and mice for regions at a distance of the order of the coil radius from the coil plane as a result of homogeneous excitation ensured by a separate volume resonator.

Especially for SI of the mouse brain, low sensitivity limits the maximum achievable spatial resolution. The SNR gain provided by the phased array CRP could be reinvested in improved spatial resolution while maintaining acceptable measurement times and an SNR at a level to ensure accurate quantitative analysis, enabling for the first time the acquisition of SI data with voxel sizes of 0.68 μl volume using a standard PRESS (Bottomley, 1985, 1987) protocol.

A method based on localization via outer volume suppression (OVS) permitting the acquisition of the free induction decay (FID) instead of a spin echo has been implemented based on previous work performed on high field clinical systems (FIDLOVS, Henning et al., 2009). This method, providing nearly full signal intensity and minimal CSDE yielded excellent results when applied to the mouse brain at 9.4T and enabled the recording of metabolite maps with higher spatial (17×17 voxels of 1 μl volume) and temporal resolution (12 min).

The increased signal intensity provided by FIDLOVS and the use of the CRP detector system enabled serial acquisitions of metabolite maps following the administration of bicuculline, a GABA-A receptor antagonist blocking the inhibitory action of GABA and producing strong and sustained seizures. Dose-dependent changes in regional levels of neurotransmitters (Glu and GABA), and metabolites involved in the energy metabolism (Lac, Cr and PCr) could be quantified, constituting the first report of fMRS in mice (Seuwen et al., 2015).

The same protocol was applied upon unilateral electrical hindpaw stimulation, a more physiological paradigm triggering discreet and localized metabolic events. The sensitivity of the SI readout was tested by applying several stimulus amplitudes (1 mA, 2 mA and 3 mA). Dose dependent changes in Glu levels could be measured specifically in the somatosensory cortex, contralateral to stimulated paw. Changes in Lac levels appeared to be less specific, showing a more widespread response and an unexpected dose dependency.
Next steps in the project go in two directions: i) using the technological solutions implemented (hardware and acquisition protocols) to further characterize the metabolic changes underlying neural activity, and ii) further methodological developments to increase the acquisition speed, the volume coverage and to combine metabolic information with other readouts of neural activity. The first aspect requires the development of discriminative stimulation paradigms, e.g. by using different stimulus qualities and quantities or by specific modulation of individual processes through pharmacological or genetic interventions. This might help disentangling the role of a particular neurotransmitter or metabolite in response to a defined state of brain activity. Experiment in mice should be compared to analogous studies using the rat as a model organism that is physiologically better to control and more stable than mice. Stimulus related responses in mice appear more prone to confounds from unspecific and arousal responses. In contrast, experimental evidence suggests that analogous studies in rats yield more specific results, which is relevant for studying the biochemical changes triggered by the targeted application of a stimulus. The correlation of metabolic (fMRS) and hemodynamic (fMRI) readouts extensively contributes to the understanding and interpretation of measured results. Efforts could be made towards establishing a protocol with simultaneous application of fMRS and fMRI sequences within one experimental run. Correlating the complementary obtained information within an individual will improve the interpretation of data significantly. Furthermore, such correlative studies are not necessarily limited to MR based method. Combination with alternative readouts of activity may also include nuclear and optical imaging targeting e.g Ca2+ signaling, measurement of intracellular glucose or lactate levels using optical sensors (Sotelo-Hitschfeld et al., 2015). In general, multimodal imaging approaches providing complementary information constitute powerful tools essential for characterizing complex systems. However building such hybrid experiments in an MR compatible manner is challenging, particularly regarding the combination with fMRSI as implemented here, when the complex and space-taking design of the cryogenic coil cannot be avoided due to SNR considerations.

The fMRSI methodology could be advanced in several directions. The current SI approach is limited to a single slice, i.e. cannot simultaneously probe the various brain regions involved in processing an applied stimulus. Extending the field of view in the third dimension therefore constitutes a next logical step. This can be achieved by full 3D SI or by multi-slice SI approaches. At this stage we favor the latter, as 3D SI is rather time consuming and therefore not suited for functional studies. In addition, it will be very challenging to achieve sufficient filed homogeneity throughout the 3d volume of interest. This could be achieved when using a multi-slice strategy: implementation of dynamic shim updates would allow optimizing the magnetic field homogeneity for each slice individually warranting the spectral quality required for accurate analysis. Another limit of the current approach is the temporal resolution, which compared to the state of technology in mouse MRS is high, yet compared to the time scale of physiological processes to be studied is still low. The fact that a phased array coil has been used in these studies allows for acceleration using parallel imaging strategies (Dydak et al., 2001;
Griswold et al., 2002; Pruessmann et al., 1999). Yet, the current hardware design and performance and the inherent loss in SNR upon acceleration may limit this potential. Improved detector designs with more, non-overlapping and smaller coil elements might be required though this may be a rather challenging task given the small overall dimensions and the anticipated difficulties encountered when decoupling the individual elements.

In summary, there is sufficient room for both technological and methodological improvements. Ultimately, these will render MRS and fMRS attractive tools for addressing basic aspect of brain metabolism at rest and in response to altered neural activity, for studying these changes under normal and pathological conditions such as animal models of psychiatric or neurodegenerative diseases, and to evaluate whether therapeutic intervention contribute to the normalization of disease related metabolic aberration. The noninvasive nature of the readouts, and hence their potential translatability into the clinics enhance their relevance.

References


Appendix

Standard Operating Procedure for Animal Preparation
Introduction

MR functional and metabolic imaging are unique tools to investigate brain function non-invasively. For animal studies, alternative methods for studying brain activity at the physiological, cellular and molecular levels are available, however techniques such as intrinsic optical imaging, in vivo microscopy, microdialysis, or electrophysiology are highly invasive, i.e. they involve exposure of brain tissue or the implantation of dialysis probes or electrodes. Brain function might be also inferred from analyzing the expression of activity-related genes such as cFos. Typically, these techniques yield only local information, which is a disadvantage particularly when investigating brain function, which may involve large-scale networks. MR imaging, being non-invasive, allows longitudinal studies with minimal interference with the biological system under investigation. While spatial and temporal resolution is insufficient to resolve network activity at a cellular level, it is sufficient to identify macroscopic functional units on the basis of activity related changes in the physiological and/or metabolic state. Therefore MRI has become a very powerful tool for fundamental research and most MR procedures available for humans in a clinical setting including fMRI and MRS have been translated to be used at higher field strengths on rodents.

However, fMRI and fMRS on mice face several severe limitations that go beyond the usual sensitivity issues typically encountered in this species (Schroeter et al., 2014). Animal preparation to achieve stable physiological conditions throughout the duration of the experiment is a critical success factor in these studies. Functional MR studies in mice are typically carried out under anesthesia, which typically is administered via a face mask (Adamczak et al., 2010; Ahrens and Dubowitz, 2001; Nair and Duong, 2004; Nasrallah et al., 2014) with minimal additional preparation steps. Yet under these conditions it is very difficult to properly control the physiological state of the mouse which may lead to fluctuations in metabolism and physiological parameters such as body temperature, respiratory status, heart rate, and blood pressure that are directly linked to the fMRI and fMRS readouts and therefore increase the physiological noise in the data. Here we propose to intubate and artificially ventilate the mice. In addition, a muscle relaxant administered via a tail vein cannula should be used. Intubation ensures reproducible physiological conditions through fixed respiration rate and allows better control over the level of anesthesia when inhalation anesthetics are used. The use of muscle relaxant avoids spontaneous breathing of the mouse and thus improves the ventilation and furthermore prevents movement during acquisition, especially when using sensory stimulation paradigms during fMRI or fMRS measurements. Even when fixing the head of the animal using stereotactic ear bars, slight shifts in head position are frequently observed during sensory stimulation. Movement reflexes produce artefacts in the fMRI signal time course and should be absolutely avoided in fMRS studies as datasets may become unusable as it will inevitably lead to reduced magnetic field homogeneity and correspondingly deterioration of the spectral quality. Hence careful animal preparation including intubation, artificial ventilation and paralysation are critical for fMRS on mice following guidelines to
warrant a high degree of reproducibility. A critical aspect is the sequence and the timing of the individual steps and the definition of abortion criteria if these criteria cannot be met.

**Animal preparation protocol**

The protocol described here contains a detailed description of the following steps: induction of anesthesia, intubation, positioning of the animal on the cradle, cannulation and paralysation in the first part (figure 1.A) and the necessary procedures for preparation of MR measurements in a second part (figure 1.B). Here, we describe a protocol in which mice are anesthetized using isoflurane. However, the same protocol (with small variations) can be used with another anesthetic.

**Preamble: Lab equipment**

The minimal setup for induction of anesthesia includes an induction box and a warming device (electric heating cushion or self-heating pads (hand warmer). Flexible polyethylene tubing (PE-50/10, ID 0.58mm, OD 0.97mm, Cat.Nr. 64-0752, Warner Instruments) cut to the desired length is used as endotracheal tube. A fiber optic surgical light is needed during the process of intubation and at least one small animal ventilator (for mice we advise the MRI-1 ventilator, Cwe, Inc) is necessary for mechanical ventilation. For ventilation on the cradle it is important to design a stable holder for the gas supply arriving from the ventilator. It should also be possible to disassemble it for cleaning. During the MR measurement, the animal must be kept warm using for example a warm water bath system and its body temperature should be monitored with an MR-compatible temperature sensor. The catheter inserted in the tail vein is made of polyethylene tubing (PE-50 / 10, ID 0.28mm, OD 0.64mm, Warner Instruments) and assembled with a 30G needle. During the whole procedure body temperature should be maintained.

§1: Induction of anesthesia and intubation

The mouse is placed in the induction box filled with a gas mixture of 20% O₂, 80% air and 3.5% isoflurane. Time is measured from this time point. Once the respiration frequency has dropped to approximately 30 breaths per minute (bpm, holds only for anesthesia with isoflurane), the animal can be prepared for orotracheal intubation. The mouse is placed in a supine position on a platform. The upper jaw is fixed with a rubber band to facilitate access to the animal’s larynx. A thin tube connected to the isoflurane vaporizer is inserted into the mouth to maintain the sedation. A surgical light is then positioned above the throat illuminating the trachea (figure 2). A flexible polyethylene tube (PE-50/10, ID 0.58mm, OD 0.97mm, Warner Instruments, Hamden, CT, USA) of ~4cm length is inserted 2.5 cm deep (measured from the upper incisors) into the trachea. Thereafter, the tube is cut to the minimal length required for connection to the ventilation system in order to minimize the dead volume.
Accurate animal preparation includes intubation, cannulation and paralysis of the animal. These steps need to be executed in a controlled way: each procedure should be completed within a certain amount of time, otherwise the experiment is terminated. When animals are prepared according to this protocol, the MR preparation is straightforward allowing an important gain of time. The goal is to start the functional experiment less than one hour after the induction of anesthesia in the animal.

Following intubation, the animal is immediately transported to the cradle for the MRI measurement and connected to the ventilation system.

Induction of anesthesia and intubation should be executed efficiently to:

- Minimize the time in which the animal is inhaling 3.5% of isoflurane in a rather uncontrolled way. The animal must be deeply anesthetized during intubation in order to avoid the swallowing reflex and stress responses. However, the breathing frequency is low and unphysiological.
- Avoid an unphysiological drop in body temperature.

Figure 1: Timeline of animal preparation (A) and MR preparation (B) for fMRS on mice.
Figure 2: Orotracheal intubation of mice. Mice are first anesthetized using isoflurane. When the respiration frequency drops to ~30 bpm, the mouse is placed in supine position on a platform (at eye-level for the experimenter) and gently fixed at its tail and teeth (A). A tube placed underneath the elastic band holding the teeth is continuously providing the animal with anesthesia (B). A fiber-optic surgical lamp is placed on the throat of the animal, illuminating the trachea (B). A tube is inserted 2.5 cm deep into the throat; a mark previously drawn on the tube indicates the position of the teeth relative to the length of the inserted tube. (Photos by Giovanna Ielequa)

§2: Positioning, cannulation and paralysation

Once connected to the ventilation pump the level of isoflurane is at a controlled level of 2%, the respiration rate has been set to 80 bpm. Head and body of the animal are placed as straight as possible before fixing the head using ear bars. A rectal temperature probe is inserted; the body temperature should be maintained at above 35°C, body temperature being maintained using warm water circulation. If the experiment requires sensory stimulation, the stimulation device is mounted. For tail vein cannulation, a short polyethylene tube (~25 cm, PE-50 / 10, ID 0.28mm, OD 0.64mm, Warner Instruments, Hamden, CT, USA)) is filled with physiological saline and connected to a 30G needle and the needle inserted into the tail vein. The muscle relaxant pancuronium bromide, (1mg/kg; Sigma-Aldrich, Steinheim, Germany) is then administered intravenously (i.v.). The animal will be paralyzed 1-2 minutes following pancuronium administration. The catheter remains inserted into the tail vein for further drug administration if required by the experimental protocol. Once the preparation of the mouse is completed, the cradle is inserted into the magnet such that the head of the animal is positioned at the center of the pre-mounted rf receiver coil (see below, §3). The level of isoflurane is then reduced to 1.2 to 1.5 %). Following the MRS experiment, the mouse needs to stay connected to the ventilation pump until it has started to breath spontaneously.

§3: Preparation of MRS measurements

MRS studies require several preparatory steps including adjustments of the ref coils (tuning and matching), acquisition of anatomical images and field maps, adjustments of shims gradients, water suppression pulses and outer volume suppression (OVS). Reproducible positioning of the mouse allows reducing the optimization steps as pre-adjusted experimental parameters (power of rf pulses, shim sets, placement of OVS slices) parameters can be used as starting values.
A C57BL/6 mouse was anesthetized with isoflurane and intubated before being positioned on the mouse bed (optimized for measurements with a cryogenic coil). Warm water is circulating through tubings integrated in the cradle in order to maintain the core temperature of the animal above 36°C. Electrode needles were placed in both hind paws for electrical stimulation. A catheter was inserted in the tail vein serving for injection of muscle relaxant. Great care is taken in installing the mouse in a comfortable but straight position (the tail, the limbs and the body of the animal must be straight) in order to avoid inclination of the head. Earbars are used to further stabilize the head of the animal.

The coil setup used for fMRS studies described in Chapters 5 and 6 is a cryogenic coil prototype, which is positioned in the magnet isocenter using a guiding tube. The animal cradle is inserted in the magnet with the coil already in place. The position of the animal is fine adjusted with sub-millimeter precision on the basis of scout images. The measurements will be continued only when the conditions are judged as optimal. Accurate animal positioning is critical with regard to data quality and efficiency of fMRS measurements. Also we put emphasis on keeping to a strict time regimen during these preparatory steps.

![Image](attachment:animal_prepared_for_fmrs.png)

**Figure 3: Animal prepared for fMRS** A C57BL/6 mouse was anesthetized with isoflurane and intubated before being positioned on the mouse bed (optimized for measurements with a cryogenic coil). Warm water is circulating through tubings integrated in the cradle in order to maintain the core temperature of the animal above 36°C. Electrode needles were placed in both hind paws for electrical stimulation. A catheter was inserted in the tail vein serving for injection of muscle relaxant. Great care is taken in installing the mouse in a comfortable but straight position (the tail, the limbs and the body of the animal must be straight) in order to avoid inclination of the head. Earbars are used to further stabilize the head of the animal.

![Image](attachment:exact_position_magnet_bore.png)

**Figure 4: Exact position of animals in the magnet bore and relative to the coil.** The mouse bed is inserted in the magnet when the coil has already been mounted. The exact position of the animal in the magnet and relative to the coil can be adjusted with sub-millimeter precision (A). The head of the animal should not be tilted (B). The black line indicates the position of the magnet isocenter.

**Evaluation of quality and criteria for abortion of an experiment**

Adherence to the protocol described aims at reducing the physiological variability observed in fMRS data. This is illustrated e.g. by reduced variability in baseline lactate levels (figure 5, A). Lactate, as a metabolic product of glycolysis can indicate brain activity (Mangia et al., 2009) and is therefore one of the metabolites of interest in an fMRS study. Elevation in brain baseline lactate levels might therefore indicate stress and arousal. However, it could also reflect oxidative stress or prolonged exposition to...
high isoflurane levels due to prolonged preparation (Horn and Klein, 2010). Regardless of the cause, variability in baseline lactate levels indicates variability in the general physiological state of animals, and consequently influences the metabolic response to stimulation. Under those conditions, small changes in lactate levels induced by weak physiological stimuli may become difficult to detect. Figure 5B shows glutamate and lactate level in the right somatosensory cortex during electrical stimulation of the right hind paw (Chapter 6). Changes in metabolite signals are indicated for four groups of animals exposed to stimulus amplitudes of 0, 1, 2, and 3mA, respectively. The individual groups were measured over a period of several months, during which the protocol described above has become more rigorous. The 3mA group was measured last following the guidelines. As a result, less variability is observed in this dataset and a significant difference between activated region (right somatosensory cortex (S1_HP_L)) and non-activated region (left somatosensory cortex (S1_HP_R)) could be measured. We have therefore defined a set of criteria for data quality (Fig. 6). Experiments will be terminated upon a failure of an individual preparatory step or when the timing constraints for those steps could not be met. Sticking to the timing is considered critical as different levels of anesthesia are used during preparation. This ensures that the total exposure to the anesthetic is comparable across animals. A second abortion criterion is related to the body temperature. Body temperature should be maintained in the physiological range whenever possible. If it falls below 33°C, preparation is aborted and the animal allowed recovering. The experiment will be carried on if the spectra quality seems reasonable according to the measured line width (~ 15 Hz) and the efficiency of the water suppression (residual water signal ≤ NAA signal), and OVS (absence of unwanted lipid signals).

Figure 5: Non-consistent baseline lactate levels and the influence of animal preparation on data variability. Mice prepared for fMRS have to be intubated, cannulated and paralyzed. If these procedures are not executed in a controlled way, animals might begin the MR examination in very different physiological conditions, as reflected by the lactate levels (A). As a consequence, high variability is observed in the metabolic response to an electrical stimulus (B) in the groups stimulated with 1 and 2 mA. A protocol with very strict guidelines has been applied in the last group (3mA), and variability could be significantly reduced.
**Animal recovery**

The use of isoflurane at a moderate level allows rapid emergence from anesthesia, and no complications have been observed due to orotracheal intubation or cannulation. Animals must be ventilated and therefore should remain anesthetized until they are able to breathe spontaneously. During this phase the degree of paralysis is regularly evaluated by testing muscle twitch responses. The necessary recovery time can extend until 2 hours after the last administration of pancuronium.

![Diagram](image)

**Figure 6:** Criteria to assess the quality of animal preparation, impacting baseline metabolite values and their evolution during fMRS experiments. Several criteria would lead to the abortion of the experiment. Strict timings need to be respected especially during the first phase of preparation, when isoflurane is set to 3.5 %. High isoflurane levels are necessary for intubation; however they lead to unphysiological respiration rate and serious hypothermia if the animal is not properly warmed. The temperature cannot be monitored during this phase, and will always drop by 2-3°C. At this stage, the experiment should be strictly aborted if timing cannot be observed. In the second phase, the experiment is immediately aborted if the temperature drops below 33°C, which is unlikely to happen if previous timings have been respected, or if cannulation fails (as paralysation is mandatory for fMRS). In general, if the second phase extends over 15 min (often because several attempts are made for cannulation), the experiment should be aborted. If the head is not perfectly straight after several corrections, the experiment will be continued but the data might be discarded later, depending on the spectra quality. In perfectly positioned animal thinner line widths and better water suppression can be achieved. Muscle relaxant should be re-injected before stimulation. If not, movement is often observed and the data must be discarded.
Discussion and conclusion

The physiological status of the animal in the MR scanner has an important effect on detectability as well as quantification of functional brain activation and metabolic processes using MRI techniques. Particularly in mice it is difficult to maintain physiological variables within the narrow boundaries required for measuring hemodynamic responses or metabolic changes elicited by neural activity. Therefore, many studies suffer from effects caused by variation of measurement conditions, which has a negative impact on reproducibility. Small animals generally require anesthesia for immobilization and reduction of stress effects during MRI protocols. Type of anesthesia and anesthetic dose should be chosen such that the animal is appropriately immobilized. Nevertheless, responses or processes of interest should be only minimally affected mimicking the awake state as close as possible. For anatomical measurements or for simple functional paradigms, ventilation of the mouse via face mask seems appropriate as preparation time of the animal for the measurement will be short reducing potential interference with physiology. However, reproducible assessment of small changes in signal amplitude in response to a challenge requires a high stability of the reference state (typically the baseline state). As physiological instability is the major source of signal fluctuations any step to minimize this contribution should be considered. Our data show that artificial ventilation and proper fixation of the animal using a ‘pseudo-stereotactic’ setup improves the data quality. This includes adherence to the time schedule, i.e. the protocol has to be standardized with regard to the sequence and the timing of the individual steps. Anesthetic level, general stress responses, and in particular, body temperature, heart beat and blood gases affect the hemodynamic response, which is the measurement variable for brain activation in fMRI, but they also strongly determine metabolism. Hence, it is critical to expose animals to reproducible conditions, i.e. standardized procedure.

References


List of Publications

Journal articles


**Book Chapter**


**Conference papers**


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