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WHY PRECLINICAL MRS ?

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MRI Practicals on CIBM preclinical imaging systems

EPFL

PRECLINICAL MRS: WHAT?

- MRS applied on animal models for scientific questions that cannot be probed directly with human MRS
- The scope of this presentation will focus on rodent models (rats and mice), and on applications to brain studies.

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PRECLINICAL MRS: WHAT?

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SPECIAL ISSUE REVIEW ARTICLE

NMR IN BIOMEDICINE WILEY

Magnetic resonance spectroscopy in the rodent brain: Experts' consensus recommendations

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NMR in Biomedicine Special Issue
Advanced Magnetic Resonance Spectroscopy
Experts' Consensus Recommendations

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C I B M . C H

PRECLINICAL MRS: WHY?

- Usefulness of preclinical MRS (rats and mice in most cases)
  - Ethical considerations (ultimately, the goal is not to find ways to cure rodents…)
- Usefulness in terms of biological aspects
  - Animal models
- Usefulness in terms of MR physical aspects
  - New MR acquisition approaches

PRECLINICAL MRS: WHY?

- Usefulness in terms of biological aspects
  - Rats and mice are good biological models of tissue development, function and metabolism

  - Mimicking multiple aspects of the human disease (Transgenic mouse models)
  - *In vivo* state : leaving tissue works as a whole, not as the sum of its components.

    Cell cultures can be used for basic cellular/metabolic questions, while *in vivo* studies are key for complex organ functions.
  - Non invasively characterize disease progression, effect of treatments (longitudinal studies)

PRECLINICAL MRS: WHY?

- Usefulness in terms of MR physical aspects
  - The availability of MRI/MRS systems at magnetic field strength of 9.4 T and higher enables studies with high sensitivity and spectral resolution
  - Use of anaesthetics enables long experimental acquisition with minimal motion artefacts and instabilities
  - Opens the way to study deeper biochemical pathways with dynamic MRS (e.g. ^{13}C MRS)
  - Lower restrictions on the gradients and RF limits enable the developments of new MR approaches at lower risk

ANIMAL PHYSIOLOGY AND ANESTHESIA

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Effective and standardized rodent MRI/MRS studies require attention to many aspects of the experimental design.

- Anaesthesia is critical for *in vivo* preclinical MRI and MRS
  - (+) decreases the stress of the animals, potential pain in case of surgical intervention, biological motions
  - (-) decreases respiratory and cardiac activities
- MRS in awake rodents (very few studies)
  - MRS with awake rodents is challenging and requires a relatively long training period[1,2]
  - For awake-rodent MRS studies, monitoring serum cortisone levels and heart rate of the animals is recommended

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[1] Xu et al., MRM 2013 [2] Low et al., Pain. 2016

ANIMAL PHYSIOLOGY AND ANESTHESIA

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TABLE 2 Characteristics of commonly used anesthetics and their impact on brain metabolites

	Physiological effects	Side effects	Effect on brain metabolites↓↑* statistically significant changes($p < 0.05$)	Type	References
Propofol	Rapid and short-acting anesthesia effect, fast recovery time	Muscle twitching, apnea, hypotension, decreased cardiac output	Lactate ↓, glutamate ↓* (compared with isoflurane)	Injectable	[20,21]
Halothanes (e.g. isoflurane, sevoflurane)	Rapid and short-acting anesthesia effect, fast recovery time	Respiratory depression, dose dependent hypotension, increased cerebral blood flow, immune suppression	Lactate ↑, GABA ↑, choline-containing compounds ↑, *myo*-inositol ↑, glucose ↓, NAA ↑, total creatine ↑, creatine ↑, glutamate ↑, glutamine ↓, alanine ↑ * (compared with without isoflurane)	Inhaled	[22,23]
Thiopental	Ultra-short acting	Severe tissue necrosis (if administered via non-i.v. routes), prolonged recovery if the animal has low body fat, myocardial depression, decreased cardiac output, hypotension	Glucose ↑* (compared with light alpha-chloralose)	Injectable	[20,24]
Pentobarbitone	Poor analgesia characteristics (more reliable for rats than for mice)	Hyperexcitability, significant cardiovascular depression in mice, hypotension in rats	GABA ↓ glucose ↓, taurine ↓, propylene glycol ↑* (compared with isoflurane), glucose ↑ (compared with light alpha-chloralose)	Injectable	[20,25–27]
Ketamine	Rapid analgesia but less muscle relaxation	Respiratory depression, pain in injection side (due to low pH), increased cardiac output, heart rate, blood pressure	Glutamate ↓* (^{1}H-^{13}C NMR study; 80 mg per kg ketamine treated group compared with saline treated group)	Injectable	[18,20,28]
Xylazine/ketamine	The synergistic effect causes anesthesia with extended analgesia	Body temperature may decrease, increased urination, defecation, salivation, ocular lesions, hypoglycemia	Alanine ↓, ascorbate (or vitamin C) ↑, aspartate ↑, GABA ↑, glycine ↑, PCr ↑ (compared with isoflurane)	Injectable	[27,29]
Urethane	Provides long-lasting anesthesia	Mutagenic and carcinogenic in experimental animals	Lactate ↑ (compared with no urethane group)	Injectable	[30,31]
Alpha-chloralose	Provides long-lasting light anesthesia	Poor analgesic properties, prolonged and poor recovery	Unknown	Injectable	[32]

PRE-ANESTHETIC CONSIDERATIONS

FOR IN VIVO MRS STUDIES

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Stress, strain, sex, circadian cycles, weight and age of the animals affect:
- effectiveness of anaesthesia
- direct impact on MRS measurements of the neurochemical profile

- The correct dosage of the anaesthetic should provide adequate sedation but also adequate analgesia and less variability in physiological parameters during MRI/MRS experiments
- Monitoring and recording the respiration rate and temperature of animals under anaesthesia is essential (if available, pulse oximetry and electrocardiography can provide further control)

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SUBJECT FEEDBACK

Major practical difference for the experimentalist

- Human MRS scans are rarely performed over longer periods (>45 minutes) and typically split into 10 minutes scans blocks

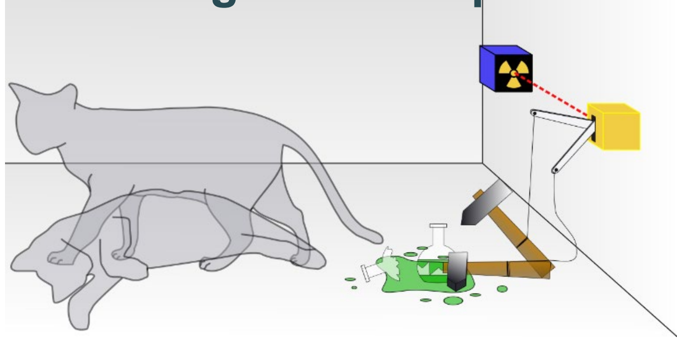
  BUT -> You can have a direct feedback from the subject

- Rodent MRS scans can be performed over extended periods (> 4 hours) and are less prone to motion

  BUT -> You cannot have a direct feedback from the subject

  -> The animal is invisible in the magnet

Schrödinger's cat experiment



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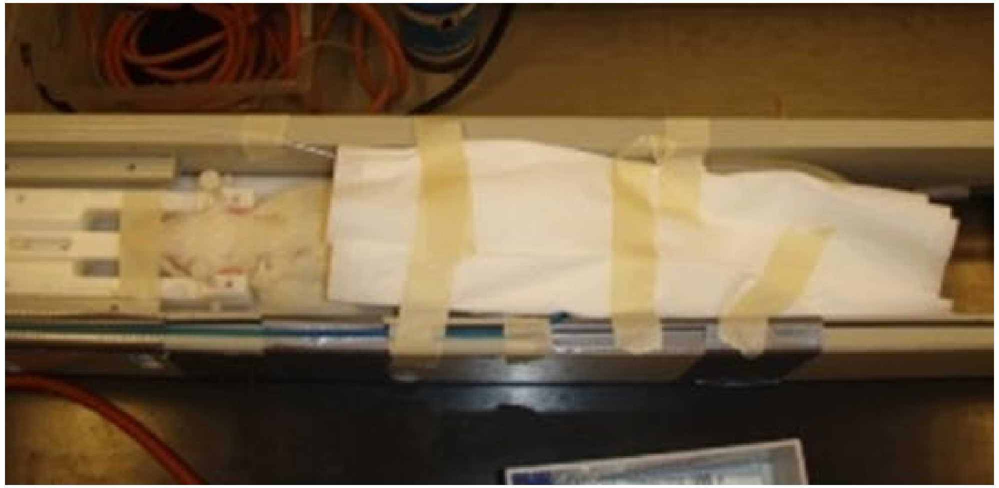
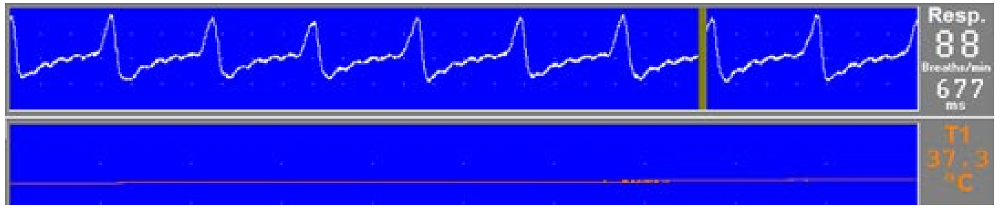
ANIMAL PHYSIOLOGY MONITORING AND REGULATION

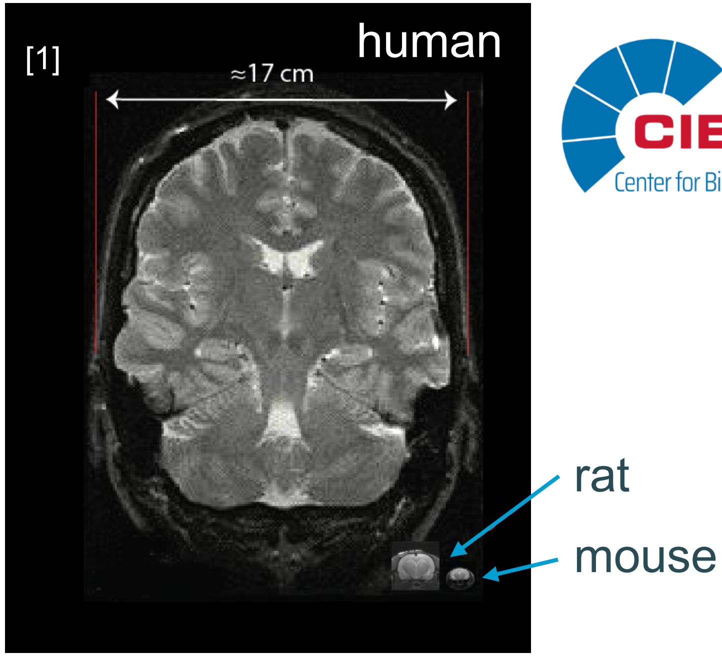




HARDWARE

Main differences with human MRS:

- small brain size
- strong B_0 inhomogeneity induced in the brain by the air/tissue interface

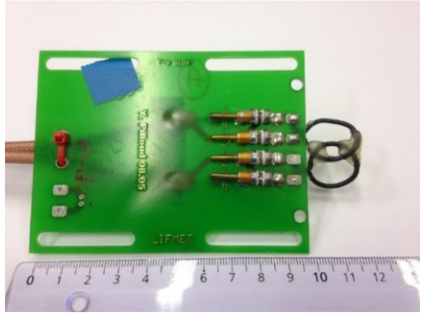


- Small VOIs (rats 50-150 µL, mice 2-15 µL) benefit from ultra-high field (UHF) (≥ 9.4T)
- higher requirements on gradient strength (ideally ≥400 mT/m) compared with that for human systems (typically 70 mT/m for 7 T clinical MR systems)
- Ultra-high field -> increased chemical shift dispersion but increased B_0 inhomogeneity requires efficient shimming methods and shim system (FAST(EST)MAP[2,3] or 3D B_0 mapping[4])

[1] in 't Zandt, Proc. Intl. Soc. Mag. Reson. Med. 24 (2016) [2] Gruetter, MRM 1993 [3] Gruetter and Tkac, MRM 2000 [4] Miyasaka et al., MRM 2006

HARDWARE

RF aspects



Surface Tx/Rx

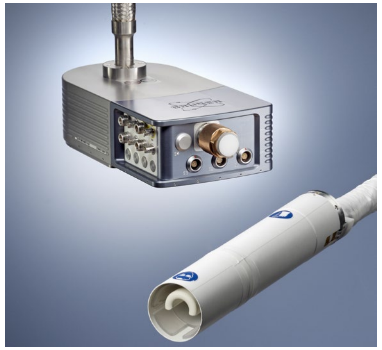
Volume Tx, array Rx

- Lower RF power requirements (much smaller and more efficient coils)
- Surface coils provide much higher SNR from regions close to the RF coil and higher B_1 efficiency than volume coils, but B_1 field is spatially inhomogeneous
- adiabatic RF pulses can mitigate B_1 inhomogeneity
- Contrary to humans, legally unlimited B_1 and strong gradients enable the optimum RF coil choice for optimal SNR and chemical shift displacement error

The problem of tissue heating is still present and should be considered, especially at UHF

HARDWARE

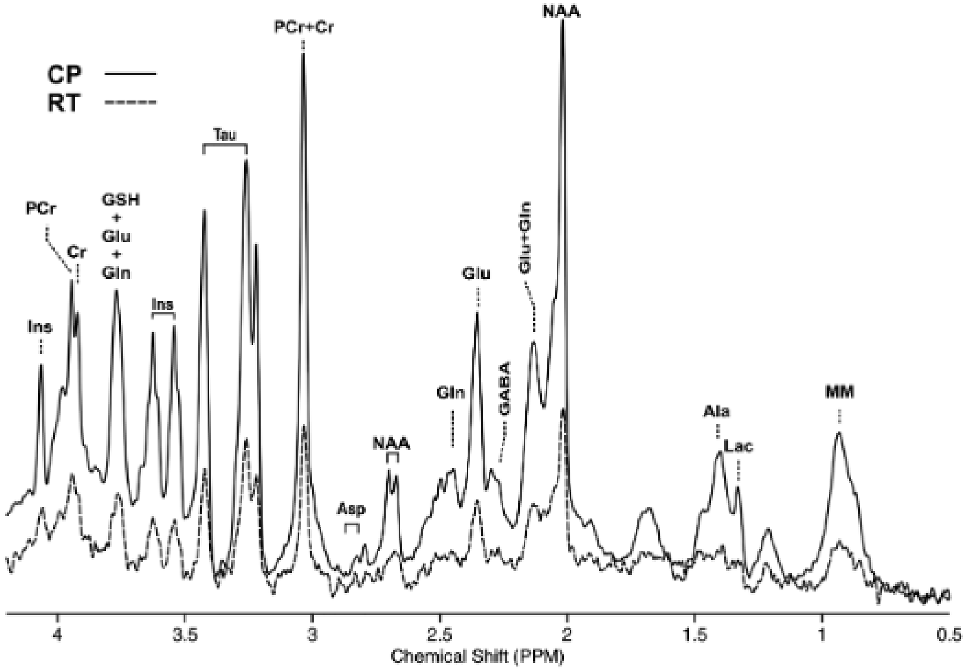
RF aspects



Cryo-coils

- For small sample volumes, the thermal noise in the coil and the receive pathway is the dominant noise source
- Cryogenically cooled RF coils can be used[1-4]

FIGURE 1 Spectra acquired with the STEAM sequence (TR/TE = 5000/3.5 ms, 384 averages) in a 2.0 × 1.1 × 2.0 mm^3 VOI located in the mouse frontal cortex. A cryogenically cooled ^{1}H two-element phased-array transmit/receive coil was employed for excitation and signal reception (solid line). As a comparison, a 72 mm diameter birdcage quadrature volume resonator was used for excitation and a ^{1}H receive-only 2 × 2 surface array coil was used for signal reception (dotted line). A 5.2-fold higher SNR was obtained with the cryoprobe (CP) compared with the room-temperature probe (RT)



[1] Kovacs et al., Prog Nucl Magn Reson Spectrosc. 2005 [2] Baltes et al., NMR Biomed. 2009
[3] Mispelter et al., Imperial College Press; 2006 [4] Junge, *eMagRes*. 2012

SEQUENCES AND ACQUISITION PROTOCOLS:

^{1}H MRS

- ultra-short-TE (≤10 ms) spectroscopic localization sequences are usually possible to achieve
- preferentially used because they provide the most accurate quantitative information (minimal J-evolution and T_2 losses)
- TR of 4-5s are typically used (minimizes signal attenuation due to T_1)

SEQUENCES AND ACQUISITION PROTOCOLS:

^{1}H MRS

localization performance of a ^{1}H MRS sequence is very important

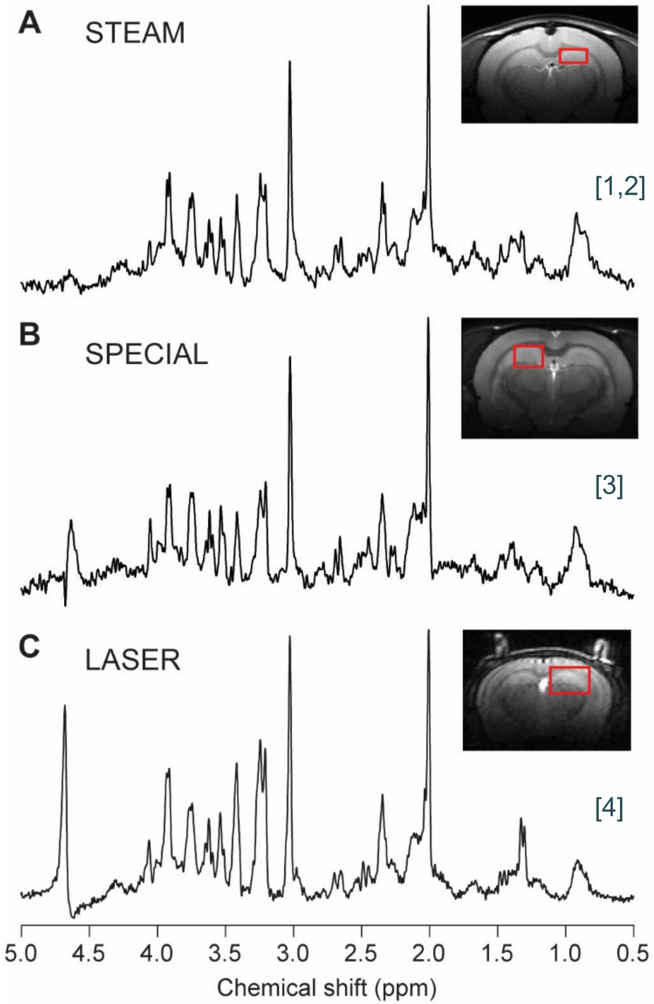
(1) an ability to detect signals originating from the VOI

(2) an ability to suppress signals from outside of the VOI

(3) minimal CSD error related to the bandwidth of the localization pulses

(4) insensitivity to B_1 inhomogeneity, especially when using surface coils

(5) efficient water suppression is important to eliminate the strong water signal



@ 9.4 T
(A) STEAM spectrum: rat brain, 2.3 x 1.3 x 2.5 mm^3 voxel placed in the hippocampus, TR = 5 s, TE = 2 ms, TM = 20 ms, number of averages = 448. Spectrum is shown with Gaussian factor = 0.15.
(B) SPECIAL spectrum: rat brain, 2 x 2.8 x 2 mm^3 voxel placed in the hippocampus, TR = 4, TE = 2.8 ms, number of averages = 160.
(C) LASER spectrum: mouse brain, 1.7 x 2.25 x 2.25 mm^3 voxel placed in hippocampus, TR = 4 s, TE = 27 ms, number of averages = 384.

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[1] Frahm et al., J Magn Reson. 1987 [2] Tkac et al., MRM 1999 [3] Mlynarik et al., MRM 2006 [4] Garwood and DelaBarre, J Magn Reson. 2001

SEQUENCES AND ACQUISITION PROTOCOLS:

^{1}H MRS

TABLE 3 Comparison of features of ^{1}H MRS localization pulse sequences used in preclinical studies.

Sequence characteristics	STEAM [1,2]	SPECIAL [3]	LASER [4]
Fraction of available signal (%)	50	100	100
Single-shot method	yes	no	yes
Localization performance	++	++	+++
Sensitivity to B_1 inhomogeneity	––	––	–
Sensitivity to motion	–	–––	–
TE (ms)	2	2.8	15-28
CSDE/ppm in 3 directions at 9.4 T	(9, 9, 9%)[a]	(4, 12, 4%)[b]	(2.4, 2.4, 2.4%)[c]
Flexibility for spectral editing	+++	+++	++
Requirement of T_2 or $T_{1\rho}$ decay knowledge for quantification	no	no	yes

For this table, the original form of SPECIAL is considered rather than semi-adiabatic form of SPECIAL. STEAM refers to the in-house implementation of the typical vendor provided STEAM sequence with improved features, such as shorter TE, better localization and OVS performance.

The evaluation of the localization performance considers the sequences as currently implemented, including OVS modules for STEAM and SPECIAL.

The requirement for $B_{1\ max}$ is not very different between sequences because to achieve such short TE for STEAM and SPECIAL very short localization pulses (which require high B_1) are used.

Large numbers of + signs indicate positive attributes, e.g. enhanced localization performance.

Large numbers of – signs indicate negative attributes, e.g. increased motion sensitivity.

[a] 0.5 ms 90° asymmetric sinc pulses for three directions.

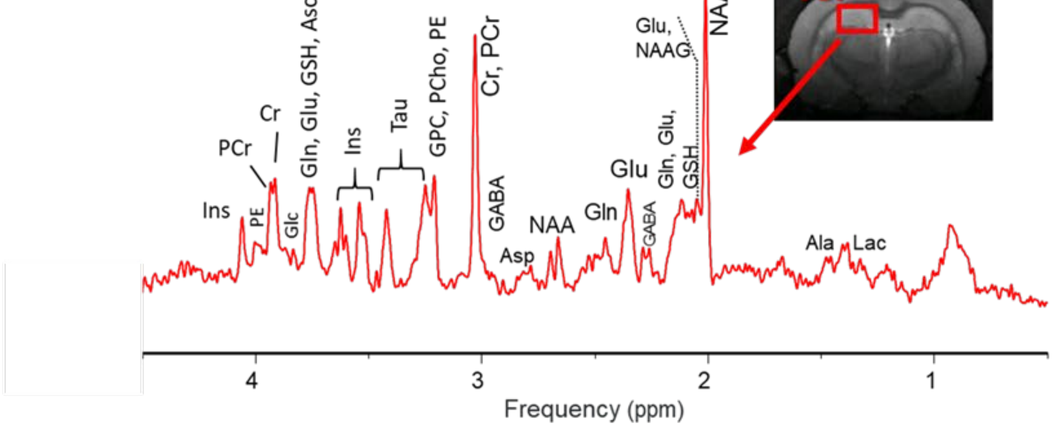
[b] 0.5 ms 90° and 180° asymmetric sinc pulses for excitation and refocusing; 2 ms AFP for inversion in the 1D ISIS.

[c] 4 ms AHP (non-selective) pulse for excitation and six 1.5 ms AFP pulses for refocusing.

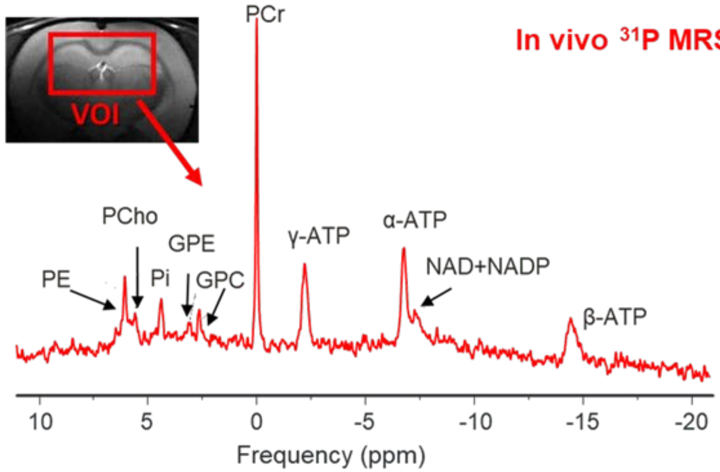
[1] Frahm et al., J Magn Reson. 1987 [2] Tkac et al., MRM 1999 [3] Mlynarik et al., MRM 2006 [4] Garwood and DelaBarre, J Magn Reson. 2001

X NUCLEI MRS – 9.4T

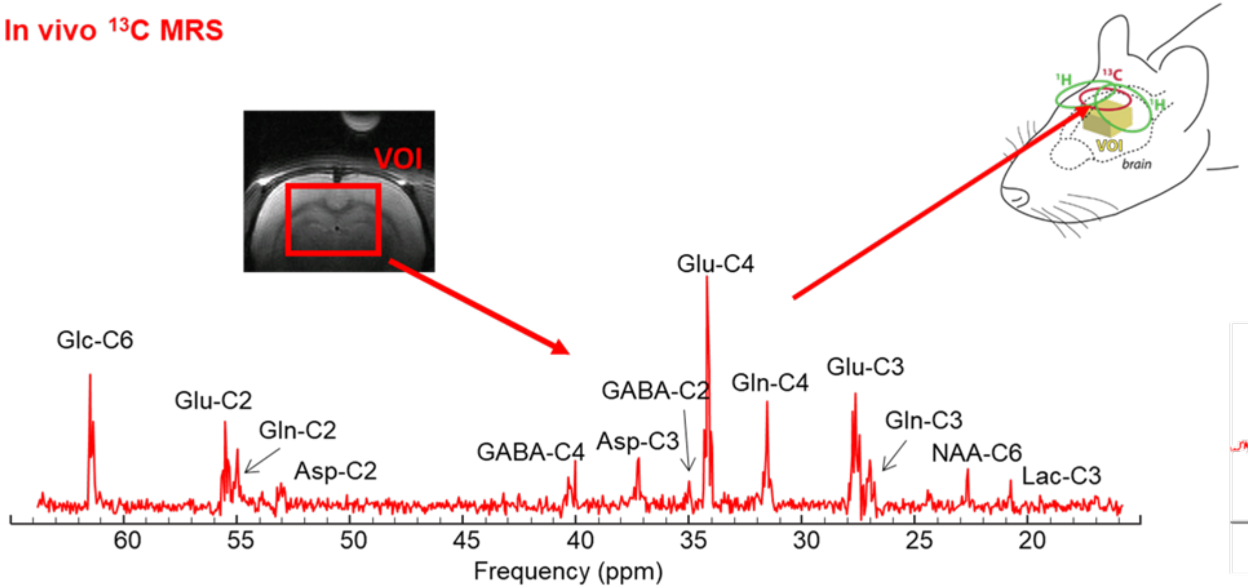
In vivo ^{1}H MRS



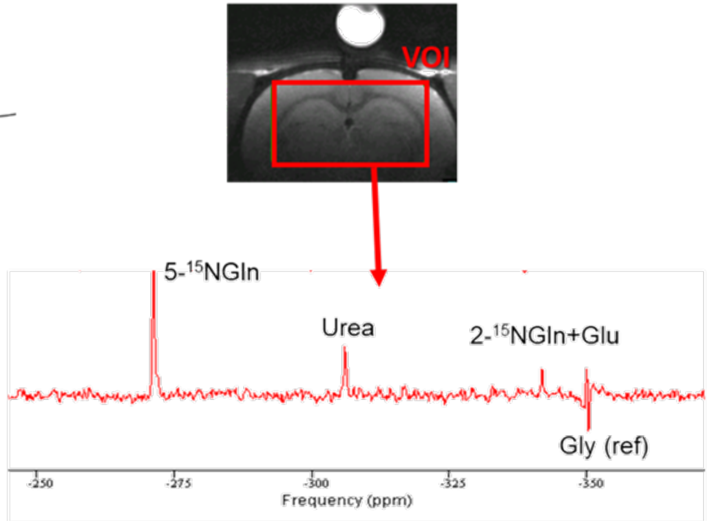
In vivo ^{31}P MRS



In vivo ^{13}C MRS



In vivo ^{15}N MRS



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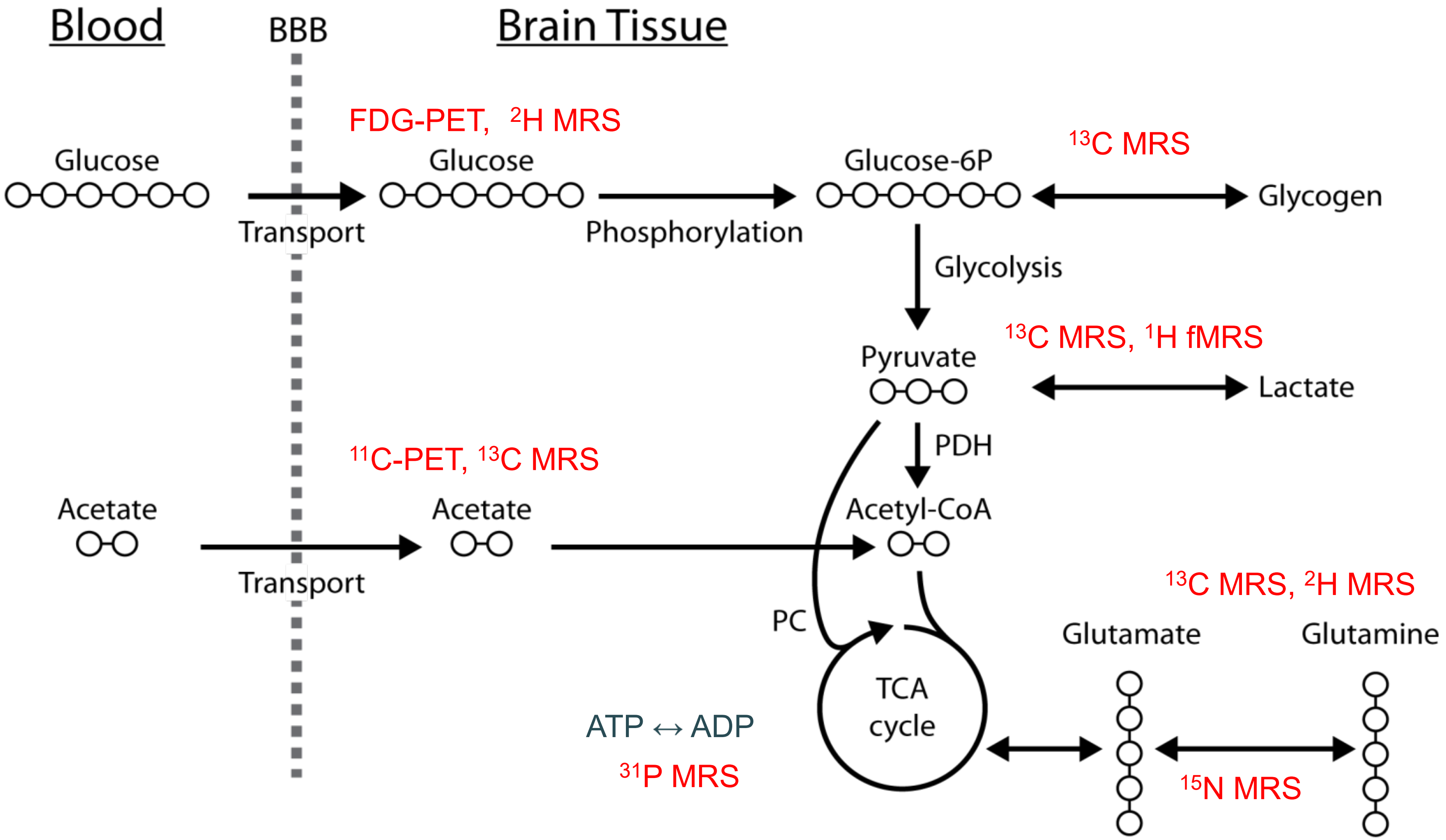
Lanz et al, 2017, 2020; Cudalbu et al 2021;

MAIN BIOCHEMICAL PATHWAYS
IN BRAIN ENERGY METABOLISM



THANK YOU FOR YOUR ATTENTION

Questions ?

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