Research topics in $^{13}$C MRS

- Neurotransmission
- $^{13}$C MRS
- Hyperpolarized $^{13}$C MRS

References
Neurons and Neurotransmission

- Neurons carry action potentials, but are not directly connected.
- Axon-dendrite connections rely on chemicals (neurotransmitters).
- Neurotransmitters can be excitatory or inhibitory.

Glutamate is the major excitatory neurotransmitter in the human brain with >80% of synapses utilizing glutamate.

Thomas C. Südhof
Stanford University

The Nobel Prize in Physiology or Medicine 2013
The brain in more than just neurons..

Complex metabolic interactions exist between glia and neurons.

Energy metabolism is coupled with neurotransmission.
Neurotransmitter Cycling

Excitatory

Neuron energy cycle

Astroglial energy cycle

Glutamate-Glutamine cycle

Probing Brain Function with Carbon

Probing Brain Function with $^{13}$C MRS

Infuse $^{13}$C-glucose (or $^{13}$C-acetate)

Collect spectra

Quantify spectra over time

Mathematical model

$V_{TCAn} =$ neuron TCA flux

$V_{TCAg} =$ glial TCA flux

$V_{NT} =$ Glu/Gln neurotransmitter flux
Metabolic Modeling

\[
\text{dGlu}_4 \quad \text{dt} = \frac{L_1}{L} \text{Vgly} + \frac{\text{Gln}_4}{\text{Gln}} \text{Vcycle} + (0)V_{dil} \quad - \frac{\text{Glu}_4}{\text{Gln}} (V_{tca} + V_{gln}),
\]

\[
\text{dGln}_4 \quad \text{dt} = \frac{\text{Glu}_4}{\text{Gln}} (V_{cycle + Vefflux}),
\]

\[
\text{dGlu}_3 \quad \text{dt} = \frac{1}{2} (1) \text{Vana} + \frac{1}{2} (0) \text{Vana} + \frac{1}{2} \text{Glu}_4 + \frac{1}{2} \text{Glu}_{tca \text{net}} + \frac{1}{2} \text{Glu}_{vca \text{net}} + \frac{1}{2} \text{Glu}_{tca \text{net}} + \frac{1}{2} \text{Glu}_{vca \text{net}} \quad - \frac{\text{Glu}_3}{\text{Gln}} (V_{tca} + V_{gln}),
\]

\[
\text{dGln}_3 \quad \text{dt} = \frac{\text{Glu}_3}{\text{Gln}} (V_{cycle + Vefflux}),
\]

\[
\text{dGlu}_{3,4} \quad \text{dt} = \frac{L_3}{L} \text{Glu}_3 + \frac{\text{Gln}_{3,4}}{\text{Gln}} \text{Vcycle} \quad - \frac{\text{Glu}_{3,4}}{\text{Gln}} (V_{tca} + V_{gln}),
\]

\[
\text{dGln}_{3,4} \quad \text{dt} = \frac{\text{Glu}_{3,4}}{\text{Gln}} (V_{cycle + Vefflux}),
\]

\[N_{V_{TCA}}, \text{rate of neuronal TCA cycle}\]

\[A_{V_{TCA}}, \text{rat of astroglial TCA cycle}\]

\[V_{cycle}, \text{rate of Glu-Gln cycle};\]

Mason, et al., Metabolic Engineering 6, 75-84, 2004
Probing Brain Function with $^{13}$C MRS

![Chemical structures of Glutamate and Glutamine]

$^{13}$C and $^1$H spectra from human visual cortex during an infusion of [1-$^{13}$C]glucose.

$^{13}$C MRS: ~45 cc, 10 min acq

$^1$H MRS: ~10 cc, 10 min acq
Hardware

de Graaf, et al, NMR Biomed 2011
Multinuclear Pulse Sequences

Direct Detection

For in vivo $^{13}$C-infusion brain studies (e.g. labeled glucose or acetate) …

- $^{13}$C spectrum: low sensitivity, excellent peak discrimination.
- Simple $^{13}$C excitation and detection is rarely used do to poor sensitivity
- Polarization transfer is commonly used: INEPT, DEPT
- Almost all methods use decoupling.

4T $^{13}$C spectrum obtained from a 45 ml volume in the human visual cortex during an infusion of 67%-enriched [1-$^{13}$C]glucose. (DEPT sequence).

Polarization Transfer

• MRI sensitivity given by

\[ \text{SNR} \propto \frac{\left( \frac{\gamma \hbar B_0}{2kT} \right) \left( \frac{\gamma}{2} \right) (\gamma B_0)}{\text{noise}} = \frac{\gamma \hbar^2 B_0}{4kT} \]

• In polarization transfer, we seek to exploit $^1\text{H} - ^{13}\text{C}$ J coupling to find a pulse sequence with sensitivity given by...

\[ \text{SNR} \propto \frac{\left( \frac{\gamma_H \hbar B_0}{2kT} \right) \left( \frac{\gamma_C \hbar}{2} \right) (\gamma_C B_0)}{\gamma_C B_0} = \frac{\gamma_H \gamma_C \hbar^2 B_0}{4kT} \]

• Given $\gamma_H \approx 4\gamma_C$, this will yield a 4x sensitivity increase!
Methods: INEPT

Human studies, occipital lobe, 4T

de Graaf, et al, NMR Biomed 2011
A Key Result…

- Direct linkage between neuroenergetics and neurotransmitter flux
- 1:1 relationship between neuronal TCA and Glu/Gln cycling rates

Applications

• $^{13}$C MRS provides the only noninvasive measurements of neurotransmitter cycling and cell-specific neuroenergetics.

• Major contributions to understanding…
  – Metabolic coupling between neurons and glia.
  – High neuronal activity of resting brain.
  – Alternations in neurological and psychiatric disease.

• Pathologies include: depression, drug addiction, epilepsy, metabolic disorders, hepatic encephalopathy, and neurodegenerative disorders.

Example: Aging

Ye old neurons just are firing like they used to.

Hyperpolarized $^{13}$C MR
A Complementary Method to PET for Imaging In Vivo Metabolism

Daniel M. Spielman, Ph.D.
Dept. of Radiology
Stanford University
Email: spielman@stanford.edu
PET/MR/\textsuperscript{13}C Center for Metabolic Imaging
Cancer and the Warburg Effect

Differentiated tissue

$+O_2$ Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$

Oxidative phosphorylation
-36 mol ATP/mol glucose

$-O_2$ Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate

Anaerobic glycolysis
2 mol ATP/mol glucose

Proliferative tissue

Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$

or $+/-O_2$

Tumor

Glucose $\rightarrow$ Pyruvate

Aerobic glycolysis (Warburg effect)
-4 mol ATP/mol glucose

Lactate

Energy production while preserving biomass
Metabolic Therapy Challenges

• Metabolic reprogramming represents a shifted balance towards glycolysis (GLY) from oxidative phosphorylation (OXPHOS)

• Malignant glioma are ideal candidates
  – Highly resistant to conventional treatments
  – Robustly manifest metabolic reprogramming

• Critical clinical obstacle: robust measurement of response

Proposed metric: Metabolic therapy index = \[
\frac{\text{[Glycolysis]}}{\text{[OXPHOS]}}
\]

How can we measure GLY/OXPHOS in vivo?
Hyperpolarized $^{13}\text{C}$ MRS

**Hypothesis:** Imaging of hyperpolarized [1-$^{13}\text{C}$]pyruvate can provide one such metabolic therapy index.
PET vs Hyperpolarized $^{13}$C MRS

- **Key idea**: inject a “magnetically” enhanced biological substrate and image both the substrate and its downstream metabolic products.

**Key technology**: A polarizer that magnetically prepares the substrate to boost its MR visibility by >10,000 fold.
MR Sensitivity

- Thermal equilibrium magnetization:

\[ M_0 = \rho \frac{\gamma^2 \hbar^2 B_0}{4kT} = \rho\left(\frac{\gamma \hbar}{2}\right)\left(\frac{\gamma \hbar B_0}{2kT}\right) \]

- Increasing sensitivity
  - Increase \( \rho \) (e.g. isotopically enriched \(^{13}\)C substrates vs natural abundance)
  - Increase polarization:
    - Higher \( B_0 \)
    - Lower \( T \)
    - Change “effective” \( \gamma \)??

In vivo hyperpolarized \(^{13}\)C via DNP exploits all of these effects!
Hyperpolarization

- **Hyperpolarization**: creating nuclear spin polarization much greater than that achieved at normal thermal equilibrium.

\[ \approx 10^{-5}\% \]

\[ \approx 25-50\% \text{ (or higher)} \]

MR signal proportional to population difference!
Hyperpolarization

- Increases net nuclear magnetization
- Does **NOT** change chemical properties.

Magnetic Field

Thermal equilibrium

Hyperpolarized

Nuclei are small dipole magnets

MR signal proportional to the net magnetization!
Brute Force

Boltzmann distribution at 3T

Lower range of temperatures achievable using liquid helium and vacuum pumps
Dissolution DNP

Step 1: Polarization (~2 hrs)
- $^{13}$C-labeled substrate + free radical
- 5T magnet, 1K temperature, 20 mM microwave

Step 2: Dissolution (~30 s)
- Water is heated and pressurized
- Melts the sample and fills the syringe
- Quality control checks before syringe is released

Inject and image before signal disappears (~ 3 min)!

40% polarization ~ 50,000 fold signal gain!
In vivo $T_1 = 40$ s
In Vivo Imaging Requirements

- Low toxicity (mM conc.)
- Long NMR relaxation times
- Chemical shift separation
- Rapid cellular uptake
- Rapid metabolism

Signal decays by relaxation and dilution

Focus on low molecular weight endogenous compounds.

Example: [1-^{13}C]pyruvate

25% polarization ~ 30,000 fold signal gain!
In vivo $T_1 = 30$ s
Hyperpolarized \([1^{-13}\text{C}]\text{Pyruvate}\)

C6 rat glioma model

Hyperpolarized \([1^{-13}\text{C}]\text{Pyr}\) imaging

Proposed metric: \(\text{Metabolic therapy index} = \frac{[13\text{C-Lactate}]}{[13\text{C-Bic}]}\)
First clinical trial of hyperpolarized [1-$^{13}$C]Pyr completed in 2012.
Proton Perfusion Delayed enhancement Alanine Bicarb

15 min occlusion (stunned) + 2 hrs reperfusion

45 min occlusion (infarcted) + 2 hrs reperfusion
PET + Hyperpolarized $^{13}$C

- Opportunity: currently only two Spinlab systems installed adjacent to PET/MR scanners.

- $^{18}$F-FDG and $^{13}$C-pyruvate
  - Measures glucose metabolic pathway at multiple points: uptake vs. glycolysis/oxidative phosphorylation balance
  - Tumors hot on FDG: initial studies (animals) show elevated $^{13}$C Lac labeling
  - Tumors cold on FDG: hyp $^{13}$C-pyr ?
  - Inflammation: FDG vs. hyp $^{13}$C-pyr ?

- Other tracers/substrates
PET + Hyperpolarized $^{13}$C

Rat ENU tumor model

Are there synergies to exploit with simultaneous PET/HP$^{13}$C?
Cancer and the Warburg Effect

Will reversing the Warburg Effect slow tumor growth?

Differentiated tissue

+O₂

Glucose → Pyruvate → CO₂ → Oxidative phosphorylation

- O₂

Glucose → Pyruvate → Lactate

Anaerobic glycolysis

2 mol ATP/mol glucose

Proliferative tissue or

+/- O₂

Glucose → Pyruvate → CO₂ → Aerobic glycolysis (Warburg effect)

5% 85%

5% 85%

O₂

Pyruvate → Lactate

Lactate

-4 mol ATP/mol glucose
Rat C6 Glioma Bevacizumab Study

Note: no correlations found with respect to tumor size.

Decreased Lac/Bic ratio predicts survival
Clinical Hyperpolarized $^{13}$C Studies

- FDA IND ##135767—“Hyperpolarized [1-13C]Pyruvate Injection”,
- Stanford IRB, protocol #39845—“A Pilot Study to Assess Lactate and Bicarbonate Detection within Malignant Brain Tumors Using [1-13C]-pyruvate DNP Magnetic Resonance Spectroscopy (MRS)”. 

**Step 1:** Pharmacy kit preparation 24 hrs prior to injection

**Step 2:** Polarization 2.5-3 hrs prior to injection

**Step 3:** Dissolution and quality control checks ~90s prior to injection

**Step 4:** Pyr injection and MRI scan 2-3 min
Recruitment - volunteers

Volunteers wanted for a pyruvate tracer Research Study

Looking for healthy volunteers

We are conducting a research study to assess the safety of infusing 13C-labeled hyperpolarized pyruvate (HP-pyruvate) prior to performing magnetic resonance imaging (MRI).

The study requires 3 visits:
Visit 1: Review and sign consent form, medical history review, vital signs, ECG, and blood draw (Approximately 2 hrs)
Visit 2: MRI using HP-pyruvate and observation (Approximately 2 hrs)
3-5 hours later: Vital signs and ECG (Approximately 30 mins)
Visit 3: Follow up visit or telephone call (Approximately 30 mins)

Participants will receive financial compensation for their time spent in this research study. There will be no direct benefits to you from participating in this study, but the knowledge gained from this study may help future cancer patients.

Key eligibility requirements:
- Must be 18 years old or older
- Must not be undergoing active treatment for malignancy
- No history of allergic reactions to MRI contrast (gadolinium)
- Must be able to undergo an MRI
- Volunteers cannot be pregnant or nursing
- Please note that this is an abbreviated list of study requirements.

If you are interested, please contact the research nurse below for more information

Stephanie Lewis, RN, MSN
Email: lewisste@stanford.edu
Tel: (650) 723-0381
First Stanford Human Hyperpolarized $^{13}$C Results

Subject 1

Subject 2
BPM31510 Phase II GBM Trial

Trial as planned

Proposed modification with HP $^{13}$C-pyruvate
Next lecture: Brain metabolic changes during heart/lung bypass surgery